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Combined Ozone and Ultraviolet Inactivation of Escherichia Coli

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COMBINED OZONE AND ULTRAVIOLET INACTIVATION OF *ESCHERICHIA COLI*

By

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The kinetics of *Escherichia coli* inactivation were studied using ultraviolet (UV) radiation, ozone, and UV and ozone (UVO) in combination in a batch reactor at varying pH levels (6, 7, and 8) and at a constant temperature of 25°C. The inactivation kinetics for all three treatment processes were pseudo first order, and the reaction rate constants were considered to be additive such that a combined reaction rate could be obtained by adding the kinetic rates of the processes applied and numerically small rates could be neglected in the computation of the combined rate. Statistical tests (ANOVA and student's t-test) performed on the inactivation data indicated no apparent effect of pH on the kinetics of the processes. It was found that the UVO process was the most efficient in inactivating *E. coli.* The increase in the inactivation rate with the UVO process is

attributed to synergetic activity of UV and ozone which results in the generation of hydroxyl radicals from ozone decomposition.

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DEDICATION

I would like to dedicate this work to my parents, Sumer and Saroj Savant, and my brother Saurabh. Their love, patience, and encouragement, made the completion of this project possible. I thank God for having them in my life.

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CHAPTER I

INTRODUCTION

Disinfection is the process of removing, inactivating or destroying all potential pathogenic organisms present in water for human consumption, wastewater prior to discharge and water used for food processing. Disinfection aims not only to remove the organisms present in the water but also to prevent future contamination of water by providing a residual disinfectant in the water. Until the turn of the century, waterborne diseases were a serious, ubiquitous health problem with frequent outbreaks of typhoid, cholera and dysentery. Owing to the serious health effects of disease causing-pathogens, disinfection practices have found the most prominent place in water treatment regulations and laws, with disinfection of water and wastewater being required by law not only for the destruction of pathogens which may be present in the water, but also to reduce the chances of future contamination of water with these organisms. The food processing industry has been using disinfectants to destroy organisms that reduce the shelf life of a product and cause disease in humans. Food processing industries have for long been concerned about the disinfection of process water. The meat industry in particular has a lot of concerns about the bacteria *E.coli* finding its way into the meat. The Center for Disease Control (CDC) estimates that a total of 73000 infections and 61 deaths occur each year due to *E. coli* infection (*http://web.bham.ac.uk/bcm4ght6/res.html).* In addition

to *E.coli* other organisms such as *Salmonella* and fungi cause widespread loss of life and food resources. *Anderson et al.* (1999) provided a figure of approximately 9000 deaths in the US and approximately 5billion dollars worth of loss caused by foodborne pathogens. At present the only options available to meat industry are to heat the meat to high temperatures, or the application of high doses of chlorine in the form of hypochlorite solution, however this is a costly and time consuming process. *Linton* and *Gerrard* (1999) showed that UV radiation could efficiently disinfect *Escherichia coli* and *Salmonella senftenberg* on pork skin and the muscles.

Diseases causing organisms vary considerably in their characteristics, environment as well as lethality. *Giardia*, enteric bacteria and protozoans are pathogens which have been clearly identified as having high risks of causing an outbreak. *Giardia* in particular has become important for determining baseline water supply disinfection needs, for two reasons. First, it is a prominent waterborne disease that must be controlled. Second, out of the full range of known waterborne pathogens, it is the most resistant to disinfection, making it extremely difficult to inactivate (*Hazen et.al, 1992)*. There are several pathogens which can proliferate in water distribution systems under the proper conditions including l*egionella* and *mycobacterium* as well as several others. In addition several fungi have also been found to grow in water distribution systems. Most organisms that become established within water distribution systems also act as opportunistic pathogens in humans, e.g. *legionella* causes legionnaire's disease, which is often fatal (*Muraca et. al, 1988)*. The potential for growth of naturally-occurring pathogens in distribution systems has resulted in additional criteria for disinfection beyond that

necessary to achieve pathogen removal from water at the treatment plants. This has led to the development of two criteria for disinfection: primary and secondary. Primary disinfection criteria are intended to provide for the removal and/or inactivation of raw water source-related pathogens at the water treatment plant, whereas secondary disinfection criteria are intended to provide a disinfecting residual in distribution systems that will prevent subsequent growth of naturally-occurring, opportunistic organisms.

In the past, the primary emphasis of disinfection was to control water borne diseases through the control of associated bacterial indicator organisms. Two events in the 1970s resulted in significant reevaluation of this long established disinfection practice. The first was the discovery that disinfection byproducts, formed in the reaction of disinfectants with certain source water organic matter, may be harmful to human health. The second was the emergence of newly recognized waterborne disease causing organisms (*Hazen, et. al.; 1992)*. Engineers and scientists consequently started exploring disinfection alternatives to chlorine as well as methods to reduce the several harmful disinfection byproducts (DBPs) that it formed.

Wide spread usage of chlorine as a disinfectant in the US began in 1904. In those early years, chlorine was applied as liquid hypochlorite. The use of chlorine gas as a disinfectant started in 1913 with the development of equipment to permit its dissolution in measured amounts (*White; 1986)*. However, recent concerns about the risks associated with chlorine DBPs has caused a shift towards alternative disinfectants, chlorine-based and otherwise. Not withstanding the recent moves towards alternative disinfectants,

chlorine remains the disinfectant of choice for most water and wastewater treatment plants in the US.

In most water treatment plants today chlorine remains the disinfectant of choice because it is cost effective, it is easy to apply and control, and it leaves a residual to protect against future contamination. However, disinfection processes using chlorine produce some undesired organic compounds such as trihalomethanes (THM). Chlorine is also capable of participating in several chemical reactions even in its Cl^+ form (*Hazen et al.*,1992). Reactions in which chlorine is added or substituted in the Cl^+ form result in the production of not only chloramines but also other disinfection byproducts which are of concern. Chlorine DBPs are of concern because they generally bioconcentrate, they are persistent in the environment and can have an adverse impact on the aquatic life as well as public health. DBPs of principal concern, due to abundance and toxicity, are THMs and HAAs (haloacetic acids). Several chlorinated byproducts, such as 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, have been shown to be mutagenic in the ovary (*Holbom et al.*,1984), and chloramines adversely affect dialysis patients (*Hazen et al.*, 1992).

In addition to chlorine based alternative disinfectants such as chloramines and chlorine dioxide, ozone and ultraviolet radiation have been used at several places to achieve disinfection goals and also to eliminate the risks involved with chlorine DBPs. Ozone is the strongest of the common disinfectants available commercially, and was used as early as 1983 for disinfecting water in Europe (*US EPA,1986).*While the ozone molecule in itself is a very strong disinfecting agent, it is also capable of forming highly

reactive chemical agents that contribute to additional oxidizing power. The most common of these is the free hydroxyl ($OH^{\bullet}$) radical, which is more reactive than ozone itself. In spite of the highly oxidative nature of ozone, its use as a primary disinfectant has been extremely limited throughout the US because of uncertainty in the reaction pathways of aqueous ozone decay as well as its low solubility in water (*US EPA,1986)*. The public health significance of ozone DBPs relative to other disinfectants has not been fully determined, however ozone by-products are generally of lesser concern than chlorine DBPs (*Noot, et. al., 1989)*. In contrast to chlorine ozone disinfection byproducts are predominantly organic compounds that incorporate oxygen in their structure (*Richardson et al., 1999*) such as aldehydes and ketones. The biological effect of these byproducts has not been determined conclusively and further research is needed (*Hazen et.al, 1992).*Ultraviolet (UV) radiation, another disinfecting agent, achieves bacterial and viral inactivation by damaging the microorganisms genetic, and produces no DBPs. Despite this benefit, the use of UV radiation in disinfection has been extremely limited because of its high operational and maintenance cost, and its limited effectiveness in the presence of color and turbidity. Ozone and UV radiation when used in combination enhance the decomposition of ozone to form hydroxyl radicals.

Over the years the Environmental Protection Agency has been tightening regulations concerning chlorine DBP's. Concern for these byproducts began to surface in the 1970s as newer analytical methods were applied for identifying constituents of drinking water. Two studies completed in 1974 showed wide spread chloroform occurrence in water supplies throughout the US (*Brass et. al., 1975)*. Public concern over

the presence of THMs in water supplies persuaded the EPA to include regulatory intent for THMs and other DBPs in the first drinking water priority list (US *EPA, 1989)*. Subsequently the 1998 amendments to the Safe Drinking Water act imposed specific and very stringent limits on DBP concentrations in drinking water. These regulatory pressures could eventually force water treatment facilities to abandon the use of chlorine as primary disinfectant in favor of alternatives that generate fewer, less hazardous DBPs.

Ozone and UV radiation have been selected for the current research because they possess the following advantages over chlorine:

1. Ozone DBPs are thought to be harmless to the environment and UV does not produce DBPs of concern (*Hazen et. al., 1992)*.
2. Ozone has microbial inactivation rates that are considerably higher than those of chlorine or its derivatives and when utilized in concert with UV radiation these are increased even further (*US EPA, 1989)*.
3. Chlorine gas has a higher toxicity towards human beings than ozone, ozone only produces mild itching in the eye and only in extreme cases of exposure to high concentrations of ozone for extended periods of time does it begin to effect the human lungs.
4. Chlorine has to be transported to site whereas ozone is produced onsite, hence eliminating transportation costs and chances of accidents.
5. EPA standards regarding chlorine DBPs are getting more stringent, making chlorine less attractive as a primary disinfectant.

Ozone has disadvantages of spontaneously decaying into oxygen and causing potential lung damage to exposed individuals, while UV radiation can cause cancer if safety regulations are not met. Both of these also suffer from the fact that operators would have to specially train to apply both or either of these. This disadvantage of ozone precludes its use as a secondary disinfectant and chlorine or other disinfectant that is capable of providing adequate residual will have to be used as a secondary disinfectant.

A research study funded by the American Water Works Association (AWWA) research foundation in 1990 deemed the determination of ozone and ultraviolet disinfection kinetics as insufficient for sound engineering judgment on the application of either of these for disinfection (*Singer,1995)*. AWWA strongly recommends that further research be done to understand the disinfection kinetics of ozone and UV. However, AWWA also states that the kinetics of ozone might never be completely understood owing to its unstable nature and therefore the need for research into the nature of ozone disinfection kinetics is even greater.

CHAPTER II

LITERATURE REVIEW

The typical response of microorganisms to exposure to a disinfectant is the lysis of its nuclei and cell membrane, or a change in its nucleic acids so as to render it unviable. This response may be temporary or permanent, depending upon the type and intensity or concentration of disinfectant used. An important aspect of disinfection by ozone and UV radiation simultaneously is the spontaneous decay of ozone and its interaction with UV radiation. This chapter focuses on presenting the available literature on the subject of disinfection by either ozone or UV radiation or both.

INDICATOR ORGANISMS

Indicator organisms have been widely used to determine the efficacy of various disinfection process and agents. Indicator organisms are biological indicators of the presence of potential pathogens in water or wastewater. According to *Keith et al.(1999)* indicator organisms should have the following characteristics:

1. should be present of feces of warm blooded organisms,

2. potential growth in environment should be minimal and always be less than that of pathogens, this ensures that the indicator organism will not pose a threat to the ecological balance in the environment.
3. should be readily detectable by simple means and have characteristic reactions to particular agents,
4. should always be present whenever pathogens are present,
5. should show more resistance to disinfectants than pathogens, and
6. should not have an extremely adverse impact on the ecological balance of the environment.

In 1986, the *United States Environmental Protection Agency* (US EPA) stated that *Escherichia coli* based standards would best serve the public health (*EPA total coliform rule, http://www.epa.gov/ogwdw000/smallsys/ndwac/coliform.html)*.

Research done by *Keith et al.(1999)* showed that *Escherichia coli* is a suitable indicator organism for water as well as wastewater, though there are a few organisms such as *Klebsiella* which interfere with the recovery of fecal coliform as well as *E. coli* if membrane fecal coliform (MFC) agar tests are used.

Thompson and *Watling (1985)* used the development of bacterial inhibition zones in test cultures as an indicator of toxicity, and concluded that the size of the *E. coli* inhibition zone can be used to determine the concentration of toxic and heavy metals in effluents. The correlation between the size of inhibition zone and the concentration of toxic material can be determined from diffusion charts which are constructed by the study of inhibition zone when the bacteria are exposed to known concentrations of the

compound. *Lund (1996)* indicated that *E. coli* is an effective indicator organism for enteric pathogens such as *Campylobacter jejuni* and *Yersinia enterocolitica.*

Based on the results of research it can be safely assumed that *E. coli* can be used to indicate the efficacy of using UV radiation and/or ozone gas for disinfection, notwithstanding the fact that *E. coli* is particularly susceptible to ozone as well as UV radiation. *E. coli* has all the characteristics of an ideal indicator organism except that it is not resistant to disinfectant action, however its response to exposure to disinfectants serves as a valuable indicator towards understanding the response of pathogens to disinfectant exposure.

DISINFECTION KINETICS

Disinfection kinetics have been widely studied throughout the 19th century. *Finch* et.al. *(1998)* compared alternative kinetic models for disinfection. All disinfection design parameters are selected based on the knowledge of disinfection rates and the concentration-time product (CT) for the disinfectant and the organism. The CT values for all disinfectants are based on the Watson-Chick law or a derivative of the law. The Watson-Chick model adequately represented the kinetics of ozone disinfection of anthrax spores and *E. coli* (Strain ATCC 11229) in completely mixed stirred reactors, semi batch reactors and, to a lesser extent in batch reactors. In case of other models they determined that the Multiple Target model was not suitable for describing microbial deactivation,

whereas the Series-Event model adequately described the deactivation of microorganisms and reduced to Watson-Chick model under special conditions.

The Watson-Chick law assumes disinfection to be a first order process whose rate can be represented mathematically as:

$$\frac{dN}{dt} = k_d \times N \tag{2.1}$$

where, N = count of organisms present at time t., k_d = first order disinfection or deactivation rate constant. *Morris (1975)* developed the concept of lethality coefficient for a given disinfectant.

$$\Delta = \frac{4.6}{Ct_{99}} \tag{2.2}$$

where, ? is the lethality coefficient, C is the residual concentration, and t_{99} is time in minutes for 99% destruction of the test organism, and 4.6 is a constant. With the introduction of lethality coefficient the Watson-Chick law was modified to include a concentration-time term, the modified form is represented in the equation below (2.3).

$$\ln\left(\frac{N}{N_o}\right) = -\Delta C^n t \tag{2.3}$$

where, N_o is the number of microorganisms present at time t=0, and n is a constant which depends on the type of disinfectant. The Watson-Chick law is simple and generally gives good fits to the experimentally observed data and is therefore widely used in the design of disinfection systems.

The Selleck model is primarily used to describe coliform inactivation with chlorine (*Selleck, et al., 1978)*

$$\frac{dS}{dt} = -\frac{d}{dt}\left(\frac{N}{N_o}\right) = \frac{k_s CS}{1 + KCT} \tag{2.4}$$

where, S is the survival ratio, C is the disinfectant concentration, k_s and K are rate constants and T is the time. The Selleck model has been widely used to describe survival curves having an initial shoulder and a declining inactivation rate (*Haas and Karra, 1984)*. The mechanistic rationale behind this behavior is explained on the basis of reactions between the bacterial or viral proteins and the disinfectant causing the disinfectant permeability to reduce (*Hiatt, 1964)*.

The series-event model rationalizes the survival curves and inactivation kinetics of micro organisms on the basis of cumulative effect of chemical disinfectants during the contact time.

$$\frac{dN_k}{dt} = k_{se} C N_{k-1} - k_{se} C N_k \tag{2.5}$$

where, k = organism site, and k_{se} = rate constants. The inactivation of a single microorganism is idealized as occurring in a series of damaging reactions that occur in integer steps (*Severin et.al, 1984)*, and the rate at which an organism passes from one level to the next is considered first order. This model assumes that a finite number of hits are needed for the organism to be inactivated, and organisms taking less than the required number of hits survive.

The multiple target model postulates that each organism or a clump of organisms possess a definite number of identical critical targets, all of which must be hit at least once to inactivate the organism (*Hiatt, 1964*):

$$P_t = \log[1-(1-e^{-kCT})^{n_c}] \quad (2.6)$$

where, k = inactivation rate constant, l/mg.s, C = chemical disinfectant concentration, T = contact time, P_t = survival probability, and n_c = number of critical targets. This model is generally used to describe the initial shoulder observed in survival curves for most microorganisms when they are exposed to a constant dose of radiation or other disinfectant. The multiple target model is not considered suitable for describing microbial inactivation kinetics because it is highly improbable that clumps of equal size and cell damage will be randomly distributed among the targets (*Wei Chang, 1975)*.

The rational model has been used in the past to model viral inactivation kinetics in systems with constant ozone residuals.

$$\frac{dN}{dt} = -kN^x C^n \quad (2.7)$$

where, x, and n are constants and C is the constant ozone residual. Rational model has not been successful with modeling bacterial inactivation due to complexities in determining the values of constants, x and n.

CHLORINE DISINFECTION

Chlorine had been used as a disinfectant as early as the mid-eighteenth century, when it was used to control an outbreak of cholera in London (*White, 1986)*. In the early days of disinfecting water sodium hypochlorite (NaOCl) a derivative of chlorine was

widely used. NaOCl when introduced into water reacts with water molecules to form hypochlorous acid (HOCl)

$$NaOCl + H_2O \rightarrow HOCl + NaOH \quad (2.8)$$

Chlorine gas if used directly reacts with water to form hypochlorous acid.

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad (2.9)$$

Hypochlorous acid is a weak acid, which means that it undergoes partial dissociation into hypochlorite ion (OCl).

$$HOCl \leftrightarrow H^+ + OCl^- \quad (2.10)$$

Hypochlorite is an oxidant too, but a weaker one than hypochlorous acid. HOCl is the most effective of all chlorine residuals and is known as free available chlorine (*Frakas et. al., 1988).*The germicidal efficiency of HOCl is due to the relative ease with which it can penetrate cell walls, this penetration is comparable to that of water, and is attributed to its low molecular weight and electrical neutrality (*Anon, 1951).*

Concerns with Chlorine

At present chlorine is the disinfectant of choice in American and Asian water and wastewater treatment plants. The use of chlorine is based on the assumption that the risk from using chlorine as a disinfectant is more than offset by its advantages, principally the ease of application and a residual power. As information was collected on the effects of chlorine by-products its continued use as a primary disinfectant became the subject of

intense debate, and there is a growing opinion that the use of chlorine as a primary disinfectant should be abandoned in favor of safer, more effective disinfectants.

One of the primary reasons for the use of chlorine in disinfecting water is that organisms inactivated with it are considered completely inactivated or dead. *Sartory (1985)* studied the recovery of chlorine deactivated coliform and found that if the deactivated organisms were contacted with nutrients for an extended period of time, the organisms became viable. He also concluded that if the nutrients included a small proportion of pyruvate, the time required for the organisms to regain viability was substantially reduced, by as much as 4 times.

One if the greatest advantage of using chlorine as a disinfectant is thought to be its residual effect, which provides protection against contamination during transit. *Payment (1999)* has shown however, that the chlorine residual is ineffective against all but the most sensitive of microorganisms and is completely ineffective against viruses, such as *Polio* virus, and *Giardia cysts.* The residual was efficient in inactivating indicator organisms such as *E. coli*, but was largely ineffective against the most resistant of organisms, which also happen to be the most pathogenic.

The potential health effects of chlorine disinfection byproducts (DBPs) have been extensively studied. Amendments to the Clean Drinking Water Act in 1998 set the maximum contaminant levels for total THMs at 80 μg/l and at 60 μg/l for HAAs (*EPA Envirofacts website: http://www.epa.gov/OGWDW/mdbp/dbpfr.html; Federal Register 1998, Vol. 63, No. 241).*The excess risk of cancer at 1000 m downstream of a discharge containing chlorine and chloroform was estimated at 10^{-6} (*Mills et. al., 1998)*; this level

is considered medically significant and is a cause of concern (*LaGrega et. al., 1994).* Trichloroacetic acid (TCAA) is a wide spread nonmutagenic, non volatile but lethal DBP (*Nestmmann et. al., 1980)* which severely hampers the oxygen uptake by the respiratory system in living beings. The oxygen uptake rates of Dragonfly nymphs exposed to a TCAA concentration range from 1-1000 μg/l increased by upto 2.5 to 5 times, indicating that TCAA decreases the oxygen uptake efficiency of the respiratory system, possibly by interfering with mitochondrial enzymes and by uncoupling oxidative phosphorylation (*Calabrese et. al., 1987). Hu et.al. (2002)* studied the effects of interaction of chlorine with bisphenol A (BPA) a known endocrine disruptor. BPA comes into contact with chlorine in the form of the epoxy liners of concrete tanks and water pipes. The chlorination of BPA produces an assorted range of compounds, the most medically significant of which is trichlorophenol, also an endocrine disrupting compound.

In summary, the scientific literature clearly indicates not only is chlorine ecologically harmful, but also that several of the advantages attributed to it do not stand up to scientific scrutiny.

OZONE DISINFECTION

Stratospheric ozone is primarily produced from exposure of molecular oxygen to ultraviolet (UV) radiation. The high energy impact of UV radiation with the oxygen (O_2) molecule splits the molecule into two oxygen atoms which then combine with molecular

oxygen to form ozone (O_3). The reaction can be represented by the following chemical reaction:

$$O_2 + UV / Disch\arg e \rightarrow 2O \tag{2.11}$$

$$O + O_2 + M \rightarrow O_3 + M \tag{2.12}$$

where, M is the momentum of the molecules, and discharge is the UV radiation.

