

# **Studies on ozone initiated inactivation of pathogenic bacteria in aqueous systems**



**Favourite N. Zuma**

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for the degree of Master of Science  
in the School of Chemistry,  
Faculty of Science and Agriculture  
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## **DEDICATION**

This thesis is dedicated to my late grand mother, Mrs. Legameko Silvina Mathe (Magcwensa) for a wonderful upbringing. I deeply appreciate the love, encouragement and support that she gave me. She is the reason why I have reached this far.

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## ABSTRACT

The effect of ozone on the inactivation of two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and one Gram-positive endospore (*Bacillus subtilis*) bacteria, often present in water and the cause of some waterborne diseases was investigated as a function of ozone concentration and ozonation duration. Ozone was generated *in situ* using corona discharge methods where the ozone concentration ranged from 0.906 - 4.724 mg/L and the inactivation of the three microbes followed pseudo-first order kinetics with respect to the microbes. Three microbes were cultured and the influence of temperature and pH of the aqueous systems on the ozone initiated inactivation rate of the three microbes was also investigated. This study reports that molecular ozone is more effective than hydroxyl radicals initiated by the ozone chain reactions. Two suggested mechanisms for the antimicrobial effectiveness of ozone in water systems from the literature is discussed. The study also found that ozonation significantly decreased the Biological Oxygen Demand (BOD) value of natural water.

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Abbreviation	Parameter
AWWA	American Water Works Association
AOP	Advanced Oxidation Processes
BAC	Biological Activated Carbon
BOD	Biological Oxygen Demand
BOD <sub>5</sub>	Represents the BOD value for 5 days
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
CFU/ml	Colony Forming Unit per milliliter
cfm	cubic feet per minute
COD	Chemical Oxygen Demand
ddH <sub>2</sub> O	deionised double distilled water
DBPs	Disinfection by-products
EPA	Environmental Protection Agency
<i>E. coli</i>	<i>Escherichia coli</i>
gpm	Gallons per minute
GAC	Granular Activated Carbon
HAAs	Haloacetic acid
HO•	Hydroxyl radical
MCL	Maximum Contaminant Level
mgd	Millions of gallons per day
mg/L	Milligrams per litre
ML/d	Megalitres per day
O•	Oxygen radical
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
SWTR	Surface Water Treatment Rule
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
THM	Trihalomethanes
UV	Ultraviolet
WHO	World Health Organisation

## 1.0 Introduction

Human activities and the ever escalating pollution growth have been blamed for the adverse pressures exerted on the quality and quantity of water resources available for use over the world. These pressures are mostly felt at the interface between water supplies and human health as proliferation of infectious diseases. Infectious diseases occur as a result of interaction between pathogenic (disease-producing) microorganisms and the host (Pelczar *et al.*, 1986). Several types of microorganisms are pathogenic; people are subjected to these microorganisms through contaminated drinking water, water drops, aerosols and washing or bathing. Some waterborne pathogenic microorganisms spread by water can cause severe, life-threatening diseases, such as gastroenteritis, amoebiasis, giardiasis, salmonellosis, dysentery, cholera, typhoid fever and hepatitis A or E (Craun, 1986). About 1.2 billion people worldwide do not have access to clean and safe drinking water, and 2.4 billion people lack sanitation. Every year, 5 million people die of waterborne diseases mostly people with low resistance, mainly the elderly and young children (Berman, 2005). Many people are weakened because of waterborne diseases and, as a result, are more susceptible to other infections. Diseases are easily spread because water treatment and sewage no longer function or are lacking completely (Berman, 2005). English Physician, John Snow was the first to report about the relationship between contaminated water and disease (Paneth *et al.*, 1998). As early as 1881, Koch and Pasteur demonstrated that bacteria could be killed by chlorine. Around the same time, Frohlich (1886) used ozone to disinfect water.

In the production of potable water, it is, therefore, pertinent to completely remove these pathogens through wholesome water treatment and disinfection protocols. In water treatment all chemical and physical characteristics of a water body deemed unfit for use are removed before water can be released for use. In addition, depending on the specific use, the water supplies should be strictly disinfected before it can be used. For examples, production of water for drinking purposes, production of high quality waters for hospitals and pharmaceutical concerns, recycling of water for recreational purposes, e.g. swimming

pools and inactivation of pathogens in effluent treatment plants before it can be released into natural ecosystems.

Water supplies of the developed countries are usually adequately disinfected. The widespread adoption of stringent water disinfection protocol has been cited as the major factor in reducing waterborne diseases, and has been perceived as the major single factor in increasing average human life expectancy in these countries (CHEMRAWN XV, Perspectives and Recommendations, 2006). Effluents from any treatment plant should be thoroughly treated prior to discharge into receiving waters in order to reduce the concentration of pathogens. However, this practice has limitations as residuals of disinfectant may be environmentally detrimental and high costs are involved.

Disinfectant demand of water depends on the chemical and physical characteristics apart from microorganisms themselves. Certain dissolved chemical substances can easily be oxidized by chemical disinfectants and their oxidation competes with the disinfection reactions (EPA guidance manual, 1999). Sufficient disinfectant has to be dosed to oxidize these substances as well as to inactivate pathogens completely. In most treatment plants, chemical oxidation processes are regarded as a separate unit, independent from disinfection. By-products also play a significant role in the subsequent treatment processes. However, proper design of integrated process involving oxidation and disinfection with ozone in particular, can actually improve the quality of the product (Snyder *et al.*, 2003).

A good disinfectant should have a wide spectral activity against all forms of pathogens such as bacteria, viruses, protozoa and others. It should ideally have good solubility properties in the water media in addition to low residual toxicity and the by-products generated thereof. Since disinfection is usually the last process before water supplies are dispatched, the disinfectant should necessarily have easy riddance properties. In most modern disinfection plants, gaseous disinfectants or those that form active gaseous residuals are preferred to ensure low residual prevalence; examples include chlorination, chloramination (using ammonia and chlorine), use of chlorine dioxide and ozonation (Snyder *et al.*, 2003).

Chlorine based disinfection is currently the most widely used method in the production of drinking water because of its relatively low running costs. Although chlorination is known to have saved millions of lives, to date, it is also known to react with aquatic organic material present in natural water to form trace levels of highly toxic trihalomethanes, a group of lightweight chlorinated hydrocarbons which are suspected to be carcinogens (Li, 2004). The prevalence of trihalomethanes in chlorine disinfected drinking water has led to renewed interest in opting for chloramination as a disinfecting method, because chloramine impedes the formation of trihalomethanes (Pieterse, 1988; DeMers *et al.*, 1993). To date, there has been no explicit consideration of adverse consequences of chloramination on property and water quality in buildings, especially, the effect of chloramine on re-growth of bacteria during stagnation, plumbing failures and lead leaching. A more recent survey on the occurrence of disinfection by-products indicated that the occurrence of almost all of the by-products was significantly reduced in the systems using chloramine as opposed to chlorine (Farren, 2003). However, chloramine is not as effective as chlorine in deactivating bacteria, viruses, and *Giardia*. It is also alleged to generate chlorinated phenols, which gives a bad taste to water and some noxious gases similar to that of chlorine. Excessive dosage of ammonia could also lead to nitrification problems and chloramine takes a much longer time than chlorine for effective disinfection (Neden *et al.*, 1992).

Chlorine dioxide is another useful alternative disinfectant. Chlorine dioxide is effective in destroying phenols, yet it does not form trihalomethanes in significant amounts. Chlorine dioxide's disinfectant properties are not adversely affected by a higher pH, whereas free chlorine residues are. Consequently, chlorine dioxide is a useful disinfectant at higher pH values (White, 1992). On the other hand, the organic by-products generated from use of chlorine dioxide are not yet fully understood and it may have undesirable by-products, including aldehydes, carboxylic acids, and ketones (Lykins *et al.*, 1990). The principal inorganic by-products of chlorine dioxide reactions within water treatment are chlorite ( $\text{ClO}_2^-$ ), chloride ( $\text{Cl}^-$ ), and chlorate ( $\text{ClO}_3^-$ ), in the order listed. Both chlorate and chlorite, particularly the latter, have been implicated in the formation of methaemoglobin. Consequently, most European countries limit the level of chlorine dioxide which can be

used and the Environmental Protection Agency (EPA) in the USA has considered implementing it as well. The EPA recommendation is that the sum of chlorine dioxide, chlorite, and chlorate in the distribution system must be less than 1.0 mg/L (Lykins *et al.*, 1990).