In the laboratory and in industry, ozone is generally produced using the corona discharge method. Dry atmospheric air is passed between two parallel or concentric electrode plates separated by a dielectric. An electric potential is applied between the electrodes, and as this potential is sought to be neutralized electric discharges are produced causing the cleavage of molecular oxygen and the subsequent formation of ozone.

Decay of Ozone

The decay of ozone has been investigated since the early 1900s, but a pathway that completely explains the various reactions and decompositions that ozone undergoes before it's complete conversion to molecular oxygen has not been found. *Hoigne* and *Staehelin* (*1984*) empirically concluded that the decomposition of ozone is initiated and limited by its reactions with the hydroxide ion (OH^-), at a rate given by,

$$\frac{d[O_3]}{dt} = k[O_3][OH^-] \tag{2.13}$$

where, [] refer to concentrations in g-mol/l. Acknowledging the importance of the hydroxide ion *Grasso and Weber (1989*) suggested the following equation to represent the decay rate:

$$\frac{d[O_3]}{dt} = k_1[O_3][OH^-] + k_2[O_3]^2[OH^-] \quad (2.14)$$

where, k_1, and k_2 are reaction rate constants. They also concluded that attempts to fit ozone decomposition data into inappropriate kinetic expressions resulted in significant variations in the reported decay coefficient particularly at low pH studied .The decay rate of ozone in natural waters was found to be significantly affected by the carbonate ion concentration (*Aieta et. al. (1988); Nemes et. al. (2000)*). Carbonate is a scavenger of the hydroxide radical, which is one of the most significant chain carriers in aqueous ozone decomposition.

$$O_3 + OH^- \leftrightarrow HO_2^- + O_2 \quad (2.15)$$

$$HO_2^- + O_3 \leftrightarrow O_3^- + HO_2 \quad (2.16)$$

$$CO_3^{2-} + OH \leftrightarrow CO_3^- + OH^- \quad (2.17)$$

$$CO_3^- + O_3^- \leftrightarrow CO_3^{2-} + O_3 \quad (2.18)$$

The rate law for these reactions was represented as:

$$\frac{d[O_3]}{dt} = -1.5k_1[O_3][OH^-] \quad (2.19)$$

where, k_1 is the reaction rate constant. Numerous researchers since have various estimates of the decay coefficients for ozone, and there is as yet no unanimity on the

decay pathway. Hence it is generally recommended that researchers develop rate expressions for ozone decomposition that are applicable to their test conditions.

Germicidal Action of Ozone

Ozone is a very potent germicide owing to its high oxidative potential. Disinfection by ozone is a direct result of bacterial cell wall disintegration, or lysis, a mechanism of inactivation that is distinctly different than that of chlorine, which is thought to attack a particular enzyme group after diffusing through the cell wall (*White, 1992).*

Advantages and Disadvantages of Ozone over Chlorine

1. Ozone DBP's are thought to be harmless to the environment (*Hazen et. al., 1992).*
2. Ozone has microbial inactivation rates that are considerably higher than those of chlorine or its derivatives (*Morris, 1975)*
3. Chlorine gas has a higher toxicity towards human beings than ozone, ozone only produces mild itching in the eye and only in extreme cases of exposure to high concentrations of ozone for extended periods of time does it begin to affect the human lungs.
4. Chlorine has to be transported to site whereas ozone is produced onsite, hence eliminating transportation costs and chances of accidents.

The disadvantages of ozone compared to chlorine are the lack of a residual disinfection effect, and capital and power cost for generation, These disadvantages

preclude the use of ozone as the secondary disinfectant and hence chlorine has to be used as a secondary disinfectant.

ULTRAVIOLET DISINFECTION

Ultraviolet disinfection has been used commercially for many years in the pharmaceutical, beverage and cosmetics industries as well as in wastewater treatment. Drinking water disinfection using UV radiation was first attempted in the early 1900s, but was abandoned shortly thereafter. However, UV technology is gaining popularity once again because of environmental concerns about disinfection byproducts of chlorine and technological advances in the generation of UV radiation.

Wolfe (1990) conducted a comprehensive literature review which listed the dose required for deactivation of various bacteria and enteric viruses (Table 2.1). *Wolfe* also described the mechanism of disinfection by UV radiation which operates by irreparable damage to the nucleic acid chain of the organism (Figure 2.1), this damage is most extensive at a wavelength of 253.7 nm. One concern with UV disinfection is that several microorganisms contain the enzyme photolyase, which repairs nucleic acid damage arising from exposure to UV radiation. This effect is enhanced if the bacteria are held in nutrient free water for several hours in the dark, and then exposed to light (*Sommer et.al, 2000)*

Table 2.1

Approximate dosage for 90% inactivation of selected microorganisms by UV

Microorganism	Dosage ($\mu W.s/cm^2$)
Salmonella typhi	3,000
Vibrio cholerae	3,400
Shigella flexneri	1,700
Poliovirus 1	5,000
Giardia muris	82,000
Shigella dysenteriae	2,200
Coliphage	3,600

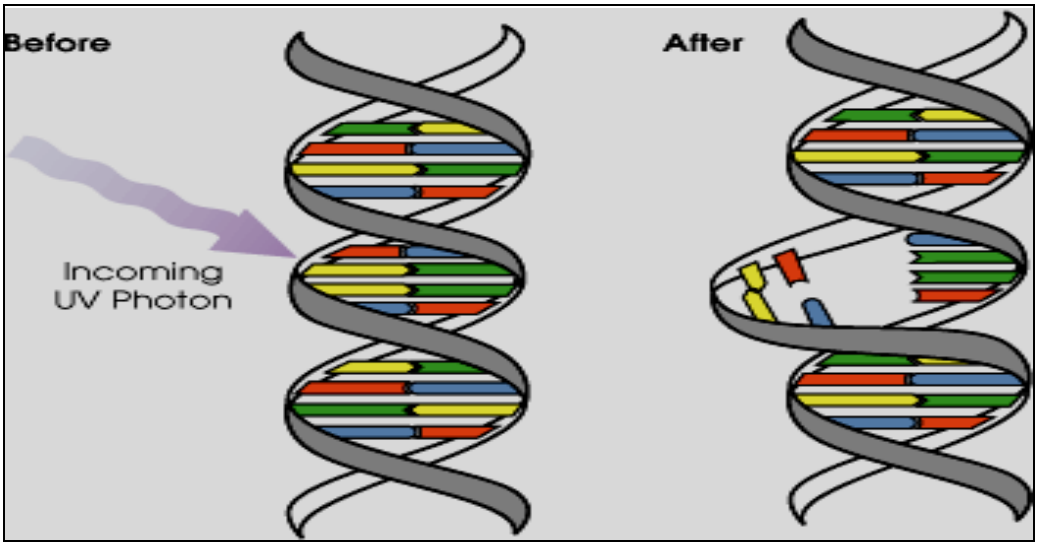

Figure 2.1, Effect of UV radiation on a DNA/Nucleic acid strand.

Photoreactivation has been observed in a number of bacterial strains, including *E. coli* B. *Villarino et al. (2000)* found that glucose uptake by E. *coli* K12S within the first 30 minutes after UV exposure was the same as bacteria which were not exposed. Furthermore even after exposure to lethal levels of UV radiation membrane integrity was maintained although no DNA or rRNA activity was observed. This might indicate a possible precursor to photoreactivation.

Anderson et al. (1999) found that high intensity UV radiation deactivated *Bacillus cerus* by 8 log units and *Aspergillus niger* by 4.5 log units. Shorter wavelengths were more lethal than longer wavelengths, which was attributed to greater sensitivity of DNA and RNA bases to the shorter wavelengths. Fungal spores were more resistant than the bacteria to UV light because their dark pigments retarded the penetration of UV radiation.

Temperatures between 20 and 40ºC did not effect the rate of *E. coli* disinfection with UV radiation did not vary with the temperature range of 20-40ºC. The disinfection rate increased above 40ºC, however, but this could have been caused by thermal inactivation of the bacteria (*Abu-Ghararah,1994).*

ULTRAVIOLET AND OZONE INTERACTION

Ozone photodissociates in the presence of UV radiation in the wavelength range of 200-310 nm and a number of mechanisms have been proposed to describe this interaction. *Chao* et al. (*2000)* theorized that the photodissociation of ozone by UV radiation at 254 nm was influenced by both natural and UV induced decay processes:

$$O_3 \rightarrow O_2 + O(D) \quad (2.20)$$

$$O_3 + hn \rightarrow O_2 + O(P) \quad (2.21)$$

$$O(D) + O_3 \rightarrow 2O(P) + O(P) \quad (2.22)$$

$$\rightarrow 2O_2 \quad (2.23)$$

$$O_2 + O_3 \rightarrow O(P) + 2O_2 \quad (2.24)$$

$$O(P) + O_3 \leftrightarrow 2O_2 \quad (2.25)$$

where, P = Photodissociation, D = Spontaneous decay. *Topudruti et. al.* (1993) theorized that the interaction between ozone and UV radiation results in the formation of hydrogen peroxide, which in turn reacts with UV to form hydroxyl radicals.

$$O_3 + uv + H_2O \leftrightarrow H_2O_2 + O_2 \quad (2.26)$$

$$H_2O_2 + uv \leftrightarrow 2OH\bullet \quad (2.27)$$

Bablon et. al. (1991) represented ozone decay kinetics as a series of interconnected reaction pathways, which lead to the decay as well as the generation of ozone (Figure 2.2). Degradation begins with the reaction between ozone and an initiator (UV or a chemical initiator) to form superoxide anion (O_2^-). Once this process is started, the reaction enters into a cycle in which several intermediates are formed that lead to the ultimate production of oxygen as an external end product.

As pointed out earlier, the chemistry of ozone decomposition in pure water is not completely understood, and the same is true of UV-induced ozone decay. The interaction between UV and ozone, however maybe an important determinant of their combined effectiveness as disinfectants. Limited research has been done in the simultaneous use of

ozone and UV disinfection, and results have been contradictory. *Venosa et.al. (1984)* observed that a simultaneous application of UV and ozone retarded the efficacy of ozone as a disinfectant and theorized that this was due to UV induced decomposition of ozone to molecular oxygen. *Diaz et.al (2000).* concluded that concurrent application of UV significantly enhances the disinfecting characteristics of ozone, however they do not propose a mechanism for this synergetic action.

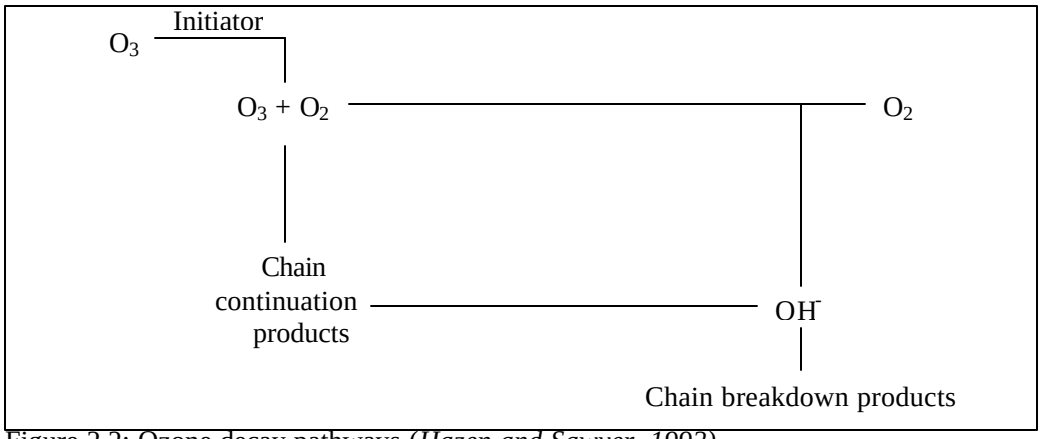

Figure 2.2; Ozone decay pathways (*Hazen and Sawyer, 1992).*

CONCLUSION

While chlorine has been widely and successfully used as a disinfectant for drinking water supplies, growing concerns over health impacts arising from chlorine DBPs has led to the examination of alternative disinfectants. Ozone and UV radiation are both known to be effective disinfectants, but are more costly than chlorine and do not provide residual disinfecting power. The combined use of ozone and UV has been studied extensively for chemical oxidation, but not for disinfection. We hypothesize that UV and ozone could work in combination to achieve higher rates of microbial deactivation than

either disinfectant alone. This work aims to obtain data to document and quantify the hypothesized synergism between ozone and UV radiation as disinfectants.

CHAPTER III

Objectives of the study

This work aims to obtain data to document and quantify the hypothesized synergism between ozone and UV as disinfectants. All disinfection design parameters are selected based on the knowledge of disinfection rates and the concentration-time product (CT) for the disinfectant and the organism.

Even though ozone and UV radiation have higher deactivation rates than most other disinfectants, neither is economical except in small plants. UV lamps are difficult to maintain and are expensive, whereas ozone is difficult to apply and control. However, the effect of simultaneous application of UV and ozone on microbial deactivation rates, which directly affects the economics of the process, has not been thoroughly examined.

Food processing industries have for long been concerned about the disinfection of process water. The meat industry in particular has a lot of concerns about the bacteria *E.coli* finding its way into meat. The Center for Disease Control (CDC) estimates that a total of 73000 infections and 61 deaths occur each year due to *E. coli* infection (*http://web.bham.ac.uk/bcm4ght6/res.html).* In addition to *E.coli* other organisms such as *Salmonella* and fungi cause widespread loss of life and food resources. *Anderson et al.* (1999) provided a figure of approximately 9000 deaths in the US and ~ 5billion dollars worth of loss caused by foodborne pathogens. At present the only options available to

meat industry are to heat the meat to high temperatures, or the application of high doses of chlorine in the form of hypochlorite solution, however this is a costly and time consuming process. *Linton* and *Gerrard* (1999) showed that UV radiation could efficiently disinfect *Escherichia coli* and *Salmonella senftenberg* on pork skin and the muscles.

The current research into combined ozone and ultraviolet deactivation of Escherichia *coli* is an attempt to:

1) Determine the first order reaction rates for UV deactivation of E.*Coli.*
2) Determine the first order reaction rates for ozone deactivation of E.*Coli*.
3) Study the effect of pH of the water on the deactivation rates.
4) Determine a combined deactivation rate for ozone and UV radiation applied simultaneously.
5) Compare the deactivation rates of the three treatments to determine whether a simultaneous application results in an increased deactivation rate.

CHAPTER IV

COMBINED OZONE AND ULTRAVIOLET INACTIVATION OF *ESCHERICHIA COLI*[1]

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ABSTRACT

The kinetics of *Escherichia coli* inactivation were studied using ultraviolet (UV) radiation, ozone, and UV and ozone (UVO) in combination in a batch reactor at varying pH levels (6, 7, and 8) and at a constant temperature of 25°C. The inactivation kinetics for all three treatment processes was pseudo first order, and the reaction rate constants were considered to be additive such that a combined reaction rate could be obtained by adding the kinetic rates of the processes applied and numerically small rates could be neglected in the computation of the combined rate. Statistical tests (ANOVA) performed on the inactivation data indicated no apparent effect of pH on the kinetics of the processes. It was found that the UVO process was the most efficient in inactivating *E. coli*. The increase in the inactivation rate with the UVO process is attributed to synergetic activity of UV and ozone which results in the generation of hydroxyl radicals from ozone decomposition.

INTRODUCTION

Chlorine has been the disinfectant of choice in American and Asian water and wastewater treatment plants for almost a century, and has been widely recognized as one of the best and the cheapest disinfectants available commercially. The use of chlorine is based on the assumption that the risk from using chlorine as a disinfectant is more than offset by its advantages, i.e. ease of application and residual disinfection power. This residual chlorine

provides protection against contamination during transit, however chlorine residual is ineffective against all but the most sensitive of microorganisms and is completely ineffective against viruses such as *Polio* virus, and *Giardia* cysts (*Payment, 1999).* The residual was efficient in inactivating indicator organisms such as *E. coli*, but was largely ineffective against the most resistant of organisms, which also happen to be the most pathogenic.

Recent research into the effects of chlorine by-products such as trihalomethanes and haloacetic acids has prompted the exploration of alternatives to chlorine as a primary disinfectant. An assessment of the potential health effects of chlorine and chloroform discharged into streams showed that the excess cancer risk 1000 m downstream of a discharge site is 10^{-6} (*Mills et al.,1998)*; this level of risk is medically significant and is a cause of concern. Trichloroacetic acid (TCAA) a widespread non-volatile chlorine DBP that is produced more rapidly than chloroform in an aquatic environment (*Udden and Miller, 1983)* is nonmutagenic but lethal at higher concentrations (*Nestmmann et.al., 1980).* Concern over public health effects has led the US Environmental Protection Agency to impose increasingly stringent guidelines regarding chlorine DBPs (*US EPA, 1999),* in 1998 EPA set in motion the stage 1 disinfectant/disinfection byproducts rule, which set the limits for annual allowable total trihalomethanes at 80 µg/l and at 60 µg/l for haloacetic acids (HAA) (*Stage 1. Disinfectants and Disinfection Byproducts Rule, US EPA)*. These regulations will provide important stimulus towards supplanting chlorine as the primary disinfectant. The widespread adoption of ozone as primary disinfectant in Europe was motivated primarily by concerns over chlorine DBPs (*White, 1986).* The

alternative disinfectants ozone and ultraviolet (UV) radiation achieve pathogen inactivation at rates higher than chlorine, however the highly unstable nature of ozone and the high cost of UV generation have restricted their application to drinking water disinfection.

The effect of exposure to a disinfectant on a microbial cell is the lysis of its cell wall or cellular membrane, or a change in its internal structure chemistry, nucleic acids or enzyme systems so as to render it nonviable (*Wolfe, 1990).* Inactivation may be temporary or permanent, depending upon the type and intensity or concentration of disinfectant used. The rate of disinfection likewise depends on the nature and dose of the disinfectant, and the mechanism of disinfection. The Watson-Chick model adequately represented the kinetics of ozone disinfection of anthrax spores and *E. coli* (Strain ATCC 11229) in completely mixed stirred reactors, semi-batch reactors and, to a lesser extent in batch reactors.

The Watson-Chick law assumes disinfection to be a first order process whose rate can be represented mathematically as:

$$\frac{dN}{dt} = k_d \times N \qquad (4.1)$$

where, N = count of organisms present at time t and, k_d = first order disinfection or deactivation rate constant. *Morris (1975)* developed the concept of lethality coefficient for a given disinfectant.

$$\Delta = 4.6 / Ct_{99} \qquad (4.2)$$

where, ? is the lethality coefficient, C is the residual concentration, and t_{99} is exposure time in minutes required for 99% destruction of the test organism. With the introduction

of lethality coefficient the Watson-Chick law was modified to include a concentration-time term:

$$\ln\left(\frac{N}{N_o}\right) = -\Delta C^{n} t \tag{4.3}$$

where N is the number of target organisms left at time t, N_o is the number of target organisms at time t=0, C is the concentration of the disinfectant, n is a coefficient which depends on the type of disinfectant. *Masschelein (1998)* concluded that the CT concept does not give adequate representation to the disinfection of water by ozone. He gave a number of reasons for his conclusion including the variety of organisms present, competitive ozone consumption and ozone self decomposition.

The goal of this study was to evaluate the efficacy of a combined dose of ozone and ultraviolet radiation on the deactivation rate of *E. coli*, and to quantify the kinetics of the combined action of these two disinfectants. *E. coli* was selected for this study because it has been widely used as an indicator for the presence of pathogenic organisms in drinking water as well as wastewater, and was shown to be suitable for this purpose (*Keith et al., 1999)*. Research conducted during past century showed that the UVO process (combined application of UV radiation and ozone) is highly effective in the oxidation of not only trace organics compounds but also of inorganic compounds such as ammonia and arsentie (*Gunten, 2003)*. This is in part attributed to the generation of highly reactive free radicals during UV induced decomposition of ozone. Ozone photodissociates in the presence of UV radiation in the wavelength range of 200-310 nm. However, photodissociation resulting in the generation of hydroxyl radicals takes place at a wavelength of 253.7 nm which coincidentally is also the optimum for bactericidal

action (*Sobtka, 1993;Diaz et.al, 1984)*. The catalytic decay reaction that occurs is extremely important and has led to numerous experimental and theoretical studies on the process. *Topudruti et. al.* (1993) theorized that the interaction between ozone and UV radiation results in the formation of hydrogen peroxide, which in turn reacts with UV to form hydroxyl radicals.

$$O_3 + uv + H_2O \leftrightarrow H_2O_2 + O_2 \tag{4.4}$$

$$H_2O_2 + uv \leftrightarrow 2OH\bullet \tag{4.5}$$

Literature on the effectiveness of UV and ozone as combined disinfectants is ambiguous, as both synergetic (*Diaz, et. al., 2000)* and antagonistic (*Venosa, et. al., 1984)* interactions have been reported. *Venosa et.al. (1984)* theorized on the basis of their experimental results that a simultaneous application of UV and ozone retarded the efficacy of ozone as a disinfectant by decomposing ozone to molecular oxygen. In contrast *Diaz et.al.(2000)* concluded that the concurrent application of UV significantly enhanced the disinfecting characteristics of ozone.