Ozonation can be another attractive alternative. When these other disinfectants mentioned above are compared to ozonation, their relative lower efficacy precludes their use in specialized water production units. Therefore, much attention has been drawn to the integrated approach in which ozone is applied as a primary disinfectant (Kruithof *et al.*, 1993). Few studies have reported about the inactivation behavior of ozone-based advanced oxidation process or its integrated application combined with other disinfectants.

Ozone is a powerful oxidant for a wide range of chemical pollutants in addition to its wide spectral activity against most pathogens. However, high running costs complex system control has limited its uptake to only small-capacity production and only in developed countries. Ozone finds widespread applications in water treatment and increasingly in wastewater treatment (Robson *et al.*, 1990). Ozonation can play an important role in improved water treatment and can help to achieve a better, safer water quality. Ozone can be used not only as a disinfectant, its excellent properties in aiding coagulation, promoting the removal of organic and inorganic contaminants and removal of taste and odours have made it a popular choice in modern water treatment practice (Li, 2004). Whereas in the past, drinking water plants were using ozone towards the end of the process for disinfection, the current use worldwide has shifted towards using ozonation as a pretreatment reagent. With few exceptions, chlorination is used as a final disinfection stage.

Ozone is reported to be the strongest disinfectant and oxidant for water treatment (Wojtenko *et al.*, 2001). Contact times for antimicrobial action are much less than when using chlorine. Ozone rapidly attacks bacterial cell walls and is more effective against the thick-walled spores of plant pathogens and animal parasites than chlorine, at practical and safe concentrations (Suslow, 2003). In comparison to the potential negative effects of

residues and organic reaction products formed with chlorine applications, ozone does not form deleterious chlorinated hydrocarbons, trihalomethanes and other chlorinated disinfection by-products (Li, 2004).

Studies for the implementation of ozonation have been conducted for the Gold Coast City Council and the Armidale City Council. At three water treatment plants in Southern Africa, i.e. the Windhoek Water Reclamation Plant, Western Transvaal Regional Water Company and a large pilot scale for disinfection of mine service water for Gold Fields use ozonation as a means (Van Leeuwens *et al.*, 1992).

The literature data regarding the effect of control factors such as pH, temperature and dosage rates on ozonation are contradictory. In one study, it was shown that the decomposition of ozone in a basic medium is much faster than in an acid medium wherein it was concluded that when the pH of the medium increases, the formation of  $\text{OH}^-$  radicals increases, therefore, generating more hydroxide ions which act as initiators for the decomposition of ozone (Kasprzyk-Hordern *et al.*, 2003). In another study done under acid conditions, the decomposition of ozone was found to be much faster than predicted from the  $\text{OH}^-$  initiation. It was suggested that ozone reacts with water to produce radicals. In that study thermal or hydrogen peroxide initiated decomposition of acidic aqueous ozone is described as a chain process propagated by  $\text{OH}$  and  $\text{O}_2^-/\text{HO}_2$  radicals (Sehested *et al.*, 1998).

Gram-negative *Escherichia coli* are a type of fecal coliform bacteria that is commonly found in the intestine of animals and humans. Its presence in water is a strong indication of recent sewage or animal waste contamination (U.S EPA, 2006). Sewage may contain many types of disease-causing organisms. Its infections often cause severe bloody diarrhea and abdominal cramps and also hemolytic uremic syndrome in elderly and infants (U.S EPA, 2006).

Gram-negative *Pseudomonas aeruginosa* is a typical opportunistic pathogen, isolated within hospital settings from equipments that contain or use water such as sink drains,

toilets, showers and others. Even disinfected instruments, utensils, bathroom fixtures, and mops have been incriminated in hospital outbreak (Botzenhart *et al.*, 1993). It is a chronic nosocomial pathogen that is difficult to control. Its most common nosocomial infections occur in patients with severe burns and neoplastic diseases (Moore, 1997).

Gram-positive endospore, *Bacillus subtilis* is one of the most commonly encountered saprobic species of aerobic spore forming *bacilli* and is widely distributed in soil, water, and air as its spores are so resistant, its control is of great economic concern in the food processing industry and in the preparation of all sterile products. It is not considered a human pathogen, it may contaminate food but rarely cause food poisoning (Ryan *et al.*, 2004). *B. subtilis* produces the proteolytic enzyme subtilism. *B. subtilis* is responsible for causing ropiness (a sticky, stringy consistency caused by bacterial production of long chain polysaccharides in spoiled bread dough) (M'hir *et al.*, 2007).

In view of the above, the objectives of this study are to:

- (i) Investigate the rate of ozone initiated destruction and inactivation of representative Gram-negative pathogenic bacteria, *Escherichia coli* in detail.
- (ii) Probe the scope of ozone in disinfection of two other selected strains, Gram-negative *Pseudomonas aeruginosa* and Gram-positive endospore, *Bacillus Subtilis*.
- (iii) Establish the optimal conditions for the ozone initiated inactivation of microorganisms in natural river water.

In this study the effect of flow rate, ozone dosage, temperature and pH on the rate of microbial inactivation were investigated.

## 1.1 Waterborne Bacterial Diseases

Common water and sanitation-related diseases such as diarrhea is caused by a variety of microorganisms including viruses, bacteria and protozoan. Diarrhea causes a person to lose both water and electrolytes, which leads to dehydration and, in some cases, to death. About

4 billion cases of diarrhea per year cause 1.8 million deaths, of which over 90 per cent of them (1.6 million) are children under five years of age (Berman, 2005). Other infectious disease such as cholera is an acute intestinal infection caused by the bacterium *Vibrio cholerae*. The bacteria produce a toxin that causes an infected person to dehydrate through vomiting and profuse watery diarrhea. Two strains of cholera are now associated with infection namely: *V. cholerae* serogroup 01 and *V. cholerae* serogroup 0139 (WHO, 2003). Cholera is acquired directly through contaminated water or food, or indirectly from exposure to the feces or vomit of an infected person. In many tropical countries, cholera outbreaks are common. New outbreaks can occur sporadically in any part of the world where water supplies, sanitation, food safety and hygiene are inadequate. The greatest risk of cholera occurs in overpopulated communities and refugee settings characterized by poor sanitation and unsafe drinking water (Weir *et al.*, 2004). Cholera continues to plague many parts of the world, but has largely been concentrated in Africa, which contributes more than 80% of the total cases worldwide. Case fatality rates are high, and we are not anywhere near curbing new cholera epidemics, especially in Africa. It is thus imperative to renew discussions about the nature of this deadly disease, measures for prevention and control.

Other water- related diseases such as cyanobacteria, occur worldwide especially in calm and nutrient-rich waters. Some species of cyanobacteria produce toxins that affect animals and humans. People may be exposed to cyanobacterial toxins by drinking or bathing in contaminated water (WHO, 1999). The most frequent and serious health effects are caused by drinking water containing the toxins (cyanobacteria) or by ingestion during recreational water contact (WHO, 1999). Humans are affected with a range of symptoms including skin irritation, stomach cramps, vomiting, nausea, diarrhea, fever, sore throat, headache, muscle and joint pain, blisters of the mouth and liver damage (WHO, 1999). Swimmers in water containing cyanobacterial toxins may suffer allergic reactions, such as asthma, eye irritation, rashes, and blisters around the mouth and nose. Animals, birds, and fish can also be poisoned by high levels of toxin-producing cyanobacteria (WHO, 1999).

Typhoid fever is a bacterial infection caused by ingesting contaminated food or water. Symptoms are characterized by headaches, nausea and loss of appetite. About 12 million people are affected by typhoid every year.

A hygienic environment, clean water and adequate sanitation are key factors in preventing opportunistic infections associated with HIV/AIDS, and in the quality of life of people living with the disease. AIDS-affected people are more susceptible to water-related diseases than healthy individuals due to the weaker immune systems. Maintaining a healthy environment is essential to safeguarding the health, quality of life and productivity of people living with HIV/AIDS

([www.unicef.org/wes/index\\_wes\\_related.html](http://www.unicef.org/wes/index_wes_related.html) , 2007).