MATERIALS AND METHODS

E. coli (Strain ATCC 25922, clinical isolate, human source) was used as the test organism for this research. The bacteria were cultured in a nutrient broth (2006-11-30, Millipore Corp, Billerica, MA) consisting of beef extract and peptone, and incubated at 35°C. At 24 hour intervals a 5ml volume of the culture (approximately $3.5x10^5$ cfu per 50 ml) was centrifuged for 5 minutes at 7000g in a bench-top centrifuge(Model Z300:

Barnstead/Harvey, Boston, MA). The resultant pellet was washed once with deionized water, and then resuspended in 75 ml of growth medium. These serial transfers were repeated every 24 hours for the duration of the research. *E. coli* enumeration was performed in accordance with *Standard Methods* 922B (*Clesceri et.al., 1998).* Coliform and *E.coli* colonies were identified and counted as red and blue colonies, respectively, after 24hr of incubation at 35ºC.

Batch inactivation experiments were performed in a jacketed reactor with a working volume of 100 ml (Model no. 7844, Ace Glass, Vineland, NJ) and provided with a central well to accommodate the UV source. The temperature was maintained at 25ºC using a refrigerated circulator (Model 1006S, Fisher Scientific, Pittsburg, PA), and mixing was continuously provided using a teflon-coated stir bar.

Batch inactivation tests were conducted with UV, ozone, and UV and ozone simultaneously (UVO) as disinfectants at pH levels of 6.0, 7.0 and 8.0. Each treatment was run in triplicate at each pH level, control runs that did not receive any disinfectant dose were also performed. Ozone was produced using extra dry oxygen (>99%) using an air cooled corona discharge generator (PCI-WEDECO Environmental Technologies, West Caldwell, NJ). Ozone concentrations were varied by adjusting the generator voltage and the sparging time of the gas stream through the test media, and quantified using *Standard Methods* 2350E (*Clesceri et.al, 1998).* The UV source was an air cooled 450W high-pressure mercury vapor lamp (Ace Glass Inc., Vineland, NJ). The high-pressure lamp provides 67% higher luminous efficiency than a low-pressure lamp, and has an

output spectrum with about 60% of its power at 254 nm, higher than either low or medium-pressure lamps. UV intensity in the reactor was rated at 2000 $\mu W/cm^2$.

Medium for the batch inactivation tests consisted of 0.05M phosphate buffer at the desired pH, prepared in deionized water (≈ 18MΩ/cm). For the ozone and UVO inactivation tests, the medium was sparged with ozone-enriched oxygen for 5 to 10 minutes. When UV was provided the source was allowed to warm up and attain normal operating temperature. The medium was then transferred to the test chamber and inoculated with 10 ml ($3x10^5$ cfu) of *E.coli*. Samples were then withdrawn periodically from the reactor and analyzed for the concentration of *E.coli* and, when appropriate, ozone.

RESULTS AND DISCUSSION

Ultraviolet Disinfection

Experimental results were analyzed based on pseudo-first order rate kinetics (*Finch et.al, 1998)* and the rate expression expressed as (*Abu-Ghararah, 1994*)

$$N = N_0 \exp(-k'_{UV} t) \tag{4.6}$$

where, $k'_{uv} = k_{uv} I$, t = time, min; N = bacterial density at time t, cfu/ml; N_0 = initial bacterial density, cfu/ml; k_{uv} = inactivation rate constant (Figure 4.3), $cm^2/(\mu W.sec)$; and I = light intensity, $\mu W/cm^2$

Microbial enumeration data from each experiment was converted to survival rates (N/N_0), while the UV exposure was quantified in terms of the dose, i.e. the product of UV intensity and exposure time. Inactivation rate constants were obtained at each pH by plotting the logarithmic survival data against the UV dose. The heat generated by the UV source resulted in small but observable temperature variations, but the impact of temperatures between 20 and 40ºC on UV disinfection kinetics was shown to be minimal (*Abu-Ghararah, 1994),* hence temperature was not considered a factor in this analysis.

ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC) (α=0.05, p=0.0018) that pH indeed affects the inactivation rate, with pH 7.0 being the least conducive for bacterial inactivation. This observation is attributed to the fact that pH 7.0 coincides with the natural growth pH of the bacteria and hence with the greatest resistance to inactivation.

Ozone Disinfection

The kinetics of ozone inactivation of *E. coli* were studied using pseudo first order kinetics (*Finch et.al, 1998*) and the concentration expressed as an average of the ozone concentrations over the 180 sec experiment time.

$$N = N_0 \exp(-k_O' t) \tag{4.7}$$

where, $k'_o = k_o C$, t is the time in minutes, k_o is the pseudo first order reaction rate constant (Figure 4.4) and C is the ozone concentration in mg/l.

The ANOVA analysis on the reaction rate constants showed that pH has no effect on the observed rates and hence, the results are presented on the basis of the average

ozone concentration for the experiments performed. The reason for this variation from the UV inactivation behavior is attributed to the fact that ozone is a much more powerful disinfectant than UV and hence, masks any effects pH might have on the reaction kinetics. However, pH does effect the maximum ozone concentrations that can be attained in the water. This observation is in concurrence with the conclusions drawn by *Finch et. al. (1998)* and EPA (*Disinfection Profiling and Benchmarking Guidance Manual, US EPA)* that pH has no effect on the inactivation kinetics of an organism using ozone.

Combined Ultraviolet and Ozone Disinfection

The result of a combined dose of UV radiation and ozone was analyzed using first order reaction kinetics; the equation took the following form

$$N = N_0 \exp(-k'_{IC} t) \tag{4.8}$$

where, $k'_{IC} = k_{uv} * I * C$; C is the ozone concentration in aqueous phase, I is the UV intensity, and k_{uv} is the pseudo first order reaction rate constant for inactivation with UV radiation. Additional kinetic models with the intensity and ozone concentration exponent as 2 were also performed, however it was found that the expression with exponents as unity provided the best fit to the observed data.

Table 4.1

Comparison of Regression Runs with Various Exponents on Concentration and UV Intensity

Exponent on Conc., n	Exponent on Intensity, m	Average Coeff. of Determination
1	1	0.96
2	1	0.88
1	2	0.85

The statistical analysis ANOVA was performed, which again showed that pH, had no statistical effect on the inactivation kinetics. This non-effect of pH can again be explained on the basis of the strength of ozone as a disinfectant as well as an oxidant, however it can also be explained on the basis of catalytic effect that UV radiation has on the decay of ozone and the generation of hydroxide radicals. In the presence of UV ozone quickly decays into hydroxyl radicals following a process on which pH has no effect as long as it exceeds a pH of 4.0 (*Grasso et. al., 1989*). Experimental observations also showed that most of the suspended bacteria were inactivated within 120 seconds of exposure to UV-Ozone environment, and that the ozone concentration at the end of the 120-180 sec time period had almost dropped to 0, this can be attributed to a combined effect of ozone being used up in the inactivation process as well as the catalytic decay of ozone in the presence of UV radiation.

Table 4.2

First order inactivation rate coefficients for different treatments (min^{-1})

pH	Treatment			
	Control	UV	Ozone	UV-Ozone
6.0	0.00	0.1466±0.011	1.673±0.544	3.126±0.319
7.0	0.00	0.0866±0.011	0.955±0.153	2.580±0.072
8.0	0.00	0.1333±0.011	0.951±0.123	2.556±0.398

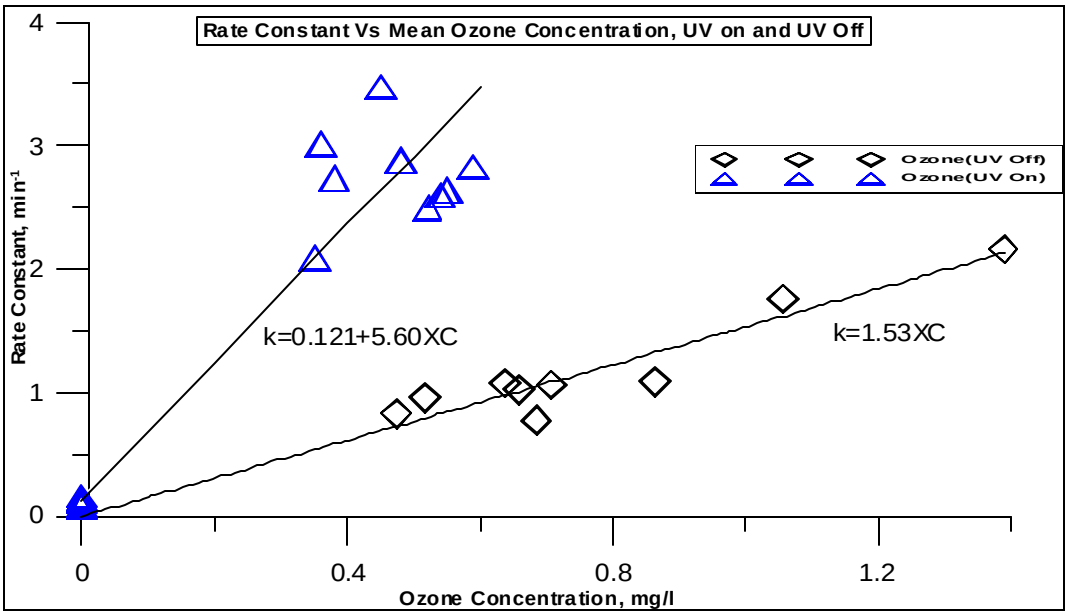

Figure 4.1; Comparison of Rate Constants with Varying Ozone Concentration and with UV either On or Off

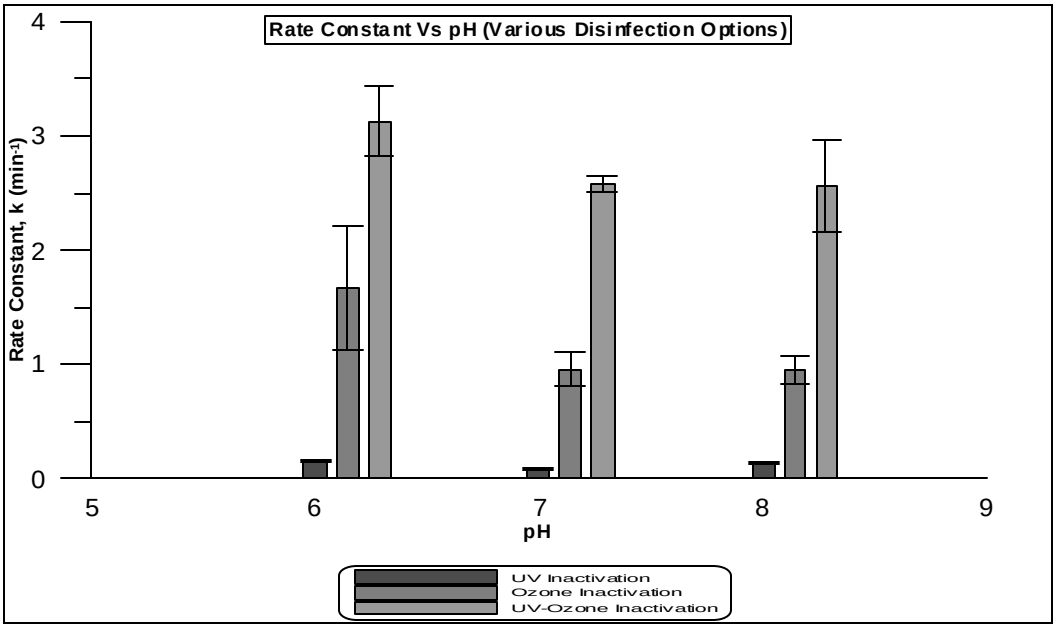

Figure 4.2; Comparison of Rate Constants for UV, O_3, and UVO_3, Vertical Bars Represent Standard Deviation

For the action of UV, ozone and UVO, it was assumed that the inactivation constant, k, is additive i.e.

$$k = k_C + k'_{uv} + k'_O + k'_{IC} \tag{4.9}$$

where k_c is the decay constant in the absence of any disinfectant, $k'_{uv} = k_{uv}I$, k_{uv} = inactivation rate constant, $cm^2/\mu W.min$, I = UV intensity, $\mu W/cm^2$, $k'_o = k_oC$, t is the time in minutes, C is the ozone concentration in mg/l, $k'_{IC} = k_{uv}IC$.

No decay in *E. coli* count was observed in 30 minutes in the absence of any disinfecting agent, which implies that k_c is 0, and hence, can be neglected. Equation 4.4 also implies that one or more of the terms can be neglected in case one or more of the constants is much larger than the others. The regressed line for the ozone (UV on) plot (figure 4.1) passes through 0.121 on the mantissa or the Y-axis, and a careful examination of this intercept shows that it is almost equal to the average of the reaction

constants for UV inactivation. In addition the slope of the regressed fit line i.e. 5.60 nearly mirrors the average value of 6.03 (Table 4.1) for the UVO inactivation process, these imply that the inactivation rate constants for the process can be determined if the rate constants for either UV or UVO processes are known. A comparison of the inactivation rate constants for the three disinfection treatments (Table 4.1 ,and Figures 4.1 and 4.2) shows that the inactivation rates for UV radiation alone are negligible when compared to either ozone or UVO process, however it is also seen that the UVO process represents an increase of almost 2-3 times from the ozone only inactivation rates. This increase is attributed by the authors to the formation of hydroxyl radicals during the decomposition of ozone in the presence of UV, this decay occurs even in the absence of UV but at a much retarded rate.

Table 4.3

Reaction Rate Constants for UV disinfection at varying pH

UV Intensity mW/cm^2	**pH**	**Run**	**Pseudo First Order Reaction Rate Constant, cm^2/µW.min**	**ANOVA, a =0.05**	
				F	**p**
2000	6	1	0.00008	22.33	0.0018
2000		2	0.00007		
2000		3	0.00007		
2000	7	1	0.00005		
2000		2	0.00004		
2000		3	0.00004		
2000	8	1	0.00007		
2000		2	0.00006		
2000		3	0.00007		

Table 4.4

Reaction Rate Constants for Ozone Disinfection at varying pH

pH	Run	Conc., mg/l	Pseudo First Order Reaction Rate Constant l/mg.min	ANOVA, a = 0.05	
6	1	1.056	1.670		
	2	1.389	1.559	F	p
	3	0.865	1.259		
7	1	0.708	1.500		
	2	0.657	1.558		
	3	0.683	1.140	3.12	0.117
8	1	0.516	1.858		
	2	0.473	1.739		
	3	0.637	1.679		

Table 4.5

Reaction Rate Constants for UV-Ozone Disinfection at varying pH

UV Intensity, mW/cm^2	Average Ozone Conc., mg/l	Pseudo First Order Reaction Rate Constant, cm^2.l/µW.l.min	ANOVA	
			F	p
2000	.45	0.00386		
2000	.36	0.00419		
2000	.48	0.00300		
2000	.54	0.00241	0.999	0.500
2000	.52	0.00240		
2000	.55	0.00240		
2000	.38	0.00360		
2000	.35	0.00300		
2000	.59	0.00239		

Conclusions

This work shows that the inactivation rates of *Escherichia coli* increase substantially when a combined dose of ozone and ultraviolet radiation is applied to a suspension of the bacteria and that the inactivation follows pseudo first order kinetics. No effect of pH was observed on the kinetics of the process other than for UV irradiation

inactivation. This study also conforms and concurs with several other studies such as those by *Finch et. al (1998)* that concluded that the inactivation of *E. coli* with ozone is not influenced by the pH of the aqueous medium, however the results dispute the conclusions reached by *Venosa et. al.(1984)* in that the current study clearly shows a significant improvement in the inactivation rates achieved by using UV and ozone simultaneously. The synergetic effect of UV radiation and ozone is attributed to the generation of hydroxyl by the decomposition of ozone in the presence of UV radiation. With respect to the kinetics of combined UV and Ozone inactivation of *E. coli* the authors conclude that first order reaction kinetics satisfy the observed experiment results.

CHAPTER V

SUMMARY

The kinetics of *Escherichia coli* (*E. coli*) inactivation with ozone and ultraviolet radiation were studied in a batch reactor maintained at a constant temperature of 25°C at pH levels of 6, 7, and 8. Three treatments, namely UV, ozone and UV-ozone, were tested at each pH to determine the efficacy of a combined dose of UV-ozone and any advantages such usage has on either UV or ozone used singularly.

Experiments were performed by inoculating the test reactor with *E.coli* and then monitoring the concentration of *E.coli* and when appropriate ozone over time. *E.coli* survival data were fit to a first-order decay model which took into account the intensity of UV radiation, ozone concentration, and the product of UV intensity and ozone concentration (UVO tests).

Table 5.1
First order inactivation rate coefficients for different treatments

pH	Control, min^{-1}	UV, min^{-1}	Ozone, min^{-1}	UV-Ozone,min^{-1}
6.0	0.00	0.1466±0.011	1.673±0.544	3.126±0.319
7.0	0.00	0.0866±0.011	0.955±0.153	2.580±0.072
8.0	0.00	0.1333±0.011	0.951±0.123	2.556±0.398

The inactivation constants as tabulated above clearly demonstrate the increase in inactivation rates achieved by the UVO process when compared to either UV or ozone

acting singularly. The inactivation rates for the UVO process are as much as 3 times faster than those achieved by ozone alone and are almost an order of magnitude higher than those achieved through UV radiation.

The model proposed to quantify the inactivation rates is additive i.e. the inactivation rates for the complete disinfection/inactivation process can be found by adding together the inactivation rates for UV alone, ozone alone and UVO process, one additional term to take into consideration the natural inactivation of *E.coli* can be added depending upon the magnitude the inactivation rate achieved for such a natural process. The current study did not encounter any inactivation of the bacterium in the absence of disinfectants and hence the model only consists of three inactivation rate terms as mentioned above.

UV decay studies with UV exposure were also performed, however the same were not taken into consideration for the kinetic equation because the kinetic equation as written includes both the UV intensity and ozone concentration terms and as such takes into consideration the ozone decay with UV exposure.

The following conclusions were drawn:

1. UV inactivation kinetics closely follow the pseudo-first order reaction kinetics.
2. Ozone inactivation did not show any statistical dependence on the pH. This can be rationalized owing to the extreme potency of ozone as an oxidant and disinfectant and hence, masks any effect the pH might have.
3. Ozone inactivation kinetics can be represented by pseudo-first order kinetic equations.

4. A combined dose of UV-Ozone achieved significantly higher inactivation rates than either UV or ozone alone.
5. UV acts as a catalyst in the decay of ozone generating hydroxyl radicals.
6. pH had no statistically appreciable effect on the inactivation kinetics of UV-Ozone, however pH did effect the decay rates of ozone in solution.

Additional research should be conducted to study the feasibility of replacing UV with peroxide or other similar compounds which have the capability of acting as catalysts in the decay of ozone. Other recommendations for future research include varying the UV intensity to probe the effect intensity of a UV source has on the inactivation kinetics, studying the process of ozone dissolution in water and researching ways to enhance the ozone concentrations which can be achieved, and methods for controlling the decomposition of ozone caused by hydroxide ions.

APPENDIX A

METHODOLOGY

Test Organism

E. coli (ATCC 25922, clinical isolate, human source) was used as the test organism for this research. The bacteria were cultured in a nutrient broth (2006-11-30, Millipore Corp, Billerica, MA) consisting of beef extract and peptone, and incubated at 35°C. At 24 hour intervals a 5ml volume of the culture (approximately $3.5x10^5$ cfu per 50 ml) was centrifuged for 5 minutes at 7000g in a bench-top centrifuge (Model Z300: Barnstead/Harvey, Boston, MA). The resultant pellet was washed once with deionized water, and then resuspended in the 75ml of growth medium. These serial transfers were repeated every 24 hours for the duration of the research.

Ozone Generation and Measurement

Ozone gas was generated from extra-dry oxygen using an air-cooled corona discharge ozone generator (Model C2P-3, PCI Ozone Corp.).The generator used throughout the research had voltage settings which were varied alongwith ozone contact time to obtain various ozone concentrations. Ozone output from generator was measured using *Standard Methods* 2350E (*Clesceri et.al., 1998).*

Ultraviolet Radiation Generation

Ultraviolet radiation was generated using an air cooled 450 W high pressure mercury vapor lamp (Model 7825-34; Ace Glass Inc., Vineyard, NJ), with additional heat removal achieved through the cooling system of the reactor. A high pressure quartz mercury arc lamp was chosen for use in this research because of the many advantages it offers over a low pressure lamp. The luminous efficiency of a Hg-Qz arc is about 5 candle power per watt as opposed to 3 candle power per watt for the low pressure lamp, and about 60% of radiation output is of 254nm which is greater than either low or medium pressure lamps.

Enumeration of *E Coli*

E. coli enumeration was performed in accordance with *Standard Methods* 922B (*Clesceri et.al., 1998). E. coli* enumeration was performed using the m-ColiBlue24 Broth (Hach Co., Loveland, CO), a nutritive, lactose-based medium, containing inhibitors that selectively eliminate non-coliform bacteria. The *Coliforms* become visible by reducing a non-selective dye, TTC (2,3,5-triphenyltetrazolium chloride) present in the medium. The reduction of TTC results in the formation of red colonies which can be easily seen on the membrane filter. *E. coli* become visible by a blue color which forms in *E. coli* colonies. This color formation is the result of enzymatic cleavage of a substrate, BCIG (5-bromo-4-chloro-3-indolyl-ß-D-glucoronide), by the enzyme ß-glucoronidase produced by *E. coli* (*Hach company technical literature*).