To improve the economic progress of developing countries, water contamination and spread of infectious diseases must be contained. This is achieved through (drinking) water treatment, sewage, waste and sewage water treatment and education on personal and food hygiene. Even when water treatment is applied, one has to watch out for outbreaks of waterborne diseases. Water that is used for drinking purposes can be prepared from surface water, groundwater or recycled water. This water can be contaminated by pathogenic microorganisms and other pollutants. Sufficient disinfection is needed to prevent diseases. Some of the most common waterborne diseases prevented by disinfection, those associated with bacteria are: typhoid fever, para-typhoid, childhood bacterial diarrhea and Legionnaires' disease, and which are viral are: hepatitis and rotavirus diarrhea and those which are parasitic are: amoebiasis, giardiasis and cryptosporidiasis (Walmsley *et al.*, 2000).

## **1.2 The Bacteria**

Bacteria are prokaryotic unicellular microorganisms. Most bacteria are small, rod-like (*bacilli*), cocci (spheres), or filaments that range from 0.5 to 1  $\mu\text{m}$  in diameter. They have

no visible internal structure and, they lack the nucleus which is an essential feature of multicellular organisms (Buchanan and Gibbons, 1974).

Bacteria reproduce by cell division. Sometimes the two daughter cells fail to separate and they grow in pairs, cluster of chains. Some are motile; while others form spores that resist heating or drying. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction (Koch, 2002).

Bacteria require a watery environment in which to grow. However, though they may not grow out of water, they do not necessarily die when dried. Many bacteria and moulds form spores, resistant bodies which will withstand desiccation not just for years but for decades (Vreeland *et al.*, 2000; Cano *et al.*, 1995).

Bacteria are ubiquitous; they can exist in soil, underwater, deep in the earth's crust and even such environments as acidic hot springs and radioactive waste (Fredrickson *et al.*, 2004). Bacteria are vital in recycling nutrients and many important steps in nutrient cycles depend on bacteria, such as the fixation of nitrogen from the atmosphere depend on bacterial activity (Martinko *et al.*, 2005).

Some bacteria strains are parasitic; they live in and on the bodies of other plants and animals. Many can invade healthy tissue and utilize valuable food substances; they seriously injure the host in which they live. The result is disease in the infected host (Todar, 2007).

To initiate an infection, a microbe enters the tissue of the body by a characteristic route, the portal of entry, usually a cutaneous or membranous boundary. The source of the infectious agent can be exogenous, originating from a source outside the body (the environment or another person or animal), or endogenous, already existing on or in the body (normal flora or latent infection). The majority of pathogens have adapted to a specific portal of entry, one that provides a habitat for further growth and spread. This adaptation can be so restrictive that if certain pathogens enter the “wrong” portal, they will not be infectious. For

instance, contact with athlete's foot fungi in small cracks in the toe webs can induce an infection, but inhaling the fungus spores will not. Following the entry of the pathogen, the next stage in infection requires that the pathogen (i) bind to the host, (ii) penetrate the epithelial boundary, and (iii) become established in the tissues. How the pathogen achieves these ends greatly depends upon its specific biochemical characteristics. Pathogens attach by a process called adhesion in which microbes gain a more stable foothold at the portal of entry. Because this often involves a specific interaction between surface molecule on the microbial surface and receptors on the host cell, adhesion may determine the specificity of a pathogen for its host organism and in some cases the specificity of a pathogen for a particular cell type. Once attached to the pathogen it cannot be easily dislodged and is hereby poised advantageously to invade the sterile body compartments. Bacterial pathogens attach most often by mechanisms such as fimbriae (pili), flagella, and adhesive slimes or capsules (Talaro, 1993).

### **1.2.1 Waterborne Pathogenic Bacteria**

#### ***1.2.1.1 Escherichia coli***

They are facultative anaerobic rod and belong to the family *Enterobacteriaceae*, and the largest group of Gram-negative bacteria. They are catalase positive, oxidase negative and indole positive (Hocking *et al.*, 1997; Doyle, 1989). *Escherichia coli* is the best known coliform, largely because of its use as a subject for laboratory studies. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. *E. coli* bacteria are usually found in human and animal wastes, during excessive rainfalls, snow melts, or other type of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or ground water. When these contaminated waters are used as sources of drinking water and the water is not treated and disinfected, *E. coli* may end up in drinking water (U.S EPA, 2006).

One of the *Escherichia coli* strain, *E. coli* 0157:H7 is an emerging cause of foodborne and waterborne illness. Although most strains of *E. coli* are harmless and live in the intestines

of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness. *E. coli* 0157:H7 was first recognized as a cause of illness during an outbreak in 1982 traced to contaminated hamburgers (U.S EPA, 2006). Since then, most infections are believed to have come from eating undercooked ground beef. However, some strains are waterborne. In 1999, people became sick after drinking contaminated water in Washington Country, New York and swimming in contaminated water in Clark country, Washington (U.S EPA, 2006).

Infection often causes severe bloody diarrhea and abdominal cramps though sometimes the infection causes non-bloody diarrhea (American Academy of Family Physicians, 1999). These symptoms are common to a variety of diseases, and may be caused by sources other than contaminated drinking water. In children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* 0157:H7. Hemolytic uremic syndrome is a life threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5% (U.S EPA, 2006).

One of the major causes of infantile diarrhea is *Escherichia coli*. This organism may induce diarrhea by either of two mechanisms; certain enterotoxigenic strains produce an enterotoxin similar to that in cholera, while certain enteroinvasive strains penetrate the intestinal epithelium as in shigellosis. The toxin causes fluid loss in the small intestine, while the penetration occurs primarily in the large intestine. Both mechanisms lead to dehydration and salt imbalance substantial enough to be life threatening in infants. Often the infection is a nosocomial infection (Alcamo, 1994).

The rate of infection is higher in crowded tropical regions where sanitary facilities are poor, water supplies are contaminated. The immature, non-immune neonatal intestine has no protection against these pathogens. A factor that increases the likelihood of infantile

diarrhea is feeding the baby with unsanitary food or water. The practice of preparing formula from dried powder mixed with contaminated water is tantamount to inoculating the infant with the pathogen.

Traveler's diarrhea usually strikes persons visiting tropical countries and sampling the local food or drink. Despite the popular belief that "Montezuma's revenge", "Delhi belly," and other travel-associated gastrointestinal diseases are caused by exotic pathogens, a large proportion of cases are due to an enterotoxigenic strain of *E. coli*. Travelers encounter new strains to which the local population has developed immunity. The symptoms, occurring within 5 to 10 days, are profuse, watery diarrhea, low grade fever, nausea, and vomiting.

*Escherichia coli* often invade sites other than the intestine. It causes 50% to 80% of urinary tract infections in healthy persons. Urinary tract infections usually results when the urethra is invaded by its own endogenous bacterial colonists. The most common complication is called hemolytic uremic syndrome. People with this problem get hemolytic anemia, which is a low red blood cell count; thrombocytopenia, which is a low platelets count; and renal failure, which is kidney damage. Hemolytic uremic syndrome is more common in children. It can cause acute renal failure in children. This problem starts about 5 to 10 days after the diarrhea starts (American Academy of Family Physicians, 1999). It is also a complication of indwelling catheters and altered host defenses. *Escherichia coli* is the etiologic agent of a variety of other types of human and animal infections. It is one of the organisms most commonly recovered from peritonitis following rupture of the appendix or other bowel perforation, and from pneumonia following aspiration of intestinal contents. It may cause pyogenic wound infections, especially in lesions that are fecally contaminated (Laskin *et al.*, 1977).

#### ***1.2.1.2 Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* is a bacterium of the class Schizomycetes, order Eubacteriales, and family Pseudomonadaceae (National research Council, 1991). This gram negative bacillus is found in warm, moist environments, and can be frequently isolated from soil and

water and occasionally from normal human skin (Percy *et al.*, 1993). On occasion, it can be isolated from saliva or even a moist armpit or groin. Because the species is resistant to soaps, dyes, drugs, drying, and temperature extremes, it is a chronic nosocomial pathogen that is difficult to control. It is a frequent contaminant of humidifiers, ventilators, intravenous solutions, and anesthesia and resuscitation equipment. Even disinfected instruments, utensils, bathroom fixtures, and mops have been incriminated in hospital outbreak.

*Pseudomonas aeruginosa* is a typical opportunist. It is unlikely to cross healthy, intact anatomical barriers, thus its infectiousness results from invasive medical procedures or weak host defenses. Once in the tissue, *P. aeruginosa* expresses virulence factors including exotoxins, a phagocytosis-resistant slime layer, and various enzymes and hemolysins that degrade host tissues. It also causes endotoxic shock.

The most common nosocomial *Pseudomonas* infections occur in patients with severe burns, neoplastic disease, and cystic fibrosis (Moore, 1997). Complications include pneumonia, urinary tract infections, abscesses, otitis, and corneal disease. *Pseudomonas septicemia* may give rise to diverse and grave conditions such as endocarditis, meningitis, and bronchopneumonia that have a high fatality rate (80%), even with treatment. Healthy persons are subject to outbreaks of skin rashes and urinary track infections from community whirlpool baths, hot tubes, and swimming pools. This demonstrates that neither temperature nor the chlorine levels in this enclosure can inhibit the growth of this hardy pathogen. Wearers of contact lenses are also vulnerable to eye infections from contaminated storage and disinfection solutions.

The virulence of *P. aeruginosa* is multifactorial. Its cellular products (lipopolysaccharide, pilli, luekocidin and alginate) and extracellular products (neutral and alkaline proteases, elastase, phospholipase C, and a rhamnolipid hemolysin) ensure its ability to infect most hosts. Two additional proteins are excreted by *P. aeruginosa* – toxin A and exoenzyme S, with the former being the most toxic products secreted by the organism (Moore, 1997). The protease has been reported to have inhibited the chemotaxis, phagocytosis, and oxidative

metabolism of human neutrophils (Kharazmi *et al.*, 1986). The protease may also contribute to the persistence of the organism through cleavage of immunoglobulins (Doring *et al.*, 1984).

Phagocytic cells are the body's primary defence against *P. aeruginosa*. Phagocytosis is enhanced in the presence of serum factors including IgG, complement, and fibronectin (Speert, 1993). When phagocytic dysfunction occurs in diseased or immunosuppressed animal or humans, such as with neutropenia, following extensive thermal injuries/ burns, or in individuals with Cystic Fibrosis or other congenital phagocytic disorder, opportunistic infection with *Pseudomonas* can readily occur.

Other predisposing factors include previous antibiotic or corticosteroid therapy, premature birth, and immunosuppression associated with organ transplants. In individuals with normal phagocytic function, a number of *P. aeruginosa* products mentioned above and also mucoid exopolysaccharide, slime glycolipoprotein, and pyocyanin, can adversely affect phagocytes and/or phagocytosis and predispose those individuals to the pathogenic effects of the organism (Speert, 1993).

This organism can be found in fresh water sources, usually in reservoirs polluted by human or animal waste, but are not typically described as a member of ground water microflora. Within hospital settings, *P. aeruginosa* has been isolated from equipment that contains or uses water (i.e. sink drains, toilets, showers, bathroom fixtures, sanitary plumbing, and air humidifiers), with recovery of up to  $10^9$  organisms per ml after undisturbed overnight growth (Botzenhart *et al.*, 1993). This finding should prompt considerations of procedures for disinfection or decontamination of sinks before their first use of the day.

While nutrient-rich water can easily sustain growth of *P. aeruginosa*, the organism can still grow and multiply within distilled water. One study demonstrated the organism's ability to survive in autoclaved/sterilized drinking water for over 200 days following initial inoculation with  $10^6$  cells per ml (Byrd *et al.*, 1991). In contrast to other gram negative bacteria tested, which remained viable but nonculturable in the sterilized water,

*Pseudomonas aeruginosa* was readily culturable from the water for over 95 days (Byrd *et al.*, 1991). Thus, the organism's minimal growth requirements and nutritional versatility allow it to adapt to changing ecologic circumstances, ensuring its ubiquitous distribution (Moore, 1997).

*Pseudomonas aeruginosa* has been isolated in commercial laboratory animal barrier production facilities (Lindsey, 1986). It can be a constituent of the bacterial flora of the intestines of laboratory rodents, especially mice (Benirschke K, Garner F. M, and Jones T.C). In facilities with conventional housing, and minimal or no water treatment, maintenance of nasopharyngeal and intestinal colonization is associated with repeated ingestion of large numbers of the organism in the drinking water (National Research Council, 1991).

Many disinfectants or washing compounds in ordinarily recommended working solutions are sometimes ineffective in killing *Pseudomonas* sp, which are frequently found in community water supplies despite chlorination (Hoag *et al.*, 1965). Drinking water is considered important for spreading contamination within animal colonies. Macrocolonies of mucoid-encapsulated *P. aeruginosa* lining the inside of automatic water distribution pipes and rack manifolds can shed single, nonmucoid, flagellated "swarmer cells" which can contaminate inanimate objects, humans, or animals (Moore, 1997).

In animal facilities, contaminated untreated drinking water is the most common source of *P. aeruginosa*, and has been shown to be a source of cross infection in animals. Decontamination of water prior to presentation to animals can be accomplished by a number of techniques including reverse osmosis, deionization, microfiltration, reverse osmosis-deionization-ultrafiltration, autoclaving, hyperchlorination, and acidification. Additional methods include use of UV light, iodination of water, and ozonation of water (Moore, 1997). Deionization, while removing inorganic impurities, may still allow bacterial growth, and should be coupled with another procedure to ensure adequate disinfection. Microfiltration can remove particles of  $> 0.02 \mu\text{m}$ , but bacterial growth and plugging of the membrane can occur when large volume systems are used. A combined

reverse osmosis-deionization-ultrafiltration system, while effective in removing ionic and organic materials, is not an efficient method for water treatment in large facilities and may not provide sterile water, as bacteria can grow across filter. By using reverse osmosis water, with its nutrient-poor content, less bacterial growth will occur within automatic watering system pipes, resulting in a thinner biofilm for easier penetration of biocidal agents (Moore, 1997).

#### **1.2.1.3 *Bacillus subtilis***

*Bacillus subtilis* is a bacterium of the class *Bacilli*, order *Bacillales*, and family *Bacillaceae*. *Bacillus subtilis* is a gram-positive, catalase positive bacterium commonly found in soil (Martinko and Madigan, 2005), water and air. *B. subtilis* has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions, and because their spores are so resistant, their control is of great economic concern in the food processing industry and in the preparation of all sterile products (Laskin *et al.*, 1977). The basis of the spores resistance and longevity, its formation, morphology, composition, and stages of germination continue to be subjects of many investigations (Gould *et al.*, 1969; Halvorson *et al.*, 1971). The genus bacillus encompasses a great diversity of strains. Some species are strictly aerobic, others are facultative anaerobes. The bacilli also exhibit variation in temperatures of growth; some thermophiles grow from a minimum temperature of 45°C to a maximum temperature of 75°C or higher, and some psychrophiles grow at temperature from -5°C to 25°C.

Unlike several other well-known species, *B. subtilis* has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct (Nakano *et al.*, 1998).

*B. subtilis* is not considered a human pathogen; it may contaminate food but rarely causes food poisoning (Ryan *et al.*, 2004). *B. Subtilis* is responsible for spoilage in dried milk and in some fruits and vegetable products. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heating that is often use to cook food,

and it is responsible for causing ropiness- a sticky, stringy consistency caused by bacterial production of long- chain polysaccharides- in spoiled bread dough.

*B. subtilis* can divide asymmetrically, producing an endospore that is resistant to environmental factors such as heat, acid, and salt, and which can persist in the environment for long periods of time. The endospore is formed at times of nutritional stress, allowing the organism to persist in the environment until conditions become favorable. Prior to the decision to produce the spore the bacterium might become motile, through the production of flagella, and also take up DNA from the environment. *B. subtilis* has proven highly amenable to genetic manipulation, and has therefore become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation (Branda *et al.*, 2001). It is also heavily flagellated, which gives *B. subtilis* the ability to move quite quickly. In terms of popularity as a laboratory model organism *B. subtilis* is often used as the gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative rod (Sierro *et al.*, 2008).

*B. subtilis* is used as a soil inoculant in horticulture and agriculture. Enzymes produced by *B. subtilis* are widely used as additives in laundry detergents. A strain of *Bacillus subtilis* known as *Bacillus natto* is used in the commercial production of the Japanese delicacy natto as well as similar Korean food cheonggukjang (Inooka and Kimura, 1983). *B. subtilis* strain QST 713 has a natural fungicidal activity, and is employed as a biological control agent (U.S EPA, 2006). *B. subtilis* can convert explosives into harmless compounds of nitrogen, carbon dioxide, and water. It plays a role in safe radionuclide waste [e.g. Thorium (IV) and Plutonium (IV)] disposal with the proton binding properties of its surfaces (Martinko and Madigan, 2005; Ryan and Ray, 2004). Recombinants *B. subtilis* strain pBE2C1 and *B. subtilis* strain pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and that they could use malt waste as carbon source for lower cost of PHA production (Hoi- Fu Yu *et al.*, 2006).