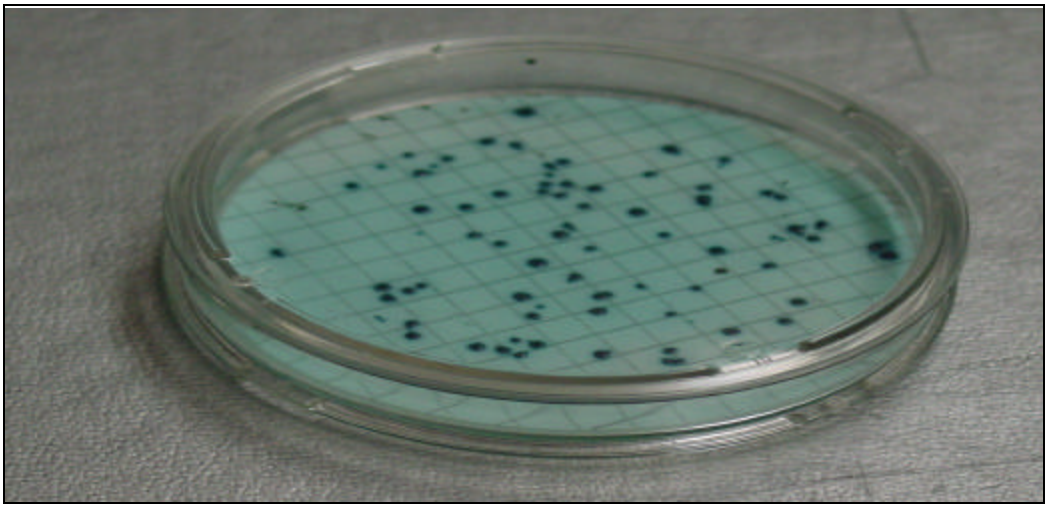

Figure 3.1, *E. coli* Colonies on m-ColiBlue24.

Experimental Methodology

The objective of the research was to investigate the kinetics of *Escherichia coli* inactivation by ultraviolet radiation and ozone, and to evaluate the effects pH has on the inactivation kinetics.

To achieve the aforementioned objective the experimental setup was divided into three separate blocks viz. UV, Ozone, and UV-ozone (UVO). These three blocks were further subdivided into experiments at pHs of 6.0, 7.0, and 8.0 to analyze the effectpH might have. The reactor (Model No. 7844, Vineland, NJ) with a working volume of 1000 l was utilized for the experiments, and was operated in a batch mode as a completely mixed reactor with constant stirring provided by Teflon coated magnetic stir bar.

The buffer system consisted of 0.05 M solution of monobasic and dibasic sodium phosphate, table 3.1 gives the composition of 1 liter of a pH buffer.

Table 3.1

Composition of the buffer system

pH	$Na_2HPO_4.7H_2O$, gm/l	NaH_2PO_4, gm/l
6.0	3.58	10.55
7.0	17.20	6.29
8.0	27.78	0.82

The deionized water (at least 18M? /cm), buffered to the desired pH, was inoculated with *E. coli* and exposed to the disinfectant for a period of 30 minutes for UV radiation and 3 minutes for ozone and UV-O_3, with 50 ml samples being taken at regular intervals and analyzed for bacterial count using the procedure mentioned before. The procedure was repeated 3 times for each pH level. Hence, 9 block experiments were performed at the 3 pH.

Ozone experiments followed the same general procedure with the difference that the concentration of aqueous ozone was varied by changing the ozone sparge time through the water column in the reactor.

Two sparging times were used, 10 and 5 minutes for all 3 pH levels. The oxygen inflow into the generator and ozone output from the generator was kept constant by regulating the oxygen flow and the applied plate voltage.

Oxygen was let into the generator at a rate of 0.67 cfm (40 scfh) and the ozone output from the generator was measured to be at 0.5 cfm (30 scfh). Ozone output was ~ 14mg/min

The ozone experiments were limited to an observation period of only 3 minutes, because the concentration of ozone in the inoculated water could not be maintained for

more that 3 minutes and neither did any measurable bacteria survive for a period more than 3 minutes.

The combined Ozone and UV experiments followed the same procedure as the ozone only experiments with the difference that UV radiation was introduced into the experiments in addition to ozone.

APPENDIX B

DATA AND TABLES

UV Experiments

Table B1

Survival Data for UV exposure, pH 6.0 Run 1

Time, min	Count/20ml	Volume, ml	Count/100ml	ln(N/No)	Time.Intensity, min-mW/cm^2
0	116	50	232	0	0
3	73	50	146	-0.46313075	6000
6	45	50	90	-0.946927701	12000
9	31	50	62	-1.319602987	18000
12	19	50	38	-1.809151212	24000
15	8	50	16	-2.674148649	30000
18	8	50	16	-2.674148649	36000
21	3	50	6	-3.654977902	42000
24	1	50	2	-4.753590191	48000
27	2	50	4	-4.060443011	54000
30	2	50	4	-4.060443011	60000

Table B2

Survival Data for UV exposure, pH 6.0 Run 2

Time, min	Count/20ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-µW/cm^2
0	125	50	250	0	0
3	110	50	220	-0.127833372	6000
6	50	50	100	-0.916290732	12000
9	27	50	54	-1.532476871	18000
12	27	50	54	-1.532476871	24000
15	17	50	34	-1.995100393	30000
18	8	50	16	-2.748872196	36000
21	3	50	6	-3.729701449	42000
24	2	50	4	-4.135166557	48000
27	1	50	2	-4.828313737	54000
30	6	50	12	-3.036554268	60000

Table B3

Survival data for UV exposure, pH 6.0 Run 3

Time, min	Count/20ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-mW/cm^2
0	120	20	600	0	0
3	80	20	400	-0.405465108	6000
6	56	20	280	-0.762140052	12000
9	38	20	190	-1.149905583	18000
12	38	20	190	-1.149905583	24000
15	20	20	100	-1.791759469	30000
18	12	20	60	-2.302585093	36000
21	15	20	75	-2.079441542	42000
24	4	20	20	-3.401197382	48000
27	3	20	15	-3.688879454	54000
30	1	20	5	-4.787491743	60000

Table B4

Survival data for UV exposure, pH 7.0 Run 1

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-mW/cm2
0	220	50	440	0	0
3	210	50	420	-0.04652002	6000
6	205	50	410	-0.07061757	12000
9	72	50	144	-1.11696143	18000
12	42	50	84	-1.65595793	24000
15	45	50	90	-1.58696506	30000
18	24	50	48	-2.21557372	36000
21	33	50	66	-1.89711998	42000
24	16	50	32	-2.62103882	48000
27	21	50	42	-2.34910511	54000
30	9	50	18	-3.19640297	60000

Table B5

Survival data for UV exposure, Ph 7.0 Run 2

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_0$)	Time*Intensity, min-μW/cm^2
0	242	50	484	0	0
3	205	50	410	-0.16592775	6000
6	172	50	344	-0.34144325	12000
9	110	50	220	-0.78845736	18000
12	112	50	224	-0.77043885	24000
15	110	50	220	-0.78845736	30000
18	60	50	120	-1.39459316	36000
21	60	50	120	-1.39459316	42000
24	44	50	88	-1.70474809	48000
27	25	50	50	-2.2700619	54000
30	10	50	20	-3.18635263	60000

Table B6

Survival data for UV exposure, pH 7.0 Run 3

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-μW/cm^2
0	200	50	400	0	0
3	165	50	330	-0.19237189	6000
6	120	50	240	-0.51082562	12000
9	115	50	230	-0.55338524	18000
12	100	50	200	-0.69314718	24000
15	78	50	156	-0.94160854	30000
18	50	50	100	-1.38629436	36000
21	45	50	90	-1.49165488	42000
24	30	50	60	-1.89711998	48000
27	26	50	52	-2.04022083	54000
30	15	50	30	-2.59026717	60000

Table B7

Survival data for UV exposure, pH 8.0 Run 1

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-μW/cm^2
0	95	50	190	0	0
3	87	50	174	-0.08797	6000
6	60	50	120	-0.45953	12000
9	44	50	88	-0.76969	18000
12	13	50	26	-1.98893	24000
15	25	50	50	-1.335	30000
18	2	50	4	-3.86073	36000
21	9	50	18	-2.35665	42000
24	4	50	8	-3.16758	48000
27	2	50	4	-3.86073	54000
30	2	50	4	-3.86073	60000

Table B8

Survival Data for UV exposure, pH 8.0 Run 2

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-μW/cm^2
0	155	50	310	0	0
3	142	50	284	-0.0876	6000
6	82	50	164	-0.63671	12000
9	80	50	160	-0.6614	18000
12	30	50	60	-1.64223	24000
15	35	50	70	-1.48808	30000
18	38	50	76	-1.40584	36000
21	16	50	32	-2.27084	42000
24	10	50	20	-2.74084	48000
27	5	50	10	-3.43399	54000
30	5	50	10	-3.43399	60000

Table B9

Survival data for UV exposure, pH 8.0 Run 3

Time, min	Count/50ml	Volume, ml	Count/100ml	ln(N/N$_o$)	Time*Intensity, min-μW/cm^2
0	106	50	212	0	0
3	75	50	150	-0.34595	6000
6	35	50	70	-1.10809	12000
9	46	50	92	-0.8348	18000
12	25	50	50	-1.44456	24000
15	15	50	30	-1.95539	30000
18	10	50	20	-2.36085	36000
21	10	50	20	-2.36085	42000
24	2	50	4	-3.97029	48000
27	1	50	2	-4.66344	54000
30	1	50	2	-4.66344	60000

Ozone Experiments

Table B10

Survival data for ozone exposure, Average conc. 0.87mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration-Time, mg-sec/l	ln(N/No)
0	1.23	290	0	0
30	1.16	115	25.94387755	-0.92495
60	1.14	65	51.8877551	-1.49549
90	0.80	45	77.83163265	-1.86322
120	0.68	32	103.7755102	-2.20415
150	0.54	26	129.7193878	-2.41178
180	0.49	10	155.6632653	-3.3673

Table B11

Survival data for Ozone exposure, Average conc. 1.05mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration-Time, mg-sec/l	ln(N/No)
0	1.16	270	0	0.00000
30	1.29	110	31.68	-0.89794
60	1.12	37	63.36	-1.98750
90	0.84	10	95.04	-3.29584
120	1.07	17	126.72	-2.76521
150	1.05	1	158.4	-5.59842
180	0.86	3	190.08	-4.49981

Table B12

Survival data for Ozone exposure, Average conc. 1.4mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration-Time, mg-sec/l	ln(N/No)
0	1.37	265	0.00	0.00000
30	1.32	80	41.68	-1.19770
60	1.25	10	83.37	-3.27714
90	0.80	5	125.05	-3.97029
120	3.15	2	166.73	-4.88658
150	1.04	1	208.42	-5.57973
180	0.80	1	250.10	-5.57973

Table B13

Survival data for Ozone exposure, Average conc.0.9mg/l

Time, sec	Conc, mg/l	Count/30ml	Count/100ml	Concentration*Time, mg-sec/l	ln(N/No)
0	1.13	95	316	0.00	0.00000
30	0.98	86	286	27.04	-0.09953
60	0.99	56	186	54.08	-0.52853
90	0.85	65	216	81.12	-0.37949
120	0.92	35	117	108.15	-0.99853
150	0.73	25	83	135.19	-1.33500
180	0.70	18	60	162.23	-1.66351

Table B14

Survival data for Ozone exposure, Average conc. 0.68mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration*Time, mg-sec/l	ln(N/N$_o$)
0	1.09	90	0.00	0.00000
30	0.80	62	20.48	-0.37268
60	0.79	25	40.97	-1.28093
90	0.66	25	61.45	-1.28093
120	0.54	20	81.94	-1.50408
150	0.49	15	102.42	-1.79176
180	0.40	9	122.91	-2.30259

Table B15

Survival data for Ozone exposure, Average conc. 0.71mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration*Time, mg-sec/l	ln(N/N$_o$)
0	1.04	80	0.00	0.00000
30	0.77	44	21.24	-0.59684
60	0.86	33	42.49	-0.87547
90	0.76	17	63.73	-1.56862
120	0.56	7	84.98	-2.48491
150	0.57	3	106.22	-3.17805
180	0.39	6	127.47	-2.58927

Table B16

Survival data for Ozone exposure, Average conc. 0.8mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration*Time, mg-sec/l	ln(N/N$_o$)
0	1.17	150	0.00	0.00000
30	1.11	95	23.72	-0.45676
60	1.00	56	47.45	-0.98528
90	0.70	35	71.17	-1.45529
120	0.60	10	94.90	-2.70805
150	0.52	6	118.62	-3.21888
180	0.44	1	142.35	-5.01064

Table B17

Survival data for Ozone exposure, Average conc. 0.5mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration*Time, mg-sec/l	ln(N/No)
0	1.07	174	0.00	0.00000
30	0.80	47	15.50	-1.31750
60	0.71	83	31.00	-0.74021
90	0.32	55	46.50	-1.15172
120	0.29	49	62.00	-1.26724
150	0.26	12	77.50	-2.67415
180	0.17	8	93.00	-3.07961

Table B18

Survival data for Ozone exposure, Average conc. 0.6 mg/l

Time, sec	Conc, mg/l	Count/100ml	Concentration*Time, mg-sec/l	ln(N/No)
0	0.86	195	0.00	0.00000
30	0.85	100	18.27	-0.66783
60	0.77	75	36.53	-0.95551
90	0.63	35	54.80	-1.71765
120	0.50	14	73.06	-2.63394
150	0.40	5	91.33	-3.66356
180	0.25	5	109.59	-3.66356

Ultraviolet-Ozone Experiments

Table B19

Survival data for Ozone-Ultraviolet exposure, average conc. 0.35mg/l

Time, sec	Conc, mg/l	Count/100ml	ln(N/No)	Time*Intensity*Concentration, mg-sec-μW/cm^2-l
0	1.28	189	0.00000	0.00
30	0.32	42	-1.50408	21500.00
60	0.13	5	-3.63231	43000.00
90	0.07	1	-5.24175	64500.00
120	0.01	1	-5.24175	86000.00

Table B20

Survival data for Ozone-Ultraviolet exposure, Average conc. 0.45 mg/l

Time, sec	Conc, mg/l	Count/100ml	ln(N/No)	Time*Intensity*Concentration, mg-sec-μW/cm^2-l
0	1.30	175	0.00000	0.00
30	0.33	35	-1.60944	27500.00
60	0.12	2	-4.47164	55000.00
90	0.08	1	-5.16479	82500.00

Table B21

Survival data for Ozone-Ultraviolet exposure, Average conc. 0.5 mg/l

Time, sec	Conc, mg/l	Count/100ml	ln(N/No)	Time*Intensity*Concentration, mg-sec-μW/cm^2-l
0	1.31	165	0.00000	0.00
30	0.51	115	-0.36101	31071.43
60	0.23	15	-2.39790	62142.86
90	0.02	3	-4.00733	93214.29

Table B 22

Survival data for Ozone-Ultraviolet exposure, Average conc. 0.60 mg/l

Time, sec	Conc, mg/l	Count/100ml	ln(N/No)	Time*Intensity*Concentration, mg-sec-μW/cm^2-l
0	1.35	245	0.00000	0.00
30	1.06	85	-1.05861	34857.14
60	0.49	15	-2.79321	69714.29
90	0.01	1	-5.50126	104571.43
120	0.00	1	-5.50126	139428.57

Ultraviolet Disinfection Result

Table B23

Reaction Rate Constants for UV disinfection at varying pH

UV Intensity mW/cm^2	pH	Run	Pseudo First Order Reaction Rate Constant, min^{-1}	ANOVA, a = 0.05	
				F	p
2000	6	1	0.00008	22.33	0.0018
2000		2	0.00007		
2000		3	0.00007		
2000	7	1	0.00005		
2000		2	0.00004		
2000		3	0.00004		
2000	8	1	0.00007		
2000		2	0.00006		
2000		3	0.00007		

Table B24

Reaction Rate Constants for Ozone Disinfection at varying pH

pH	Run	Conc., mg/l	k_o', min^{-1}	ANOVA, a = 0.05	
6	1	1.056	1.670		
	2	1.389	1.559	F	p
	3	0.865	1.259	3.12	0.117
7	1	0.708	1.500		
	2	0.657	1.558		
	3	0.683	1.140		
8	1	0.516	1.858		
	2	0.473	1.739		
	3	0.637	1.679		

Table B25

Reaction Rate Constants for UV-Ozone Disinfection at varying pH

UV Intensity, mW/cm^2	Initial Ozone Conc., mg/l	Average Ozone Conc., mg/l	k_{ouv}', min^{-1}	ANOVA	
				F	p
2000	1.29	.45	0.00386	0.999	0.500
2000	1.27	.36	0.00419		
2000	1.38	.48	0.00300		
2000	1.23	.54	0.00241		
2000	1.30	.52	0.00240		
2000	1.36	.55	0.00240		
2000	1.00	.38	0.00360		
2000	1.11	.35	0.00300		
2000	1.34	.59	0.00239		

SAS OUTPUT RESULTS

Complete Data Set

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Obs	ph	uv	o3c	o3	k
1	6	2000	0.0000	0	0.1600
2	6	2000	0.0000	0	0.1400
3	6	2000	0.0000	0	0.1400
4	7	2000	0.0000	0	0.1000
5	7	2000	0.0000	0	0.0800
6	7	2000	0.0000	0	0.0800
7	8	2000	0.0000	0	0.1400
8	8	2000	0.0000	0	0.1200
9	8	2000	0.0000	0	0.1400
10	6	0	1.0557	1	1.7736
11	6	0	1.3895	1	2.2092
12	6	0	0.8648	1	1.1104

13	6	0	0.9013	1
14	6	0	0.8537	1
15	6	0	0.8435	1
16	7	0	0.7081	1
17	7	0	0.6573	1
18	7	0	0.6828	1
19	7	0	0.7068	1
20	7	0	0.7908	1
21	7	0	0.8172	1
22	8	0	0.5167	1
23	8	0	0.4728	1
24	8	0	0.6369	1
25	8	0	0.6284	1
26	8	0	0.6088	1
27	8	0	0.7432	1
28	6	2000	0.4583	1
29	6	2000	0.3583	1
30	6	2000	0.4869	1
31	7	2000	0.5387	1
32	7	2000	0.5179	1
33	7	2000	0.5476	1
34	8	2000	0.3786	1
35	8	2000	0.3562	1
36	8	2000	0.5810	1
37	6	0	0.0000	0
38	7	0	0.0000	0
39	8	0	0.0000	0

1.5142
1.4599
1.2147
1.0622
1.0294
0.7825
1.2086
1.3997
1.2503
0.9486
0.8425
1.0738
1.3988
1.2859
1.5340
3.8500
3.0100
2.9214
2.5857
2.4857
2.6286
2.7257
2.1369
2.7886
0.0000
0.0000
0.0000

Complete Data Set (O3 class)
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The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8
uv	2	0 2000
o3	2	0 1

Number of observations 39

Complete Data Set (O3 class)
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The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	39.15745102	4.35082789	56.91	<.0001
Error	29	2.21689349	0.07644460		
Corrected Total	38	41.37434452			

R-Square	Coeff Var	Root MSE	k Mean
0.946419	21.85843	0.276486	1.264895

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	1.08776802	0.54388401	7.11	0.0031
uv	1	1.23837453	1.23837453	16.20	0.0004
o3	1	33.17315646	33.17315646	433.95	<.0001
ph*uv	2	0.00393238	0.00196619	0.03	0.9746
ph*o3	2	0.50600703	0.25300351	3.31	0.0507
uv*o3	1	3.14821260	3.14821260	41.18	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.47624223	0.23812111	3.11	0.0595
uv	1	4.35563038	4.35563038	56.98	<.0001
o3	1	25.57698855	25.57698855	334.58	<.0001
ph*uv	2	0.10170651	0.05085326	0.67	0.5218
ph*o3	2	0.50600703	0.25300351	3.31	0.0507
uv*o3	1	3.14821260	3.14821260	41.18	<.0001

Complete Data Set (O3 numeric)

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The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8
uv	2	0 2000

Number of observations 39

Complete Data Set (O3 numeric)
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The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	39.01126474	4.33458497	53.19	<.0001
Error	29	2.36307978	0.08148551		
Corrected Total	38	41.37434452			

R-Square	Coeff Var	Root MSE	k Mean
0.942885	22.56762	0.285457	1.264895

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	1.08776802	0.54388401	6.67	0.0041
uv	1	1.23837453	1.23837453	15.20	0.0005
o3c	1	24.96587781	24.96587781	306.38	<.0001
ph*uv	2	1.85469205	0.92734602	11.38	0.0002
o3c*ph	2	0.24690970	0.12345485	1.52	0.2367
o3c*uv	1	9.61764264	9.61764264	118.03	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.00892109	0.00446054	0.05	0.9468
uv	1	0.04803015	0.04803015	0.59	0.4488

```
             o3c                            1     32.26939806
32.26939806     396.01    <.0001
             ph*uv                          2      1.00293460
0.50146730       6.15    0.0059
             o3c*ph                         2      0.29472592
0.14736296       1.81    0.1819
             o3c*uv                         1      9.61764264
9.61764264     118.03    <.0001

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                                                    UV vs. No UV
11:14 Monday, March 24, 2003 460

                                      Obs    ph    uv      o3c    o3
k

                                        1    6     2000     0     0
0.16
                                        2    6     2000     0     0
0.14
                                        3    6     2000     0     0
0.14
                                        4    7     2000     0     0
0.10
                                        5    7     2000     0     0
0.08
                                        6    7     2000     0     0
0.08
                                        7    8     2000     0     0
0.14
                                        8    8     2000     0     0
0.12
                                        9    8     2000     0     0
0.14
                                       10    6     0        0     0
0.00
                                       11    7     0        0     0
0.00
                                       12    8     0        0     0
0.00

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                                                    UV vs. No UV
11:14 Monday, March 24, 2003 461

                                          The GLM Procedure

                                      Class Level Information

                                   Class         Levels    Values
```

ph 3 6 7 8

uv 2 0 2000

Number of observations 12

UV vs. No UV

11:14 Monday, March 24, 2003 462

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.03956667	0.00791333	59.35	<.0001
Error	6	0.00080000	0.00013333		
Corrected Total	11	0.04036667			