### **1.3 Requirements of a disinfectant**

The last 100 years have brought significant environmental advances. At the beginning of the 20<sup>th</sup> Century, water and wastewater were treated by one principle, “the solution to pollution is dilution (U.S. EPA, 1986).” But as population density increased, so did the spread of infectious disease. Only by the use of science and technology have we been able to identify threats to public health and find ways to overcome them. Safe drinking water has now become commonplace, ongoing research continue to make it safe, even in the light of increasing wastewater reuse. Attainment of the disinfection guidelines can only be achieved by the disinfection process, which, from a disease prevention standpoint, is the most important unit process (U.S. EPA, 1986).

Disinfection is the elimination or inactivation of harmful organisms. It should be distinguished from sterilization, which is the destruction of all life forms. A disinfectant must be able to completely destroy particular pathogens at the concentrations likely to occur, and it should be effective in the normal range of environmental conditions.

While the disinfectant should destroy pathogens, it must not be toxic to man or other higher animals, such as fish, in receiving water. Ideally, some residual disinfecting capability should be provided for a water supply to provide protection against re-infection while the water is in a distribution system. The residual, which passes to the consumer, should not be unpalatable or significantly alter the taste.

A disinfectant should be safe and easy to handle, both during storage and during addition. The availability of simple or automatic analytical procedures ensures a reliable and consistent dosing system, which can be accurately controlled.

These factors severely restrict the number of reliable disinfectants. The requirements of effectiveness in destroying pathogens, safety of handling, and non-toxicity to man in normal use, present a major challenge. For example, the addition of certain toxic metals can provide effective destruction of pathogens but the residual toxicity is harmful to

humans. Chlorine, the most common disinfectant is a dangerous chemical and requires rigorous safety handling procedures. The other disinfectants that have found large-scale use are ozone and chlorine dioxide. Others, such as heat, ultraviolet irradiation, ultrasonic vibration, ultra-filtration, silver, bromide and iodine, only find limited application because of the high costs involved. Disinfection is usually the final stage in the water treatment process in order to limit pathogens. Disinfection can be attained by means of physical or chemical means (Lewis and McIndoe, 2004).

#### **1.40 Methods of disinfection**

##### **1.4.1 Physical methods**

The technology, based on nonchemical methods, is undergoing rapid development. Some techniques are already available commercially. This category is represented by techniques employing such physical principles for disinfection as gamma radiation, ultrasound, ultrafiltration, reverse osmosis, heating, freezing, and ionizing radiation (Acra *et al.*, 1980, 1984). Disinfecting small quantities of water by pasteurizing with heat or solar energy is a technology with some potential, but requires further development. Physical methods (boiling or the use of ceramic filters), ultraviolet light and others have been recommended for such cases (Acra *et al.*, 1980, 1984).

None of these methods is entirely free from practical problems that could induce users to revert to untreated water. Fuel wood, for instance, for boiling is scarce; particularly in rural areas where its demand is high. Besides, the flat taste of boiled water discourages some customers. The diverse types of ceramic filters have a wide range of pore sizes and present difficulties in selection. They suffer frequent clogging of the ceramic candles and often leak through disguised fine cracks. Proprietary halogen preparations frequently lead to consumer complaints and rejection because of the undesirable tastes and odours imparted to the water. It is especially so if high doses are applied inadvertently or as required in cases of heavily polluted waters.

Ultraviolet Light (UV) disinfection is a means of killing or rendering harmless microorganisms in a dedicated environment. These microorganisms can range from bacteria and viruses to algae and protozoa. UV disinfection is used in air and water purification, sewage treatment, protection of food and beverages, and many other disinfection and sterilization applications. A major advantage of UV treatment is that it is capable of disinfecting water faster than chlorine without cumbersome retention tanks and harmful chemicals.

However there are factors affecting the proper functioning of the UV such as: iron and manganese, Total Dissolved Solids (TDS), turbidity, and suspended solids (U.S. EPA, 2003). Iron and manganese will cause staining on the quartz sleeve and prevent the UV energy from transmitting into the water at levels as low as 0.03 ppm of iron and 0.05 ppm of manganese (Dallan, 1998). Total TDS should not exceed approximately 500 ppm (about 8 grains of hardness). Calcium and magnesium, in high amounts, have a tendency to build up on the quartz sleeve, again impeding the UV energy from penetrating the water (U.S. EPA, 2003).

Turbidity is the inability of light to travel through water. Turbidity makes water cloudy and aesthetically unpleasant. Large solids have the potential of harboring or encompassing the microorganisms and preventing the necessary UV exposure (Wu *et al.*, 2005). An additional factor affecting UV is temperature; UV levels fluctuate with temperature levels. The optimal operating temperature of a UV lamp must be near 40°C (104°F).

#### **1.4.2 Chemical methods**

Chemical methods depend mostly on selected chemicals with biocidal as well as redox properties. Their primary functions range from killing or inactivating pathogens to removing undesirable constituents to disinfecting water supplies, wastewater treatment effluent, or industrial waters. There are two kinds of chemical disinfection namely: primary disinfection which achieves the desired level of microorganism inactivation and secondary disinfection maintains a disinfectant residual in the finished water that prevents the

regrowth of microorganisms (Technologies for upgrading existing or designing new drinking water treatment facilities, EPA/625/4-89/023). Primary methods of disinfection are chlorination, chloramination, and ozonation,. Other disinfection methods include chlorine dioxide, potassium permanganate, and nanofiltration (Environmental Pollution Control Alternatives, EPA/625/5-90/025).

#### **1.4.2.1 Chlorination**

Chlorination is by far the most commonly used method globally to disinfect wastewater. It kills pathogens, and prevents their accumulation in fish, shellfish and other aquatic organisms. It also destroys a variety of bacteria, viruses and protozoa, including *Salmonella*, *Shigella* and *Vibrio Cholerae*. Chlorine acts as a powerful disinfectant when used either on its own, as sodium hypochlorite (bleach) or as calcium hypochlorite. It can quickly kill bacteria and other microbes when added in mild concentrations. Chlorine has the major advantage of ensuring clean disinfected water right up to the point of use, whereas the actions of other disinfectants are usually only temporary. In addition to disinfecting, chlorine helps remove tastes and odours, controls the growth of slime and algae in main pipes and storage tanks, and helps to remove unwanted nitrogen compounds from water (Euro Chlor, 2006). Over 90% Europe drinking water is chlorinated (Euro Chlor, 2006).

In a recent survey (Disinfection Committee, 1992) of the disinfection practices in the USA it was found that most water utilities were making concerted efforts towards reducing halogenated by-products in potable water. Chlorine can react with organic compounds present in the water to form halogenated disinfection by-products (DBPs) (i.e. that have chlorine or bromine incorporated into their structure), most notably trihalomethanes (THM) and haloacetic acids, which may adversely affect human health (Jolley *et al.*, 1977). Some studies in human populations seem to indicate that chlorinated drinking water may cause cancers of the bladder, colon and rectum, but the studies are not conclusive (Jolley *et al.*, 1977).

The most significant change by utilities was to alter the point of application of chlorine, the dosage of chlorine used, and the addition of ammonia. Although chloramines are recommended as a primary disinfectant (Longley *et al.*, 1982) and for controlling bacterial regrowth in distribution systems (Neden *et al.*, 1992), these substances could affect kidney dialysis patients and aquarium fish if present at high residual concentrations (White, 1992).

Formation of mutagenic and carcinogenic (cancer causing) agents in water and wastewater effluent treated with chlorine has prompted research to seek alternative disinfecting methods that would minimize environmental and public health impact (Pieterse, 1988).

Although chlorination is by far the most common disinfectant in water treatment, it has many disadvantages in that its by-products can be quite toxic if residual concentrations are not maintained within its permissible limits shortcomings as a disinfectant. Thus chlorination is rapidly being supplanted, or at least supplemented by other disinfectants. Before considering each of the other in detail, it is worth comparing chlorine with alternatives.