R-Square	Coeff Var	Root MSE	k Mean
0.980182	12.59673	0.011547	0.091667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	0.00446667	0.00223333	16.75	0.0035
uv	1	0.03361111	0.03361111	252.08	<.0001
ph*uv	2	0.00148889	0.00074444	5.58	0.0427

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.00148889	0.00074444	5.58	0.0427

```
            uv                             1       0.03361111
0.03361111     252.08    <.0001
            ph*uv                          2       0.00148889
0.00074444        5.58    0.0427

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                                                    O3 vs. No O3
11:14 Monday, March 24, 2003 463

                                    Obs    ph    uv      o3c     o3
k

                                      1    6     0     1.0557    1
1.7736
                                      2    6     0     1.3895    1
2.2092
                                      3    6     0     0.8648    1
1.1104
                                      4    6     0     0.9013    1
1.5142
                                      5    6     0     0.8537    1
1.4599
                                      6    6     0     0.8435    1
1.2147
                                      7    7     0     0.7081    1
1.0622
                                      8    7     0     0.6573    1
1.0294
                                      9    7     0     0.6828    1
0.7825
                                     10    7     0     0.7068    1
1.2086
                                     11    7     0     0.7908    1
1.3997
                                     12    7     0     0.8172    1
1.2503
                                     13    8     0     0.5167    1
0.9486
                                     14    8     0     0.4728    1
0.8425
                                     15    8     0     0.6369    1
1.0738
                                     16    8     0     0.6284    1
1.3988
                                     17    8     0     0.6088    1
1.2859
                                     18    8     0     0.7432    1
1.5340
                                     19    6     0     0.0000    0
0.0000
                                     20    7     0     0.0000    0
0.0000
```

21 8 0 0.0000 0
0.0000

O3 vs. No O3 (O3 class)
11:14 Monday, March 24, 2003 464

The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8
o3	2	0 1

Number of observations 21

O3 vs. No O3 (O3 class)
11:14 Monday, March 24, 2003 465

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4.87076690	0.97415338	10.50	0.0002
Error	15	1.39118295	0.09274553		
Corrected Total	20	6.26194985			

R-Square	Coeff Var	Root MSE	k Mean
0.777836	27.68763	0.304542	1.099919

Source	DF	Type I SS	Mean Square	F Value	Pr > F

```
            ph                             2      0.54547733
0.27273866       2.94    0.0837
            o3                             1      4.23437669
4.23437669      45.66    <.0001
            ph*o3                          2      0.09091289
0.04545644       0.49    0.6220


            Source                      DF     Type III SS     Mean
Square    F Value    Pr > F

            ph                             2      0.09091289
0.04545644       0.49    0.6220
            o3                             1      4.23437669
4.23437669      45.66    <.0001
            ph*o3                          2      0.09091289
0.04545644       0.49    0.6220

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                                        O3 vs. No O3 (O3 numeric)
11:14 Monday, March 24, 2003 466

                                            The GLM Procedure

                                      Class Level Information

                                    Class         Levels    Values

                                    ph                 3    6 7 8

                                     Number of observations    21

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---------------------------------------

                                        O3 vs. No O3 (O3 numeric)
11:14 Monday, March 24, 2003 467

                                            The GLM Procedure

Dependent Variable: k

                                                    Sum of
            Source                      DF         Squares     Mean
Square    F Value    Pr > F

            Model                          5      5.94992176
1.18998435      57.21    <.0001
```

```
            Error                       15      0.31202809
0.02080187

            Corrected Total             20      6.26194985


                                R-Square     Coeff Var      Root MSE
k Mean

                                0.950171      13.11265      0.144229
1.099919


            Source                      DF       Type I SS     Mean
Square    F Value    Pr > F

            ph                           2      0.54547733
0.27273866      13.11    0.0005
            o3c                          1      5.34947497
5.34947497     257.16    <.0001
            o3c*ph                       2      0.05496946
0.02748473       1.32    0.2961


            Source                      DF     Type III SS     Mean
Square    F Value    Pr > F

            ph                           2      0.00007661
0.00003831       0.00    0.9982
            o3c                          1      4.66070607
4.66070607     224.05    <.0001
            o3c*ph                       2      0.05496946
0.02748473       1.32    0.2961

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                                          O3 - Effect of pH
11:14 Monday, March 24, 2003 468

                            Obs    ph    uv      o3c     o3       k
ko3

                              1     6     0     1.0557    1     1.7736
1.68002
                              2     6     0     1.3895    1     2.2092
1.58992
                              3     6     0     0.8648    1     1.1104
1.28400
                              4     6     0     0.9013    1     1.5142
1.68002
                              5     6     0     0.8537    1     1.4599
1.71009
```

6	6	0	0.8435	1	1.2147
1.44007					
7	7	0	0.7081	1	1.0622
1.50007					
8	7	0	0.6573	1	1.0294
1.56610					
9	7	0	0.6828	1	0.7825
1.14602					
10	7	0	0.7068	1	1.2086
1.70996					
11	7	0	0.7908	1	1.3997
1.76998					
12	7	0	0.8172	1	1.2503
1.52998					
13	8	0	0.5167	1	0.9486
1.83588					
14	8	0	0.4728	1	0.8425
1.78194					
15	8	0	0.6369	1	1.0738
1.68598					
16	8	0	0.6284	1	1.3988
2.22597					
17	8	0	0.6088	1	1.2859
2.11219					
18	8	0	0.7432	1	1.5340
2.06405					

O3 - Effect of pH (O3 class)

11:14 Monday, March 24, 2003 469

The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8

Number of observations 18

O3 - Effect of pH (O3 class)

11:14 Monday, March 24, 2003 470

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.63639021	0.31819511	3.43	0.0593
Error	15	1.39118295	0.09274553		
Corrected Total	17	2.02757316			

R-Square	Coeff Var	Root MSE	k Mean
0.313868	23.73225	0.304542	1.283239

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	0.63639021	0.31819511	3.43	0.0593

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.63639021	0.31819511	3.43	0.0593

O3 - Effect of pH (O3 class)
11:14 Monday, March 24, 2003 471

The GLM Procedure

Dependent Variable: ko3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.64372906	0.32186453	7.93	0.0045
Error	15	0.60845872	0.04056391		

Corrected Total 17 1.25218778

R-Square Coeff Var Root MSE ko3 Mean

0.514083 11.95982 0.201405 1.684013

Source DF Type I SS Mean Square F Value Pr > F

ph 2 0.64372906 0.32186453 7.93 0.0045

Source DF Type III SS Mean Square F Value Pr > F

ph 2 0.64372906 0.32186453 7.93 0.0045

O3 - Effect of pH (O3 numeric)
11:14 Monday, March 24, 2003 472

The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8

Number of observations 18

O3 - Effect of pH (O3 numeric)
11:14 Monday, March 24, 2003 473

The GLM Procedure

Dependent Variable: k

Source DF Sum of Squares Mean Square F Value Pr > F

```
             Model                          5      1.75268883
0.35053777      15.30    <.0001

             Error                         12      0.27488434
0.02290703

             Corrected Total               17      2.02757316


                               R-Square     Coeff Var      Root MSE
k Mean

                               0.864427      11.79443      0.151351
1.283239


             Source                        DF       Type I SS     Mean
Square    F Value    Pr > F

             ph                             2      0.63639021
0.31819511      13.89    0.0008
             o3c                            1      1.08500964
1.08500964      47.37    <.0001
             o3c*ph                         2      0.03128897
0.01564449       0.68    0.5237


             Source                        DF     Type III SS     Mean
Square    F Value    Pr > F

             ph                             2      0.01016530
0.00508265       0.22    0.8042
             o3c                            1      0.60508246
0.60508246      26.41    0.0002
             o3c*ph                         2      0.03128897
0.01564449       0.68    0.5237

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                                      O3 - Effect of pH (O3 numeric)
11:14 Monday, March 24, 2003 474

                                            The GLM Procedure

Dependent Variable: ko3

                                                    Sum of
             Source                        DF         Squares     Mean
Square    F Value    Pr > F

             Model                          5      0.73473776
0.14694755       3.41    0.0380
```

```
            Error                       12      0.51745003
0.04312084

            Corrected Total             17      1.25218778


                                R-Square     Coeff Var      Root MSE
ko3 Mean

                                0.586763      12.33100      0.207656
1.684013


            Source                      DF       Type I SS     Mean
Square    F Value    Pr > F

            ph                           2      0.64372906
0.32186453       7.46    0.0078
            o3c                          1      0.04467667
0.04467667       1.04    0.3288
            o3c*ph                       2      0.04633203
0.02316601       0.54    0.5978


            Source                      DF     Type III SS     Mean
Square    F Value    Pr > F

            ph                           2      0.02241976
0.01120988       0.26    0.7753
            o3c                          1      0.08460626
0.08460626       1.96    0.1866
            o3c*ph                       2      0.04633203
0.02316601       0.54    0.5978

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                                        O3 - With UV vs Without UV
11:14 Monday, March 24, 2003 475

                           Obs    ph    uv        o3c     o3       k
ko3

                             1     6     0       1.0557    1     1.7736
1.68002
                             2     6     0       1.3895    1     2.2092
1.58992
                             3     6     0       0.8648    1     1.1104
1.28400
                             4     6     0       0.9013    1     1.5142
1.68002
                             5     6     0       0.8537    1     1.4599
1.71009
```

6	6	0	0.8435	1	1.2147
1.44007					
7	7	0	0.7081	1	1.0622
1.50007					
8	7	0	0.6573	1	1.0294
1.56610					
9	7	0	0.6828	1	0.7825
1.14602					
10	7	0	0.7068	1	1.2086
1.70996					
11	7	0	0.7908	1	1.3997
1.76998					
12	7	0	0.8172	1	1.2503
1.52998					
13	8	0	0.5167	1	0.9486
1.83588					
14	8	0	0.4728	1	0.8425
1.78194					
15	8	0	0.6369	1	1.0738
1.68598					
16	8	0	0.6284	1	1.3988
2.22597					
17	8	0	0.6088	1	1.2859
2.11219					
18	8	0	0.7432	1	1.5340
2.06405					
19	6	2000	0.4583	1	3.8500
8.40061					
20	6	2000	0.3583	1	3.0100
8.40078					
21	6	2000	0.4869	1	2.9214
6.00000					
22	7	2000	0.5387	1	2.5857
4.79989					
23	7	2000	0.5179	1	2.4857
4.79958					
24	7	2000	0.5476	1	2.6286
4.80022					
25	8	2000	0.3786	1	2.7257
7.19942					
26	8	2000	0.3562	1	2.1369
5.99916					
27	8	2000	0.5810	1	2.7886
4.79966					

O3 - With UV vs Without UV (O3 class) 11:14 Monday, March 24, 2003 476

The GLM Procedure

Class Level Information

Class	Levels	Values
uv	2	0 2000
ph	3	6 7 8

Number of observations 27

O3 - With UV vs Without UV (O3 class) 11:14 Monday, March 24, 2003 477

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	15.28962377	3.05792475	29.38	<.0001
Error	21	2.18563744	0.10407797		
Corrected Total	26	17.47526122			

R-Square	Coeff Var	Root MSE	k Mean
0.874930	18.06000	0.322611	1.786330

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	1.49153425	0.74576713	7.17	0.0042
uv	1	13.66741584	13.66741584	131.32	<.0001
uv*ph	2	0.13067368	0.06533684	0.63	0.5435

Source	DF	Type III SS	Mean Square	F Value	Pr > F

```
            ph                                2      1.60801009
0.80400504       7.73    0.0031
            uv                                1     13.66741584
13.66741584     131.32    <.0001
            uv*ph                             2      0.13067368
0.06533684       0.63    0.5435

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                                    O3 - With UV vs Without UV (O3
class)     11:14 Monday, March 24, 2003 478

                                         The GLM Procedure

Dependent Variable: ko3

                                                          Sum of
            Source                        DF         Squares     Mean
Square    F Value    Pr > F

            Model                          5     131.2637370
26.2527474      75.21    <.0001

            Error                         21       7.3301188
0.3490533

            Corrected Total               26     138.5938558


                              R-Square     Coeff Var      Root MSE
ko3 Mean

                              0.947111      18.65455      0.590807
3.167094


            Source                        DF       Type I SS     Mean
Square    F Value    Pr > F

            ph                             2       4.3144464
2.1572232       6.18    0.0078
            uv                             1     118.7746051
118.7746051     340.28    <.0001
            uv*ph                          2       8.1746855
4.0873428      11.71    0.0004


            Source                        DF     Type III SS     Mean
Square    F Value    Pr > F

            ph                             2       8.0483377
4.0241688      11.53    0.0004
```

```
             uv                          1     118.7746051
118.7746051     340.28    <.0001
             uv*ph                       2       8.1746855
4.0873428       11.71    0.0004

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                                  O3 - With UV vs Without UV (O3
numeric)    11:14 Monday, March 24, 2003 479

                                       The GLM Procedure

                                    Class Level Information

                                 Class         Levels    Values

                                 uv                 2    0 2000

                                 ph                 3    6 7 8

                                   Number of observations    27

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                                  O3 - With UV vs Without UV (O3
numeric)    11:14 Monday, March 24, 2003 480

                                       The GLM Procedure

Dependent Variable: k

                                                  Sum of
             Source                       DF         Squares     Mean
Square    F Value    Pr > F

             Model                         9     16.54478302
1.83830922      33.59    <.0001

             Error                        17      0.93047820
0.05473401

             Corrected Total              26     17.47526122


                               R-Square     Coeff Var      Root MSE
k Mean

                               0.946755      13.09686      0.233953
1.786330
```

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	1.49153425	0.74576713	13.63	0.0003
uv	1	13.66741584	13.66741584	249.71	<.0001
o3c	1	0.45176752	0.45176752	8.25	0.0105
uv*ph	2	0.90375773	0.45187887	8.26	0.0031
o3c*ph	2	0.02540662	0.01270331	0.23	0.7953
o3c*uv	1	0.00490104	0.00490104	0.09	0.7684

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.07144348	0.03572174	0.65	0.5332
uv	1	0.56543853	0.56543853	10.33	0.0051
o3c	1	0.35938840	0.35938840	6.57	0.0202
uv*ph	2	0.31017855	0.15508928	2.83	0.0867
o3c*ph	2	0.03018181	0.01509091	0.28	0.7624
o3c*uv	1	0.00490104	0.00490104	0.09	0.7684

The GLM Procedure

Dependent Variable: ko3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	134.8242364	14.9804707	67.56	<.0001
Error	17	3.7696194	0.2217423		

```
            Corrected Total             26     138.5938558

                                R-Square     Coeff Var      Root MSE
ko3 Mean

                                0.972801      14.86837      0.470895
3.167094


            Source                      DF       Type I SS     Mean
Square    F Value    Pr > F

            ph                           2       4.3144464
2.1572232       9.73    0.0015
            uv                           1     118.7746051
118.7746051     535.64    <.0001
            o3c                          1       3.8496380
3.8496380      17.36    0.0006
            uv*ph                        2       4.5189774
2.2594887      10.19    0.0012
            o3c*ph                       2       0.3741463
0.1870732       0.84    0.4474
            o3c*uv                       1       2.9924233
2.9924233      13.50    0.0019


            Source                      DF     Type III SS     Mean
Square    F Value    Pr > F

            ph                           2      0.37521666
0.18760833       0.85    0.4464
            uv                           1      9.46996308
9.46996308      42.71    <.0001
            o3c                          1      1.45658361
1.45658361       6.57    0.0202
            uv*ph                        2      1.09508773
0.54754386       2.47    0.1144
            o3c*ph                       2      0.13996803
0.06998401       0.32    0.7335
            o3c*uv                       1      2.99242326
2.99242326      13.50    0.0019

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                                          UVO - Effect of pH
11:14 Monday, March 24, 2003 482

                           Obs    ph     uv       o3c     o3       k
ko3

                            1     6     2000    0.4583    1     3.8500
8.40061
```

	2	6	2000	0.3583	1	3.0100
8.40078						
	3	6	2000	0.4869	1	2.9214
6.00000						
	4	7	2000	0.5387	1	2.5857
4.79989						
	5	7	2000	0.5179	1	2.4857
4.79958						
	6	7	2000	0.5476	1	2.6286
4.80022						
	7	8	2000	0.3786	1	2.7257
7.19942						
	8	8	2000	0.3562	1	2.1369
5.99916						
	9	8	2000	0.5810	1	2.7886
4.79966						

UVO - Effect of pH (O3 class)

11:14 Monday, March 24, 2003 483

The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8

Number of observations 9

UVO - Effect of pH (O3 class)

11:14 Monday, March 24, 2003 484

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.98581772	0.49290886	3.72	0.0889
Error	6	0.79445449	0.13240908		

Corrected Total 8 1.78027221

R-Square Coeff Var Root MSE k Mean

0.553745 13.03059 0.363881 2.792511

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	0.98581772	0.49290886	3.72	0.0889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.98581772	0.49290886	3.72	0.0889

UVO - Effect of pH (O3 numeric)
11:14 Monday, March 24, 2003 485

The GLM Procedure

Class Level Information

Class	Levels	Values
ph	3	6 7 8

Number of observations 9

UVO - Effect of pH (O3 numeric)
11:14 Monday, March 24, 2003 486

The GLM Procedure

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	5	1.13012114	0.22602423	1.04	0.5200
Error	3	0.65015107	0.21671702		
Corrected Total	8	1.78027221			

R-Square	Coeff Var	Root MSE	k Mean
0.634802	16.67061	0.465529	2.792511

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ph	2	0.98581772	0.49290886	2.27	0.2505
o3c	1	0.13996779	0.13996779	0.65	0.4804
o3c*ph	2	0.00433564	0.00216782	0.01	0.9901

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ph	2	0.02916332	0.01458166	0.07	0.9363
o3c	1	0.03051996	0.03051996	0.14	0.7324
o3c*ph	2	0.00433564	0.00216782	0.01	0.9901

APPENDIX C

DATA PLOTS

Ultraviolet Experiments

pH 6.0

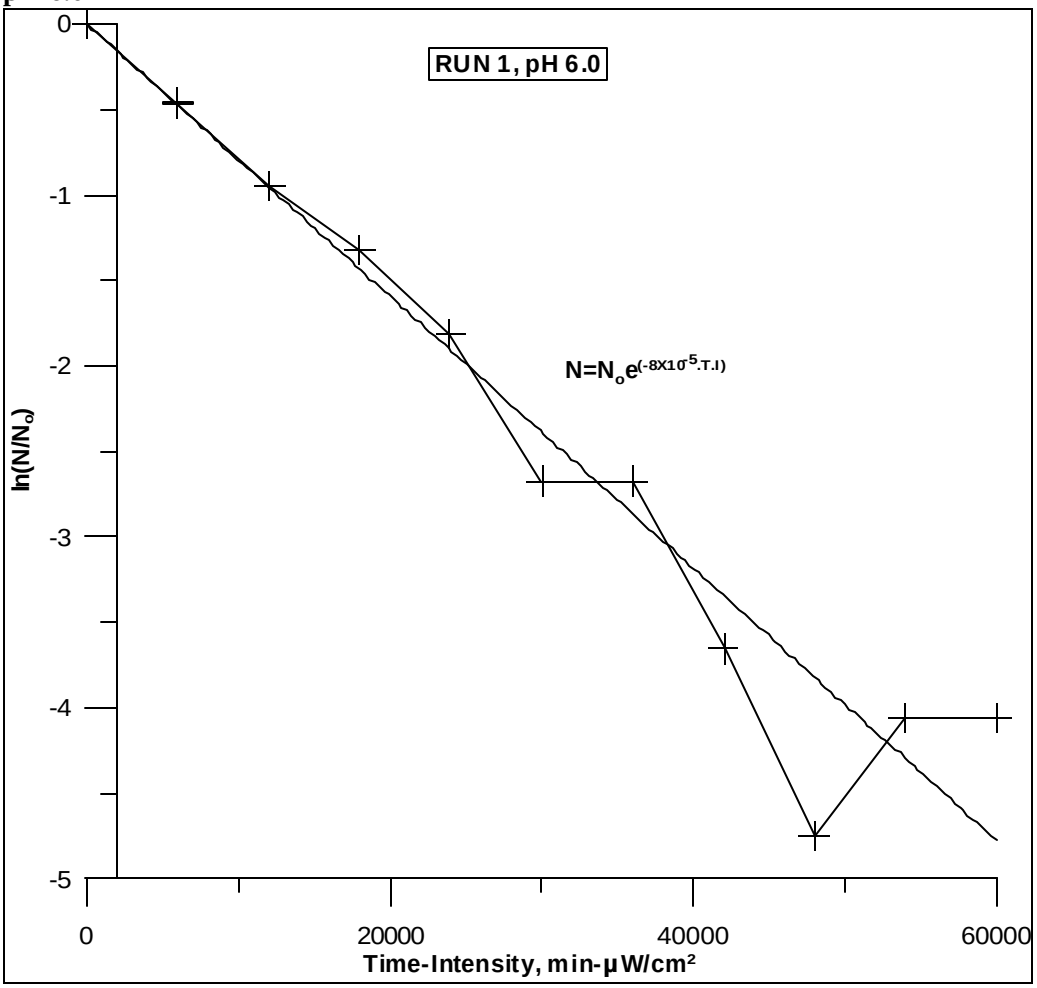

Figure C.1, Survival Plot at pH 6.0 (UV only)