#### **1.4.2.2 Chloramination**

Several towns in USA and other countries made the change from chlorine to chloramination, a combination of chlorine and ammonia to reduce the level of certain by-product of chlorination (City of Rocky Mount, 2003). By using chloramines as a disinfection method, they managed to (i) reduce the levels of trihalomethanes (THMs) and haloacetic acid (HAAs) in the drinking water supplies.(2) remain in compliance with the Federal safe Drinking Water Act. (3) continue to supply water customers with safe and aesthetically pleasing water. Chloramination however also suffers from toxicity such that water for special customers who use Town of Nashville water for kidney dialysis treatment, in fish tanks, in aquaculture, and for certain other special uses will need to make some changes (City of Rocky Mount, 2003). Chloramines are harmful when they are absorbed into the bloodstream. People with weakened immune systems, including infants, elderly people, and persons with HIV/AIDs or who are undergoing chemotherapy, etc. should

consult a health professional about whether to use specially treated water instead of water from normal public sources using either chlorinated or chloraminated drinking water (City of Rocky Mount, 2003).

A study in the USA has recently revealed that, disinfection by-product (DBP) found in drinking water treated with chloramines to be most toxic ever found. The discovery raises concerns regarding an Environmental Protection Agency (EPA) plan to encourage all U.S. water treatment facilities to change to chlorine alternatives (Melville, 2004).

They reported on the structure and toxicity of five iodoacids found in chloramines treated water in Corpus Christi, Texas, in the Journal of Environmental Science and Technology (Barlow, 2004). “*The iodoacids may be the most toxic family of DBPs to date*” Plewa said. One of the five detailed in the study, iodoacetic acid, is the most toxic and DNA-damaging to mammalian cells in tests of known DBPs.” These iodoacetic acids raise new levels of concerns. Not only do they represent a potential danger because of all the water consumed on a daily basis; water is recycled back to the environment (Barlow, 2004).

Chloramine is a weak disinfectant. It is much less effective against viruses or protozoa than free chlorine. Chloramine is appropriate for use as a secondary disinfectant to prevent bacterial regrowth in a distribution system. Nitrogen trichloride appears to be the only detrimental reaction (Neden *et al.*, 1992). It may be harmful to humans and imparts odor to the water. The use of the proper amounts of each chemical reactant will avoid its production.

#### **1.4.2.3 Chlorine dioxide**

Due to concern over trihalomethanes (THMs) in chlorinated drinking water, the U.S. Environmental Protection Agency (EPA) established a maximum contaminant level (MCL) of 0.10mg/L for total THMs. To comply with this regulation, many drinking water utilities have had to alter their treatment methods, and disinfectant such as chlorine dioxide (ClO<sub>2</sub>), is used as an alternative, primarily because chlorine dioxide does not produce the high

levels of THMs observed with chlorine treatment, it has been listed by the EPA in a subsequent amendment to the THM regulation as a suitable alternative treatment method. Chlorine dioxide is currently used in more than 300 drinking water treatment plants in the United States and in several thousand plants in Europe (U.S EPA, 1994).

Chlorine dioxide also is an excellent disinfectant; its biocidal efficiency is equal to or superior to chlorine and chloramines. Also, chlorine dioxide is effective over a wide pH range and is five times more soluble in water than chlorine. Chlorine dioxide is effective for removing iron and manganese ions, and it does not react with amines to form chloramines. Finally Chlorine dioxide produces much lower total organic chlorine levels, as compared to those obtained with chlorination (Volk, 2002).

However chlorine dioxide is an unstable and extremely corrosive gas. The chlorite ion ( $\text{ClO}_2^-$ ) is a chlorine dioxide initiated disinfection by-product. When chlorine dioxide is decomposed chlorite is formed (Lee *et al.*, 2004). Various complex reactions make up the formation of chlorite from the dissolved chlorine dioxide. Some infants and young children who drink water containing chlorine dioxide in excess of EPA's standard could experience nervous system effects. Similar effects may occur in fetuses of pregnant women who drink water containing chlorine dioxide in excess of EPA's standard. Some people may experience anemia (U.S. EPA, 2006).

#### **1.4.2.4 Potassium permanganate**

Potassium permanganate ( $\text{KMnO}_4$ ) is an oxidant that was first used in 1910 for water treatment in London (American Water Works Association, 2003). However, widespread use of  $\text{KMnO}_4$  did not occur until the 1960s, when its effectiveness for controlling tastes and odours had become recognized. Potassium permanganate may be useful in controlling the formation of trihalomethanes and other DBPs by oxidizing precursors and reducing the demand for other disinfectants (Hazen *et al.*, 1992). Although potassium permanganate has many potential uses as an oxidant, it is a poor disinfectant (Banerjee, 1950).

Potassium permanganate can inactivate various bacteria and viruses, but it is not used as a primary or secondary disinfectant when applied at commonly used treatment levels. Potassium permanganate levels that may be required to obtain primary or secondary disinfection could be cost prohibitive. A number of investigations have been performed to determine the capability of potassium permanganate as a disinfectant. The results of the study showed that high dosage rates were required to accomplish complete inactivation of bacteria in three studies (EPA Guidance manual, 1999). Early research showed that a dose of 2.5 mg/L was required for complete inactivation of coliform bacteria (Le Strat, 1944).

In using potassium permanganate in water treatment, caution should be taken to prevent overdosing, in which case, excess manganese will pass through the treatment plant. Proper dosing should be maintained to ensure that all of the permanganate is reduced and removed from the plant upstream of, or within, the filters. If residual manganese is reduced downstream of the filters, the resulting solids can turn the treated water a brown/black color and precipitate in the homes of consumers on heat exchange surfaces such as hot water heaters and dishwashers. Use of potassium permanganate can also be source of manganese in the treated water, which is regulated in the drinking water with secondary maximum contaminant level of 0.05 mg/L. Under reducing conditions, the  $\text{MnO}_2$  solids accumulated in filter backwash water and settling basins can be reduced to soluble  $\text{Mn}^{2+}$  and pass through the filters thereby remaining in the treated water (EPA Guidance manual, 1999).

Disinfection with permanganate has the following disadvantages for drinking water, long contact time is required and it has a tendency to give water a pink colour, it is also toxic and irritating to skin and mucous membranes, No by-products are generated when preparing the feed solution, however this dark purple/black crystalline solid can cause serious eye injury, is a skin and inhalation irritant, and can be fatal if swallowed. Overdosing is dangerous and may cause health problems such as chemical jaundice and drop in blood pressure (EPA Guidance manual, 1999).

#### 1.4.2.5 Ozone

Ozone was first discovered by the European researcher C.F. Schonbein in 1839. It was first used commercially in 1907 in municipal water supply treatment in Nice and in 1910 in St. Petersburg (Guzel-Seydim *et al.*, 2004). Ozone was first used in water treatment for drinking purposes in 1983 in the Netherlands. While  $O_3$  being used in Europe for drinking water disinfection and oxidation frequently, it was slow to transfer to the United States. In 1987, the Los Angeles Aqueduct Filtration Plant was placed in service and now treats up to 600 mgd of drinking water. In 1991, approximately 40 water treatment plants each serving more than 10,000 people in the United States utilized ozone (Langlais *et al.*, 1991). This number has grown significantly, with Rice reporting that as of April 1998, 264 operating plants in the United States use ozone. Most of these facilities are small: 149 plants are below 1 mgd.

Ozone is used in water treatment for disinfection and oxidation. Early application of ozone in the United States was primarily for non-disinfection purpose such as color removal or taste and odor control. However, since the implementation of the SWTR and proposal of the DBP rule, ozone usage for primary disinfection has increased in the United States (Robson *et al.*, 1990).

### 1.5 Ozone Chemistry

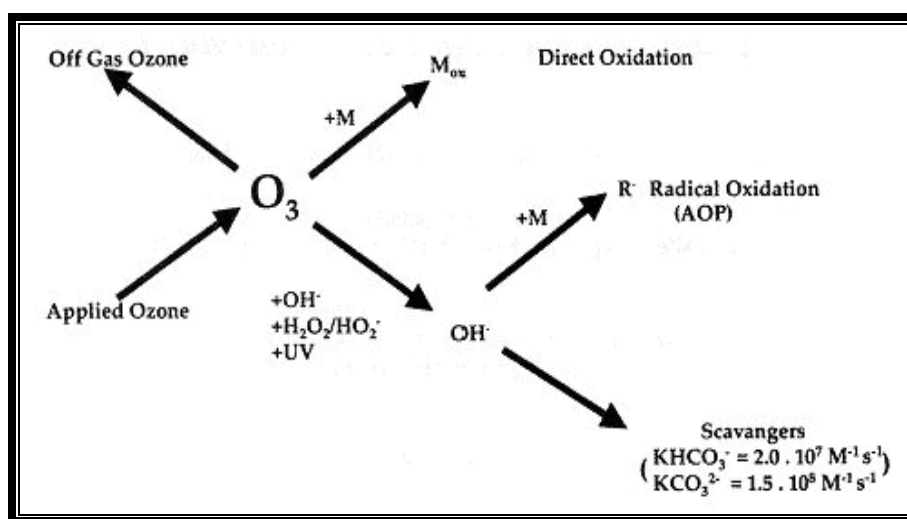
Ozonation has been used for years to disinfect water for drinking purposes in Europe. While being used frequently in Europe it was slow to transfer to the United States. Early application of ozone in the United States was primarily for non-disinfection purposes such as color removal or taste and odor control. A number of other commercial uses have been found for ozone including disinfection of bottled water, swimming pools, prevention of fouling of cooling towers, and wastewater treatment (Guzel-Seydim *et al.*, 2003).

Ozone is a faintly blue, pungent-smelling, and unstable gas with high oxidation potential. It must be generated at the point-of-use by applying energy to oxygen or dried air. Ozone is a

powerful oxidant, second only to the free hydroxyl radical. It is capable of oxidizing many organic and inorganic compounds under aqueous conditions. Reactions with organics and inorganics cause an ozone demand which should be met during water ozonation. Ozone concentration levels used in water treatment are typically below 14 %, which limits the mass transfer driving force of gaseous ozone into the water (EPA Guidance Manual, 1999).

When exposed to a neutral or alkaline environment (pH above 6), UV light, or hydrogen peroxide, ozone can decompose in water to produce more active hydroxyl free radicals (Li, 2004). The free hydroxyl radicals are among the most reactive oxidizing agents in water, with reaction rates on the order of  $10 \times 10^{10} - 10^{13} \text{ M}^{-1} \text{ s}^{-1}$ , approaching the diffusion control rates for solutes such as aromatic hydrocarbons, unsaturated compounds, aliphatic alcohols, and formic acid (Hoigne and Bader, 1976). However, the half life of hydroxyl free radicals is in the order of microseconds; and therefore their concentrations can never reach levels above  $10 \times 10^{-12} \text{ M}$  (Glaze and Kang, 1988).

In 1977 Hoigné and Bader described the reaction of ozone in aqueous solution towards other compounds in two ways, by direct reaction or by indirect reaction with radical species formed in ozone decomposition. The ozone oxidation process is represented schematically in figure 1.1.



**Figure 1.1 Reactions of ozone and dissolved solids**

Under acidic conditions, the direct oxidation with molecular ozone is of primary importance; and under conditions favoring hydroxyl free radical production, such as high pH, exposure to UV, or addition of hydrogen peroxide, the hydroxyl oxidation starts to dominate (Hoigne *et al.*, 1977). The spontaneous decomposition of ozone occurs through a series of steps. The exact mechanism and reactions associated have not been established. Mechanistic models have been proposed (Hoigne *et al.*, 1983a, 1983b; Glaze, 1987). It is believed that hydroxyl radicals forms as one of the intermediate products, and can react directly with compounds in water.

### 1.5.1 Ozone Generation (see section 1.9 for more details)

Ozone is formed by the irradiation of oxygen molecules with high energy sources to form oxygen radicals which then combine with oxygen molecules as shown in the following reactions;



These reactions occur naturally in the Earth's stratosphere under the influence of sunlight, and artificially using electrical discharge, ultraviolet light and electrolysis.

### 1.5.2 The decomposition of ozone

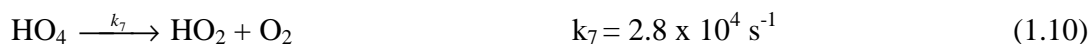
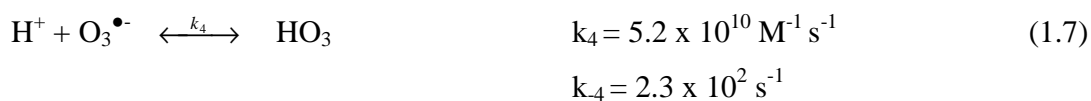
Ozone rapidly degrades to oxygen by a series of reactions involving a range of intermediate hydroxyl radicals which are utilized in some oxidation reactions. The overall reaction is:



This reaction occurs very rapidly, and approximately follows first order kinetics. In "pure" water ozone typically has a half life of approximately 20 minutes.

### 1.5.3 Properties of ozone in aqueous solution

In a pure aqueous solution ozone slowly decomposes in multiple steps involving radical formation. The depletion is a chain process and has been described by two different mechanisms, by Hoigné-Staehelin-Bader (HSB) and Gordon-Tomiyasu-Fukutomi (GTF). HSB process is described below. (Hahn *et al.*, 2000)



### 1.5.4 Transfer of ozone from the gas phase to the aqueous phase

Ozone is normally produced in air or oxygen. In order to use ozone in the treatment of water it is necessary to dissolve it in water. The solubility of ozone in water depends on the headspace gaseous phase concentration, water temperature, water vapour pressure and its rate of diffusion. The quantity of ozone, which can be transferred into water, is limited by the equilibrium between the amount of ozone in the gas phase and the amount dissolved in

water. Table 1.1 shows equilibrium concentrations of ozone dissolved in water for various gas phase concentrations.

**Table 1.1 Ozone solubility in water at equilibrium, mg/L**

Temperature °C	Ozone concentration in gas phase					
	0.0%	0.1%	1.0%	1.5%	2.0%	3.0%
15	0.006	0.60	6.04	9.05	12.06	18.1
25	0.004	0.35	3.53	5.29	7.05	10.58
30	0.003	0.027	2.7	4.04	5.39	8.09

([www.Ctre.iastate.edu/educweb/ce523/notes/oxidation%20and%20Disinfectiondoc.](http://www.Ctre.iastate.edu/educweb/ce523/notes/oxidation%20and%20Disinfectiondoc.))

The values in Table 1.1 represent theoretical maxima at ambient pressure. The solubility of ozone follows a linear relationship with concentration and pressure according to Henry's Law,  $P_i H = C_i$ , where  $P_i$  is the partial (fractional) pressure of ozone in the gas (air or oxygen) and  $C_i$  the concentration of ozone in the water. The value of  $H$  would normally be expressed as a liquid phase concentration per (partial) pressure of the ozone in the gas phase.

## 1.6 Disinfection kinetics

The major assumptions used in derivation of kinetics inactivation model are: (1) no back mixing; (2) uniform dispersion of organisms and disinfectant molecules; (3) sufficient mixture to ensure liquid diffusion is not rate limiting; (4) constant temperature and pH during the contact time (Gyürèk and Finch, 1998).

The earliest inactivation kinetic approach was used by Chick (1908) who defined inactivation kinetics as first order.

$$r_d = dN/dt = -kN \quad (1.13)$$

In Equation 1.13 known as Chick's law, where  $r_d$  is the disinfection rate (number of organisms inactivated per unit volume per unit time) and  $N$  is the concentration of viable organisms. Assuming that the rate constant,  $k$ , is actually constant, in a batch system, this results in an exponential decay in organisms, because the rate of inactivation equals  $dN/dt$ .

Watson (1908) proposed Equation 1.15 known as Chick-Watson Law, where the pseudo-first-order reaction rate assumption is not used but related the rate constant of inactivation,  $k$ , to the disinfectant concentration,  $C$ :

$$k = k' C^n \quad (1.14)$$

In Equation 1.14,  $n$  is termed the coefficient of dilution, and  $k'$  is presumed independent of disinfectant and microorganism concentration. The Chick-Watson law defines inactivation as a function of disinfectant concentration and contact time. The rate equation for the Chick-Watson law is

$$r_d = dN/dt = -k' C^n N \quad (1.15)$$

From the Chick-Watson Law, when  $C$ ,  $n$ , and  $k$  are constant (i.e., there is no disinfectant demand and decay), the above rate law may be integrated so that in a batch system the following relationship arises:

$$\ln S = \ln N/N_0 = -k' C^n t \quad (1.16)$$

In Equation 1.16,  $S$ ,  $N$  and  $N_0$  are the survival ratio, the concentrations of viable microorganisms at time  $t$  and time 0, respectively. When disinfectant composition changes with time, or when a configuration other than a batch (or plug flow) system is used, the appropriate rate laws characterizing disinfectant transformation along with the applicable mass balances must be used to obtain the relationship between microbial inactivation and concentration and time (Haas *et al.*, 1984).