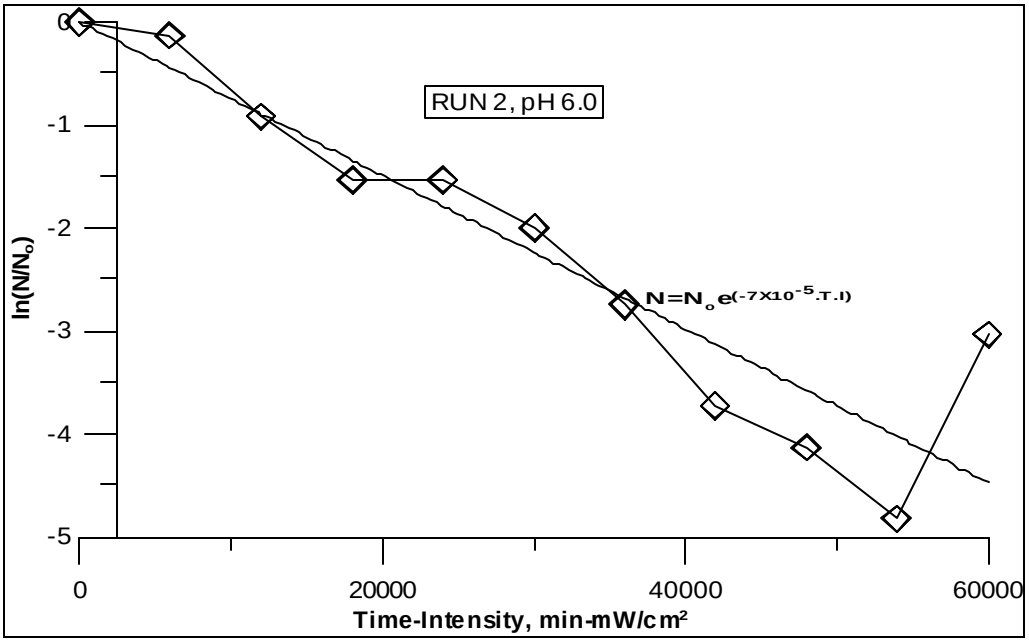

Figure C.2, Survival Plot at pH 6.0, Run 2 (UV only)

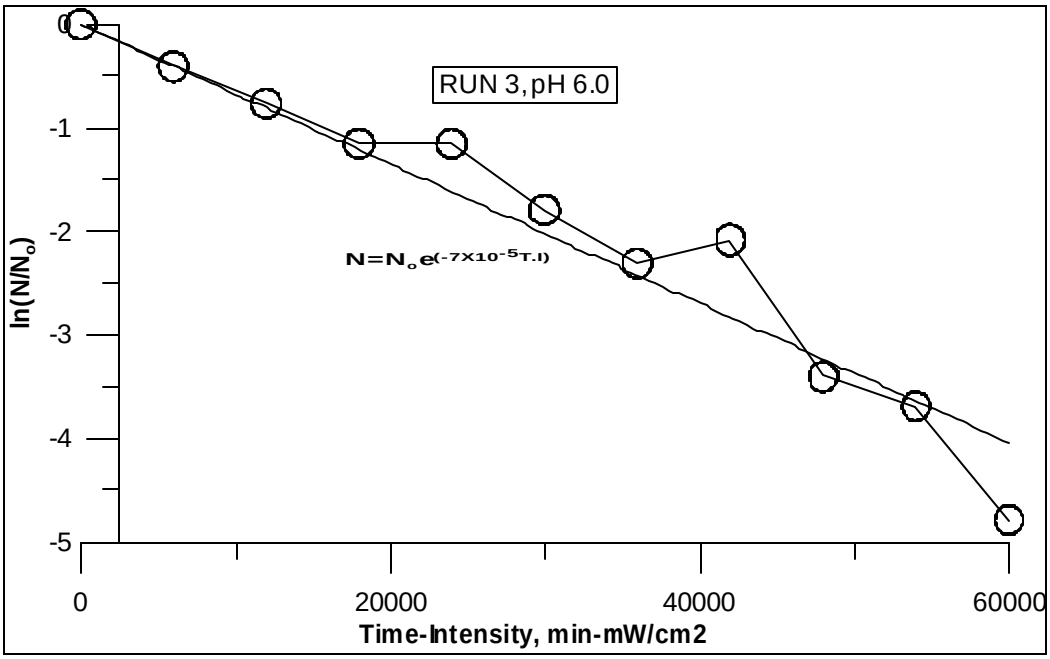

Figure C.3, Survival Plot at pH 6.0 Run 3 (UV only)

pH 7.0

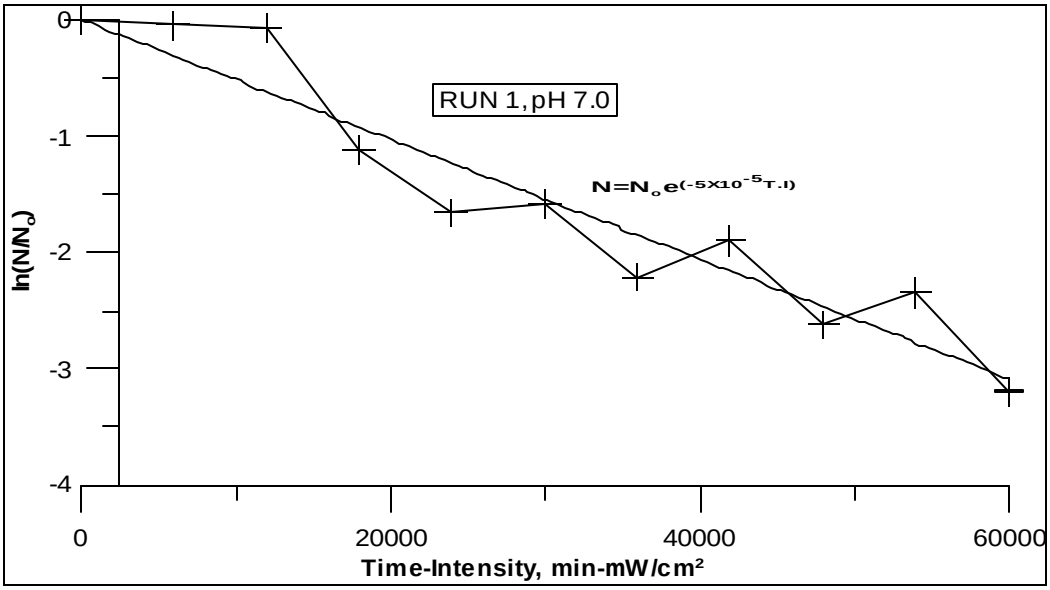

Figure C.4, Survival plot at pH 7.0 Run 1 (UV only)

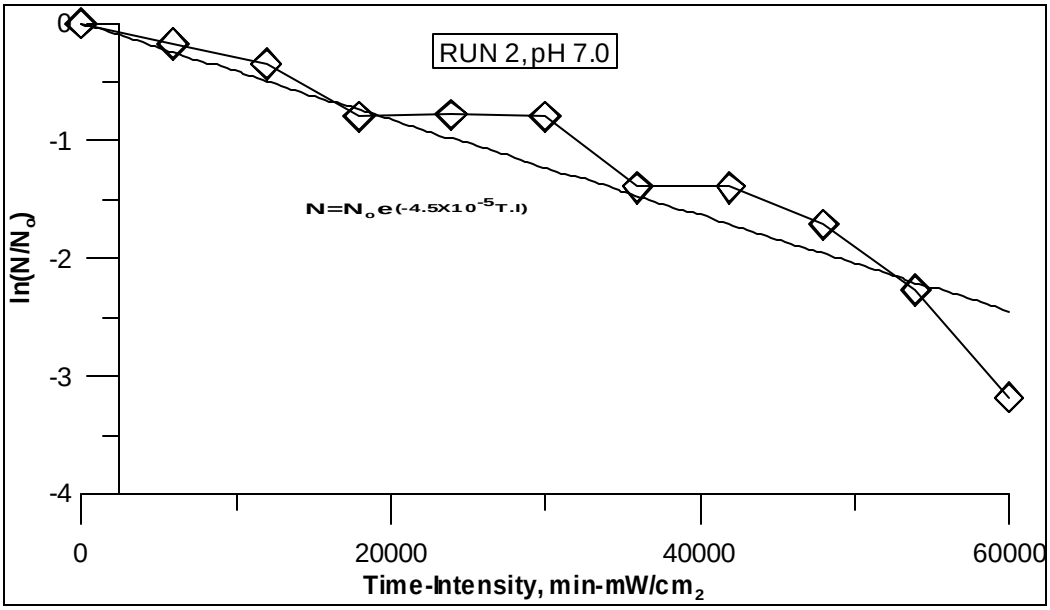

Figure C.5, Survival plot at pH 7.0 Run 2 (UV only)

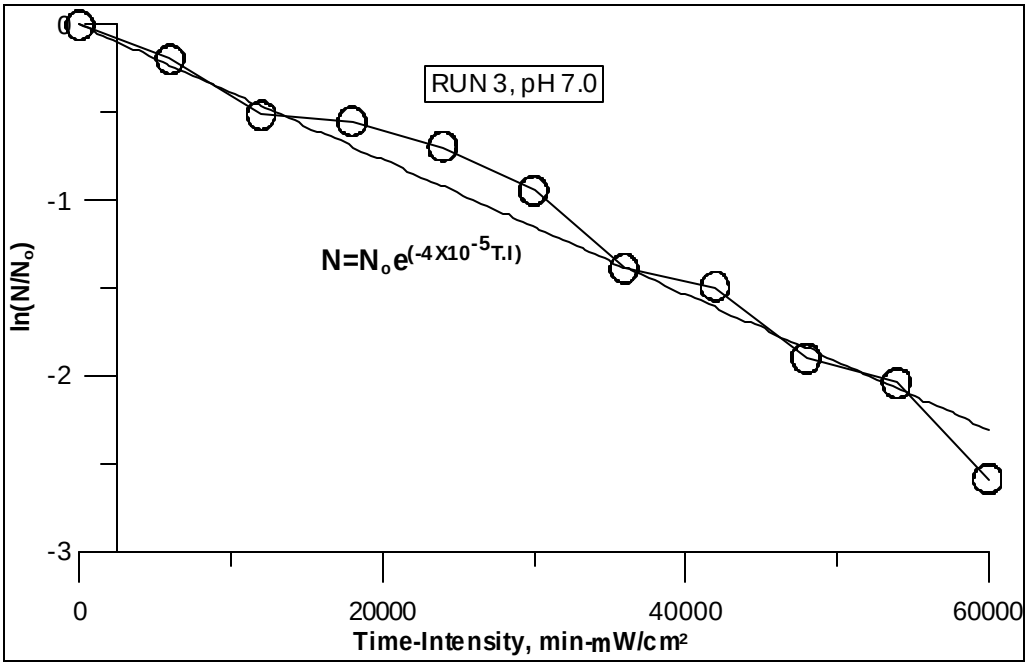

Figure C.6, Survival Data at pH 7.0 Run 3 (UV only)

pH 8.0

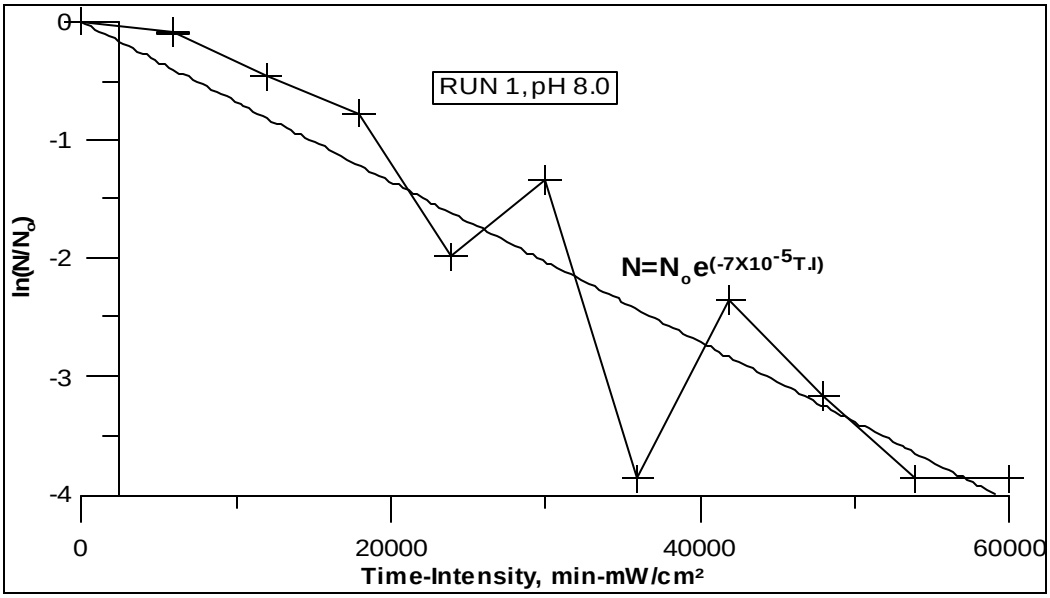

Figure C.7, Survival Plot for pH 8.0 Run 1 (UV only)

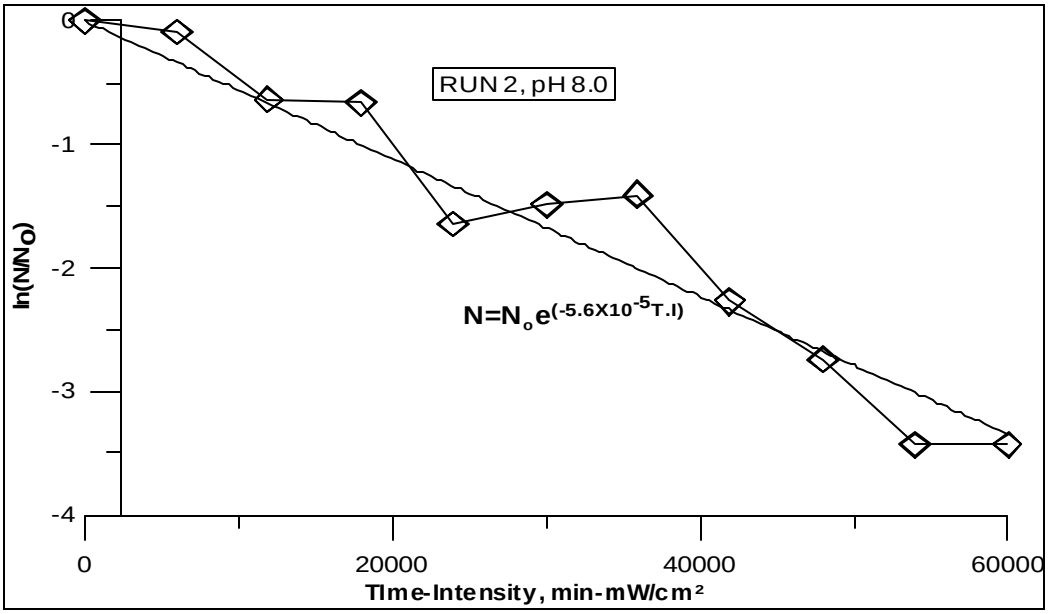

Figure C.8, Survival data for pH 8.0 Run 2 (UV only)

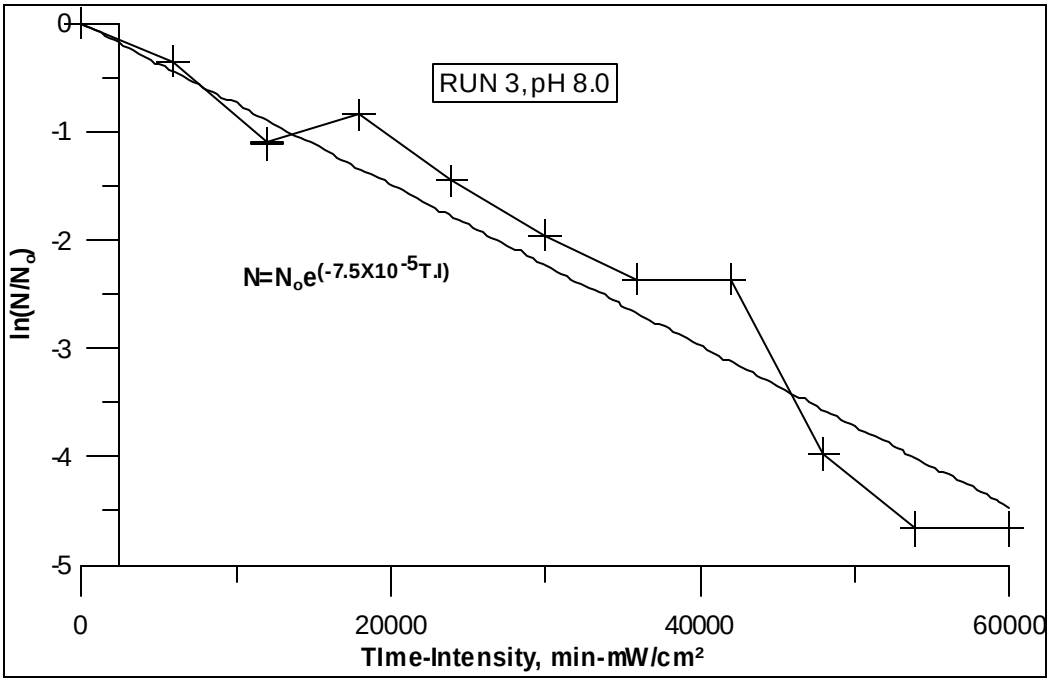

Figure C.9, Survival Data for pH 8.0 Run 3 (UV only)

Ozone Experiments

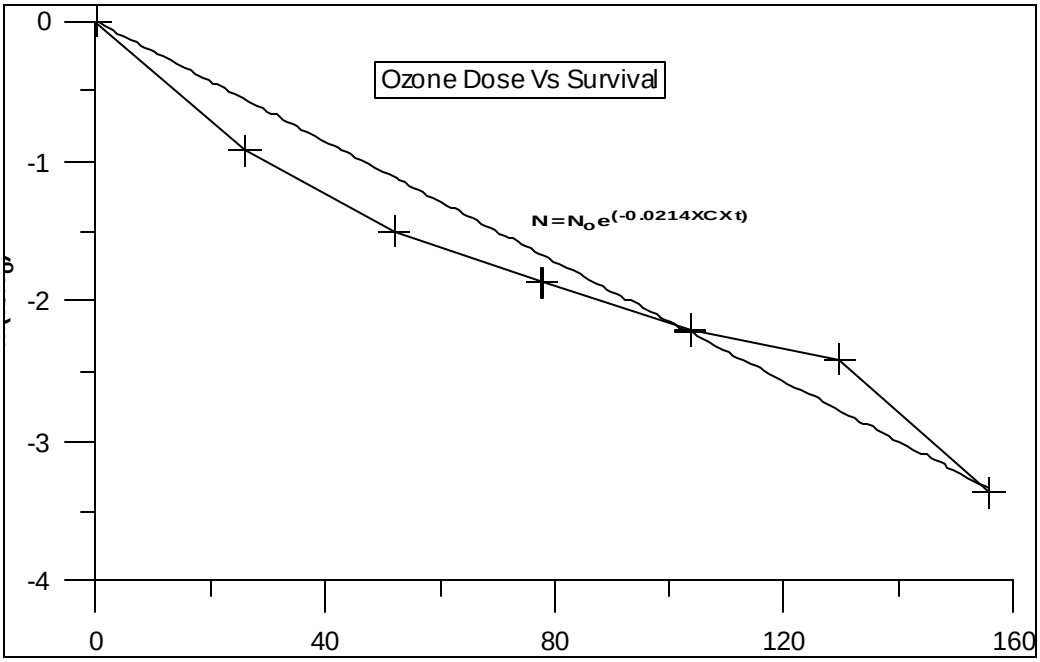

Figure C.10, Survival data for Ozone exposure, Average conc. 0.87mg/l

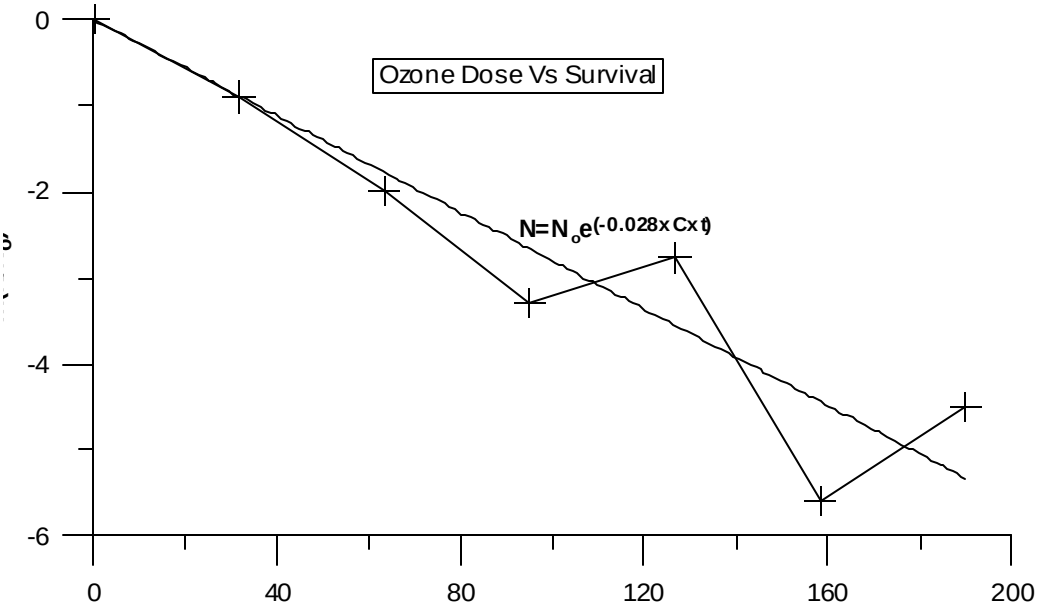

Figure C.11, Survival data for Ozone exposure, Average conc. 1.05mg/l

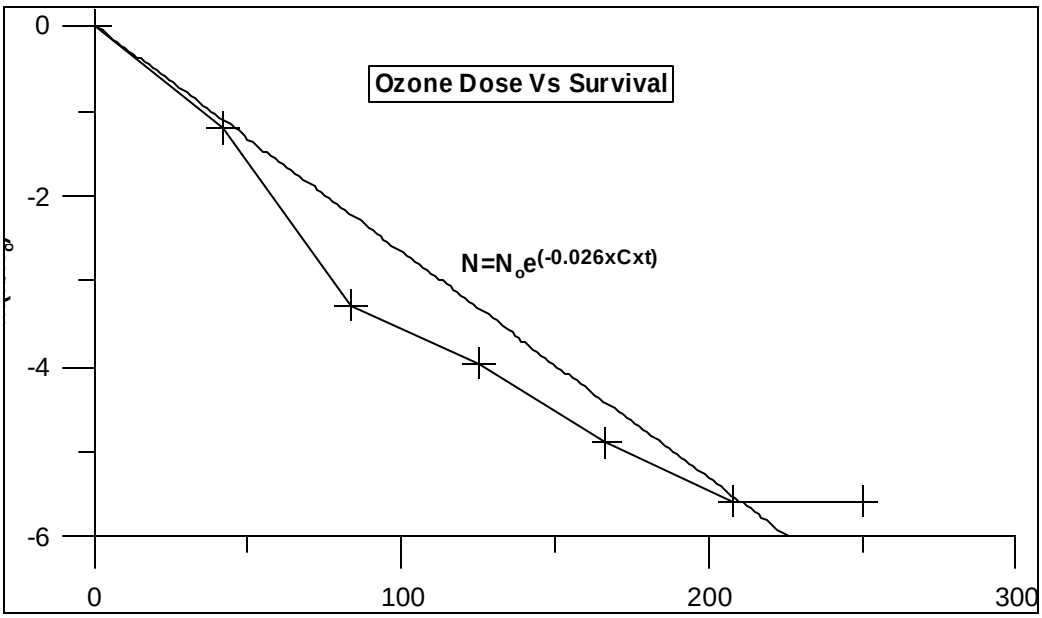

Figure C.12, Survival plot for Ozone exposure, Average conc. 1.4 mg/l

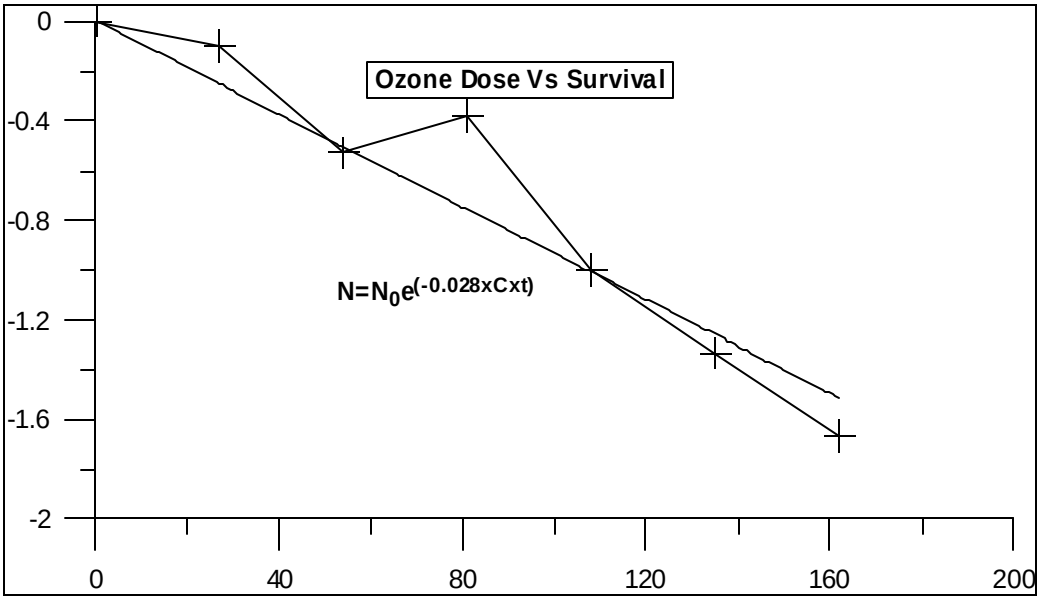

Figure C.13, Survival plot for Ozone exposure, Average conc. 0.9 mg/l

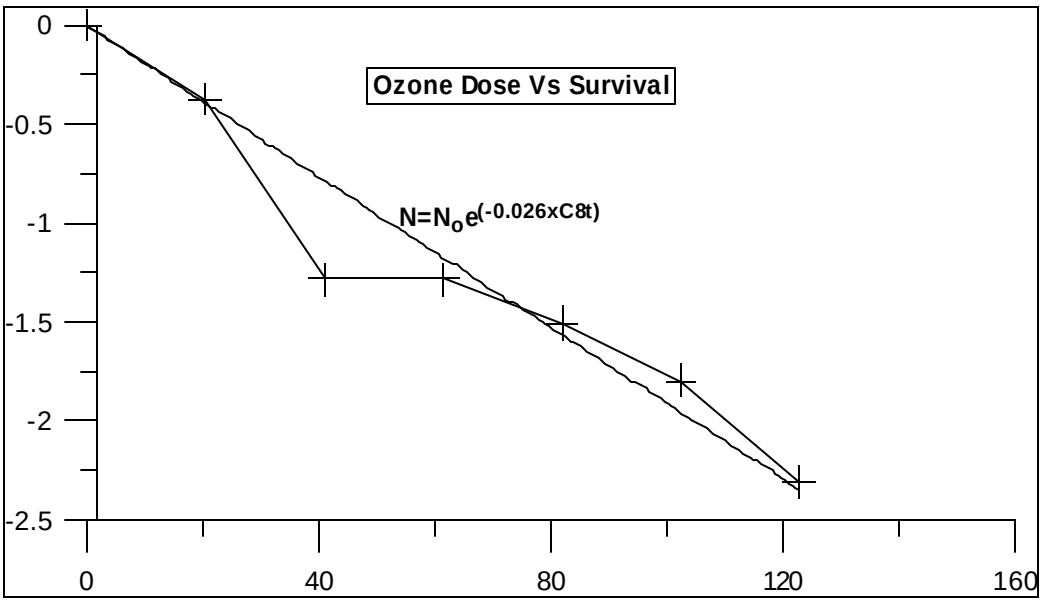

Figure C.14, Survival plot for Ozone exposure, Average conc. 0.68mg/l

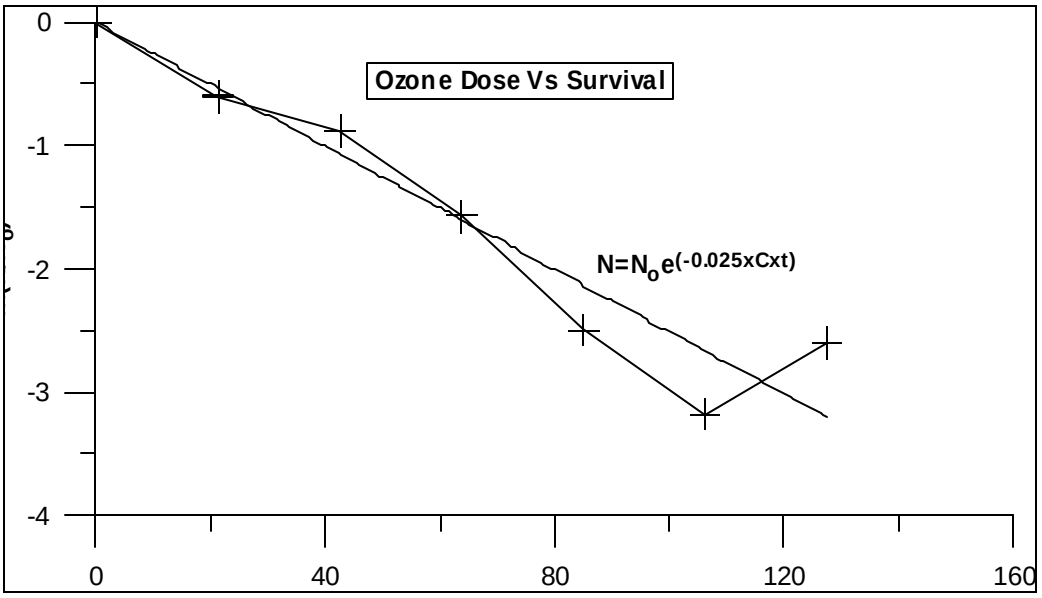

Figure C.15, Survival data for Ozone exposure, Average conc. 0.71 mg/l

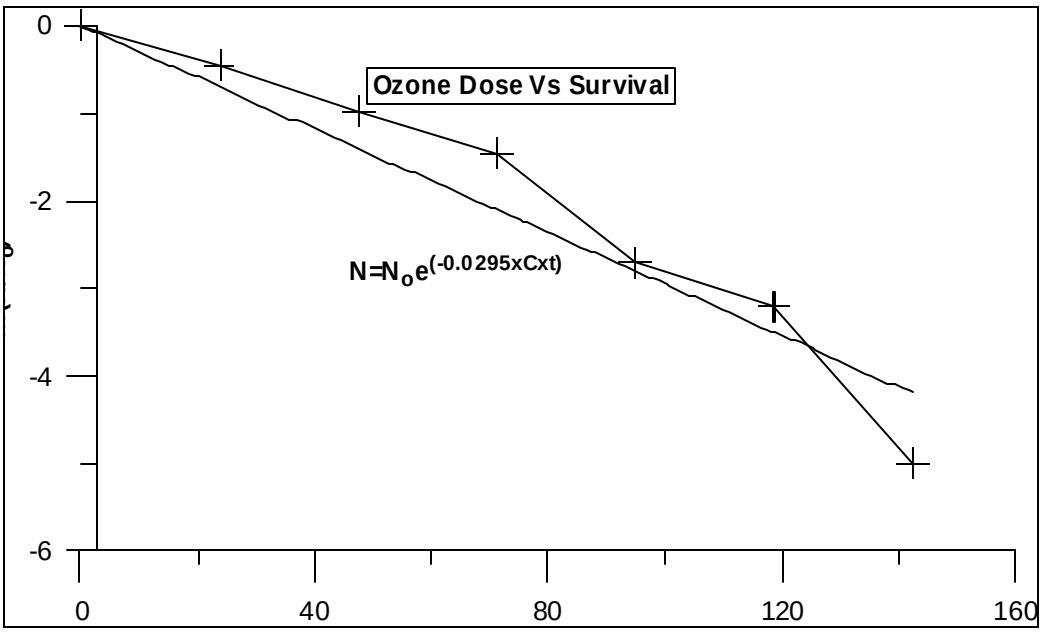

Figure C.16, Survival plot for Ozone exposure, Average conc. 0.8 mg/l

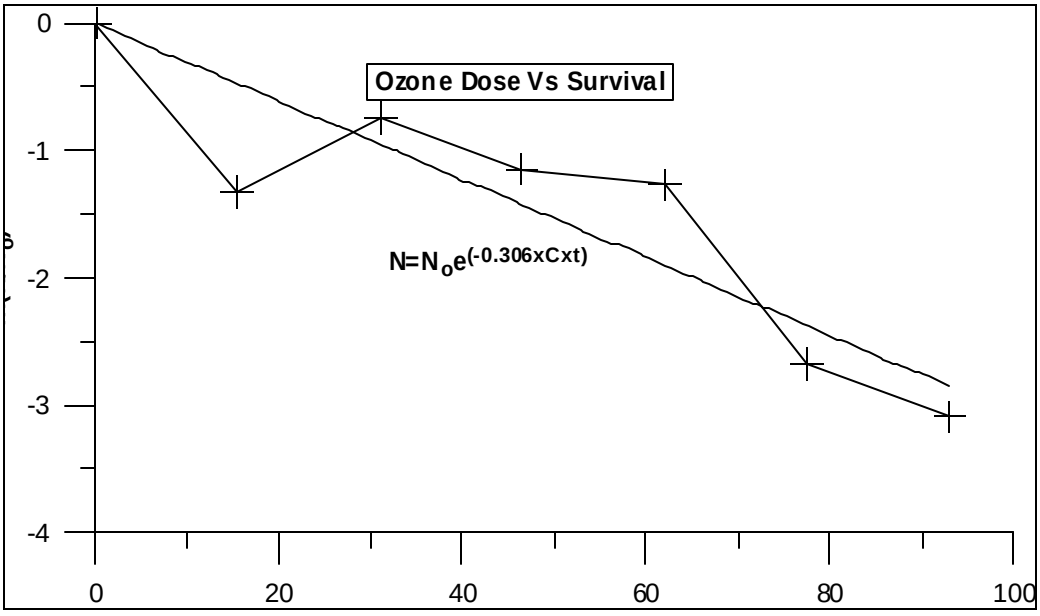

Figure B.17, Survival plot for Ozone exposure, Average conc. 0.5 mg/l

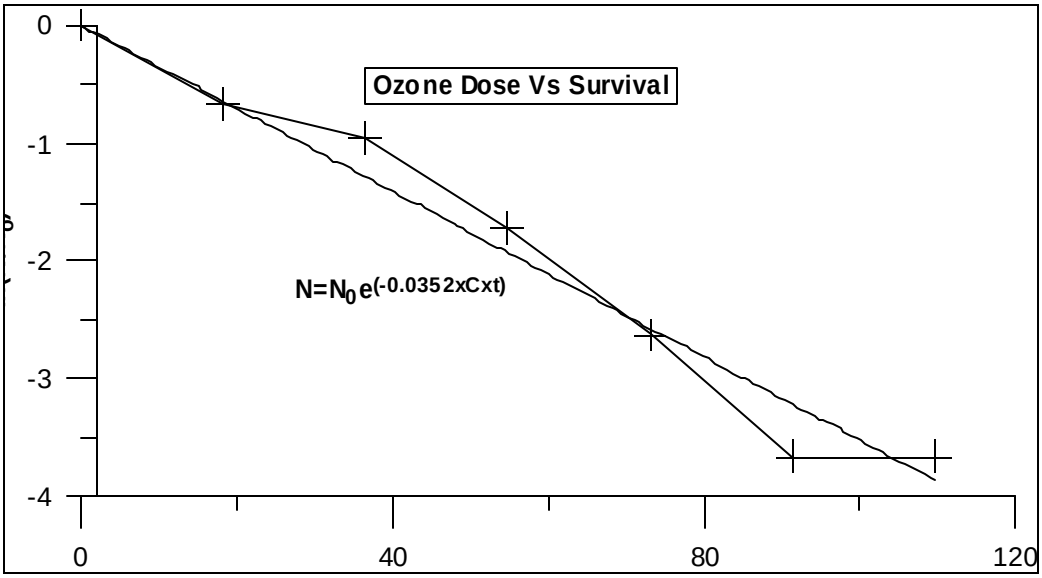

Figure C.18, Survival plot for Ozone exposure, Average conc. 0.6 mg/l

Ultraviolet and Ozone Experiments

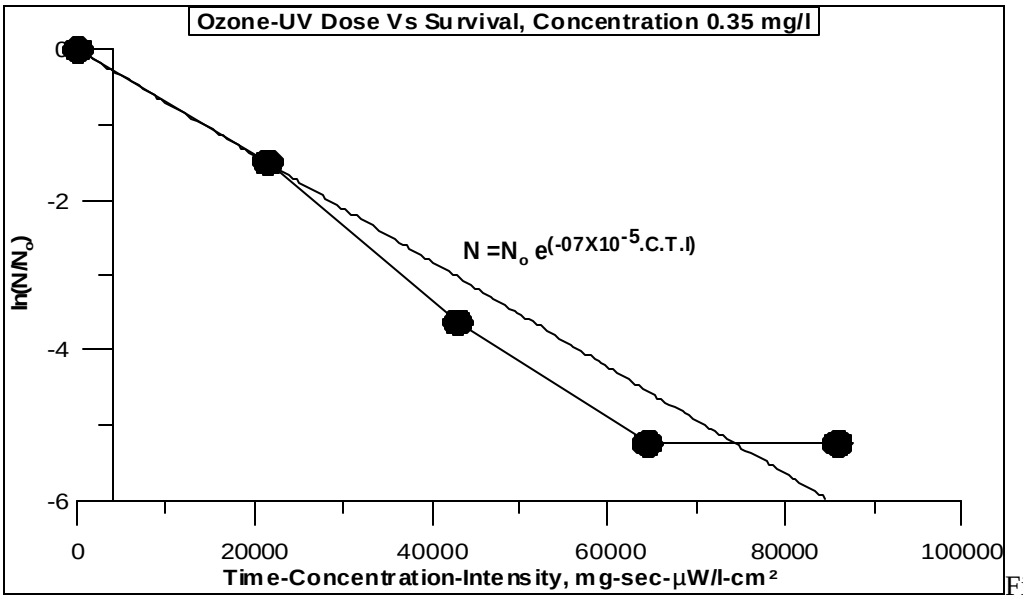

Figure C.19, Survival plot for Ozone-UV exposure, Average conc. 0.35 mg/l

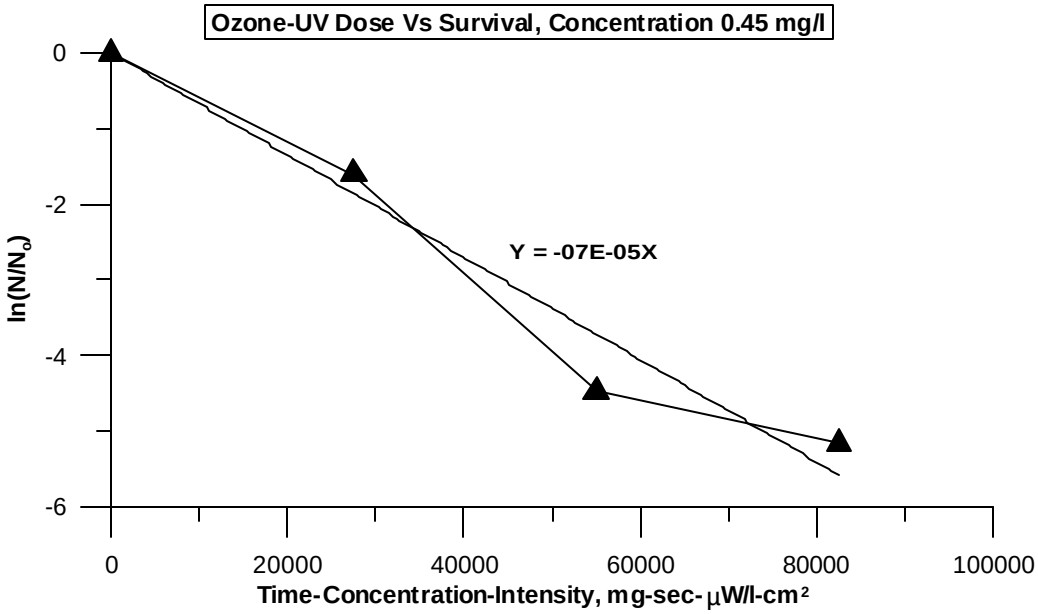

Figure C.20, Survival plot for Ozone-UV exposure, Average conc. 0.45 mg/l

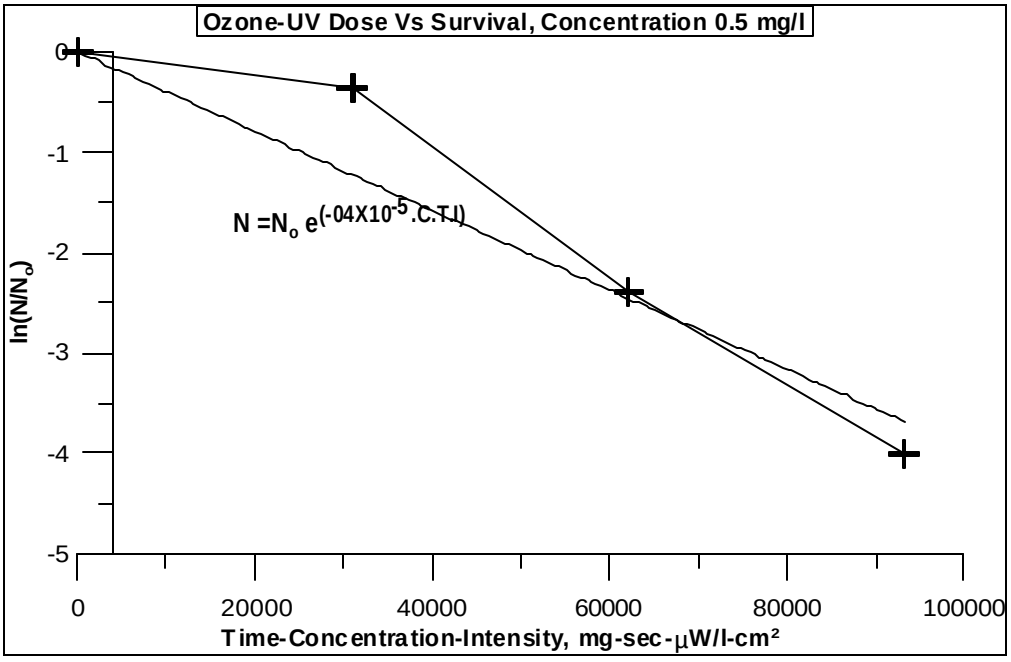

Figure C.21, Survival plot for Ozone-UV exposure, Average conc. 0.5 mg/l

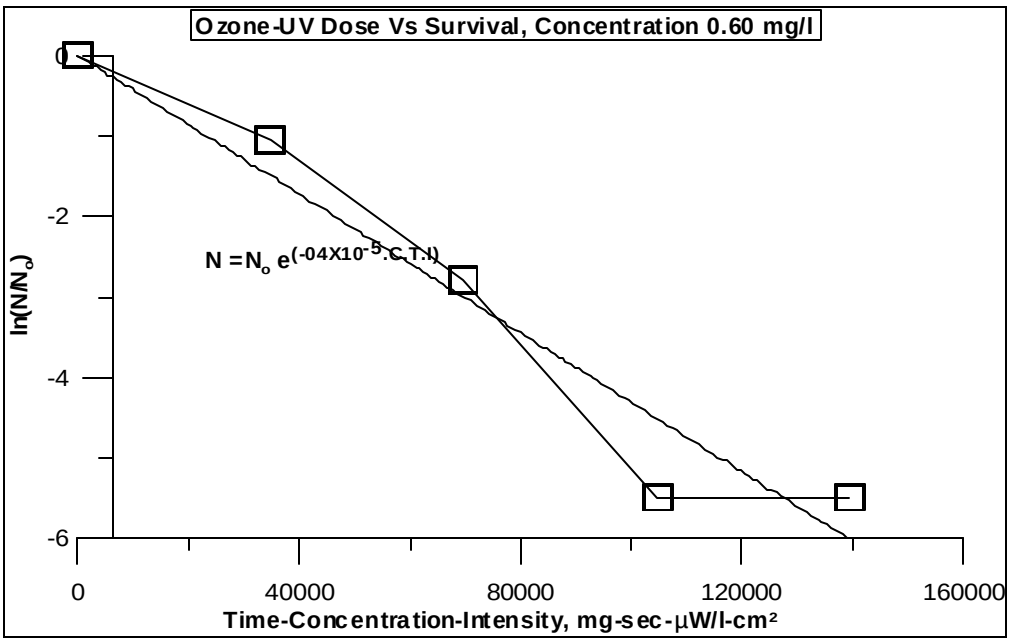

Figure C.22, Survival plot for Ozone-UV exposure, Average conc. 0.60 mg/l

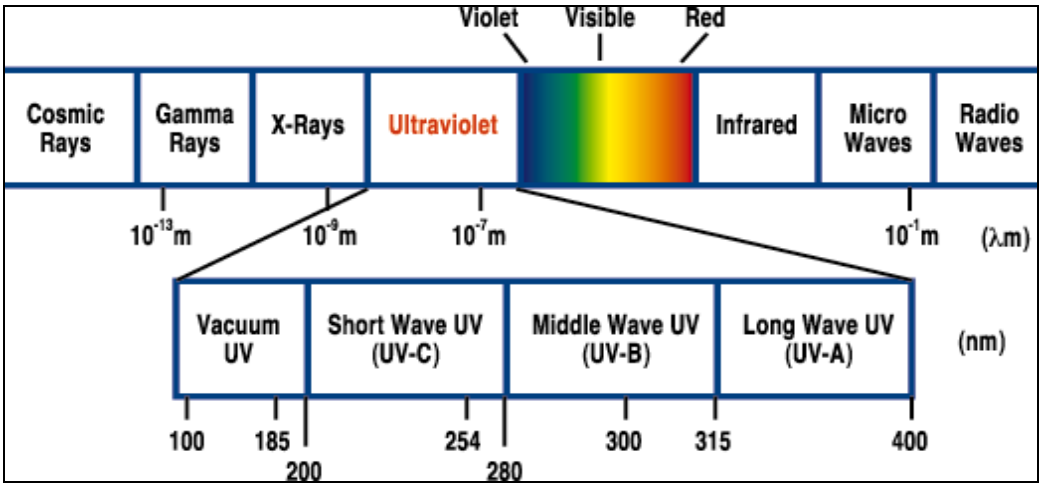

Figure C.23, UV Spectrum, Source: Fluorescent Mineral Society, http://www.uvminerals.org/spectrum.htm

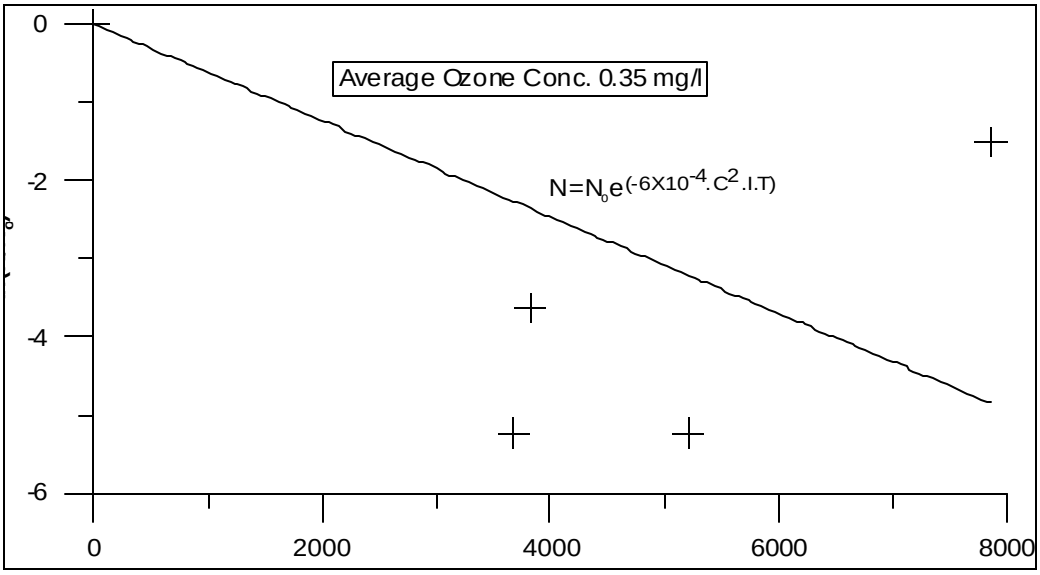

Figure C.24, Regression Run on UVO with n=2 (exponent on Concentration)

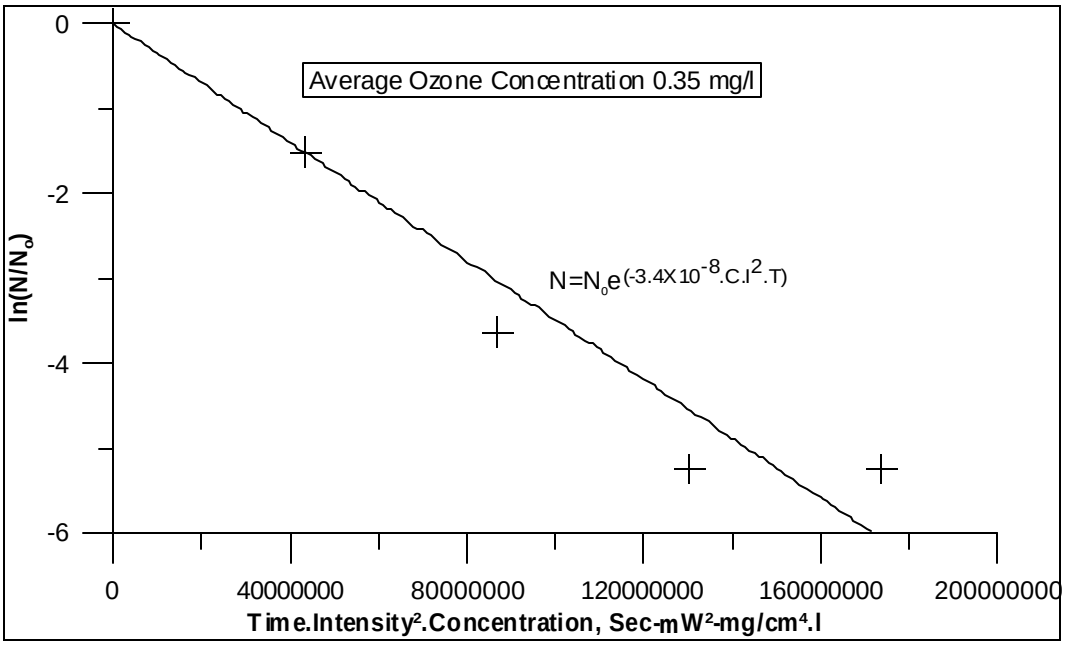

Figure C.25, Regression Run on UVO with m=2 (exponent on Intensity)

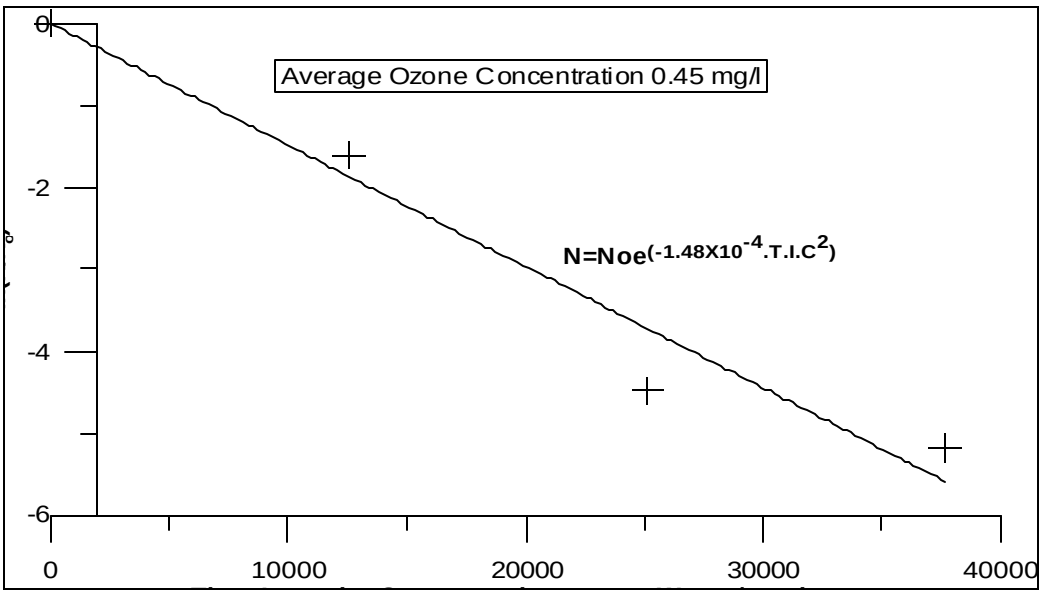

Figure C.26: Regression Run on UVO with n=2 (Exponent on Concentration)

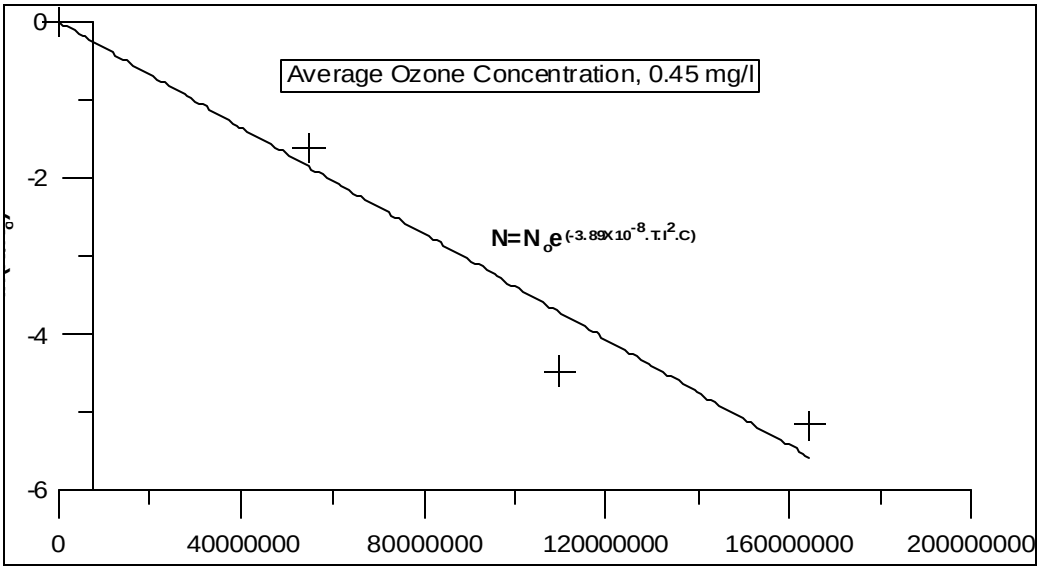

Figure C.27, Regression Run on UVO with m=2 (Exponent on Intensity)

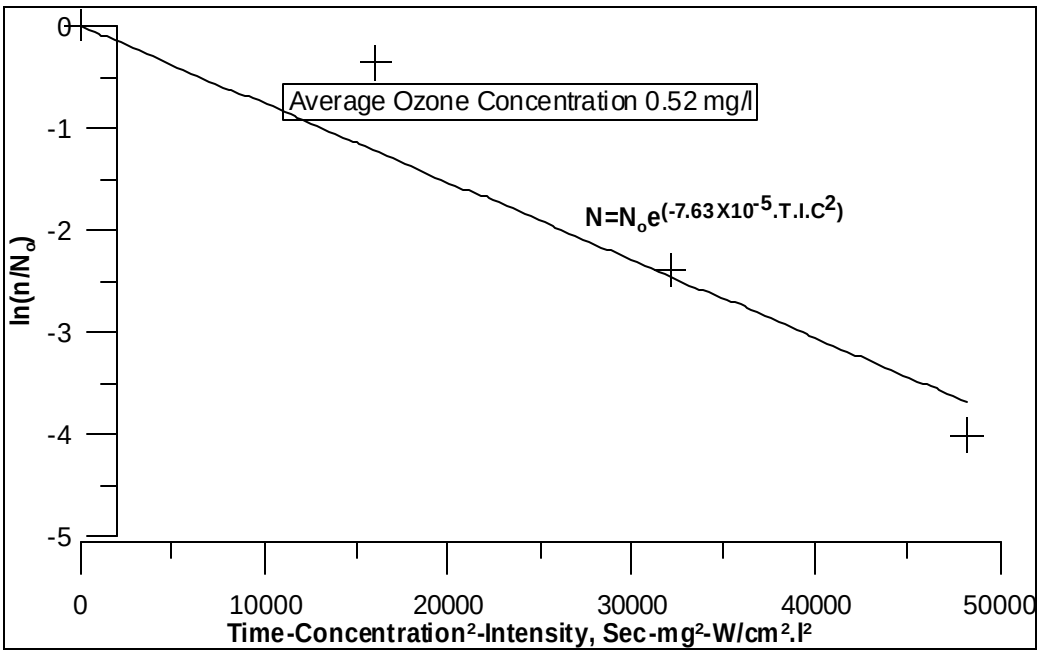

Figure C.27, Regression Run on UVO with n=2 (Exponent on Concentration)

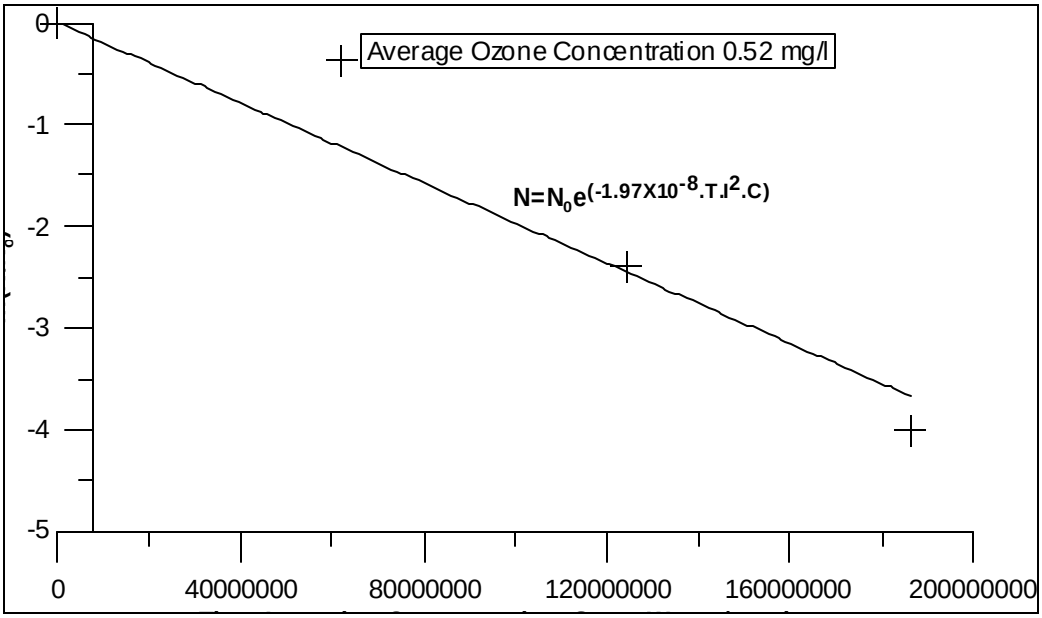

Figure C.28, Regression Run on UVO with m=2 (Exponent on Concentration)

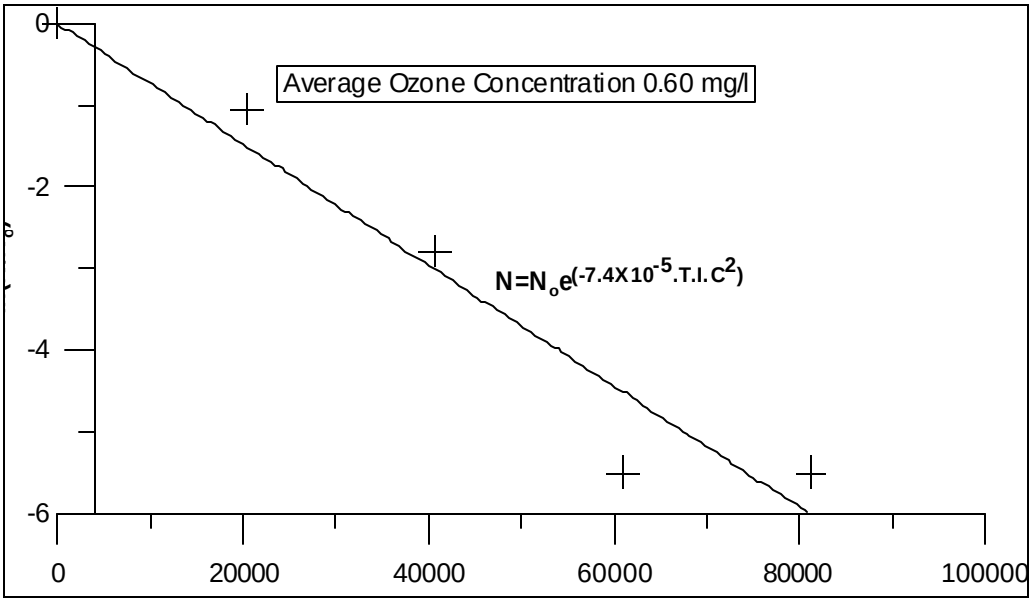

Figure C.29, Regression Run on UVO with n=2 (Exponent on Concentration)

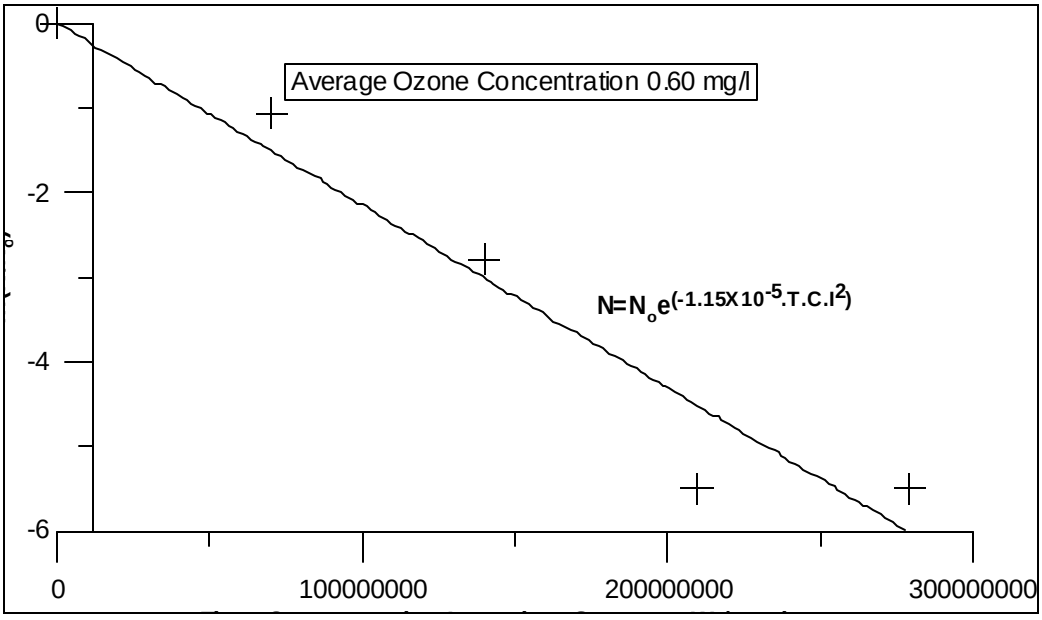

Figure C.30, Regression Run on UVO with m=2 (Exponent on Intensity)

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