## 1.7 Disinfection Parameters

Factors influencing the decomposition of ozone in water are temperature, pH, and concentration of organic solutes and inorganic constituents (Hoigne *et al.*, 1975, 1976). The following sections discuss the main influence factors for ozone decomposition and the effects that pH and temperature have on the reaction rate of ozone and pathogen inactivation. The ability to maintain a high aqueous ozone concentration is critical from a regulatory disinfection compliance standpoint. This means that factors that accelerate ozone decomposition are undesirable for inactivation because the ozone residual dissipates faster and therefore reduces the contact time credit, requiring a corresponding increase in the ozone applied, thus increasing cost ((EPA Guidance Manual, 1999).

### 1.7.1 pH

Since reaction with  $\text{OH}^-$  is the initial decomposition step, the stability of ozone solution is thus highly dependent on pH and decreases as alkalinity rises (Roth *et al.*, 1983; Tomiyasu *et al.*, 1985). At pH above 8 the initiation rate has, in the presence of radical scavengers, been shown to be proportional to the concentrations of ozone and  $\text{OH}^-$  (Eriksson, 2005). However, in acidic solutions the reaction with  $\text{OH}^-$  cannot be the only initiation step. Predicted reaction rates below pH 4 including a mechanism based only on reaction with  $\text{OH}^-$  are much lower than those determined experimentally.

There is still a contradiction on whether ozone is more powerful in microbial inactivation at higher pH values where there is a production of radicals or at lower pH values where molecular ozone predominates. Inactivation of *Giardia muris* cysts was found to improve when the pH increased from 7 to 9 (Wickramanayake, 1984a). This phenomenon was attributed to the possible changes in a cysts chemical make up making it easier for ozone to react with the cyst constituents at the higher pH levels. However, the same study found that inactivation of *Naegleria gruberi* cysts was slower at a pH 9 than at lower pH levels, thereby indicating that pH effects are organism-specific. A slight decrease has been found in the virucidal efficacy of ozone residual when pH was reduced (Roy, 1979). However, the

opposite effect was observed by Vaughn *et al.* (1987) - (cited in Hoff, 1986). They claim that changes in disinfection efficacy with variations in pH appear to be caused by the ozone decomposition rate.

### **1.7.2 Temperature**

Temperature has an important influence on the half life of ozone. Ozone becomes less soluble and less stable in water at high temperatures (Katzenelson *et al.*, 1974); however the disinfection and chemical oxidation rates remain relatively stable. Studies have shown that although increasing the temperature from 0 to 30°C can significantly reduce the solubility of ozone and increase its decomposition rate, temperature has virtually no effect on the disinfection rate of bacteria in that temperature range (Kinman, 1975). The disinfection rate was found to be relatively independent of temperature at typical water treatment plant operating temperatures despite the reduction in solubility and stability at higher temperature.

## **1.8 Mechanism of Disinfection and Bacterial Resistance to Ozone**

### **1.8.1 The Composition and Structure of Bacterial Cells and Potential Targets for Ozone.**

Although the inhibitory and lethal effects of ozone on pathogenic organisms have been observed since its discovery by Schonbein in 1840, the mechanisms for these actions still needs more clarification (Sunnen, 2005). The mechanisms of ozone disinfection include: (1) direct oxidation/destruction of the cell wall with leakage of cellular constituents outside of the wall; (2) reactions with radical by-products of ozone decomposition; (3) damage to the constituents of the nucleic acids (purines and pyrimidines); and (4) breakage of carbon-nitrogen bonds leading to depolymerization.

The Gram-positive *bacillae* (*Bacillus*), and Mycobacteria are the most resistant forms of bacteria (EPA Guidance Manual, 1999). Bacterial spores are invariably the most resistant of all types of bacteria. They have an additional spore coat, which makes them more

resistant to the action of disinfectants, the target sites of disinfectants are believed to be within the spore (Russell, 1995).

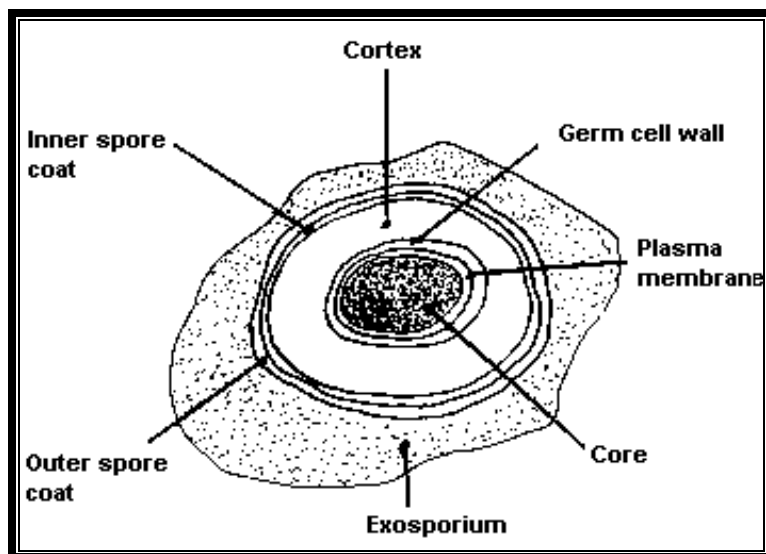
#### **1.8.1.1 Gram-positive Bacteria and Gram-negative Bacteria**

The cell wall is the first potential target of ozone. Bacteria can be divided into two basic cell wall types; Gram-positive and Gram-negative. The cell walls of Gram-positive and Gram-negative bacteria are structurally different. The backbone material of both types is the peptidoglycan layer which is composed of layers of polysaccharide chains linked by short peptides (Boyd *et al.*, 1991).

Gram negative bacteria possess a thin peptidoglycan lamella compared to the Gram-positive bacteria which is made of a very thick peptidoglycan (over 90% of cell wall) which provides a strong protective layer that protects the plasma membrane from lysis by osmotic shock and it also made of teichoic acid which are acidic polysaccharides not only permeate the peptidoglycan but also appear on the surface of the cell wall. It makes the Gram-positive cell wall acidic and is very important in regulating autolytic activity (Atlas, 1995).

#### **1.8.1.2 Bacterial Spores**

Spores are formed when conditions are unfavorable for the continued growth of bacteria and are the most resistant life forms known in bacteria (Russell, 1995). The two most important genera of bacteria producing spores are *Bacillus* and *Clostridium*. A number of chemical compounds are sporicidal under the condition of much higher concentrations and longer contact times compared with bactericidal action. Spores are the dormant, dehydrated forms of spore-forming vegetative cells. The structure of a “typical” bacteria spore includes core, plasma membrane, germ cell wall, cortex, inner and outer spore coat, and exosporium (present in some spores, but may surround just one spore coat), from inside to outside. A schematic diagram of a ‘typical’ bacteria spore is given in Figure 1.2.



**Figure 1.2** A typical bacterial spore (Russell, 1995).

#### **1.8.1.3 Virus Inactivation**

Typically, viruses are more resistant to ozone than vegetative bacteria but less resistant than sporular forms of *Mycobacteria* (Bablon *et al.*, 1991). Viruses differ in their susceptibility to destruction by ozone and there seems to be little difference between the polio and coxsackie viruses. The resistance of Polio virus type 2 was 40 times that of coxsackie AS (Roy *et al.*, 1981; Roy *et al.*, 1982). The first site of action for virus inactivation is the virion capsid, particularly its proteins (Riesser *et al.*, 1976). Ozone appears to modify the viral capsid sites that the virion uses to fix on the cell surfaces. High concentrations of ozone dissociate the capsid completely.

#### **1.8.1.4 Protozoa Inactivation**

Protozoan cysts are much more resistant to ozone and other disinfectants than vegetative forms of bacteria and viruses (EPA, 1999). Ozone can effectively inactivate *Giardia* cysts and *Cryptosporidium* oocysts which can not be killed easily by chlorine or chlorine dioxide. It has been speculated that ozone initially affects the *Giardia muris* wall and makes it more permeable (Wickramanayake, 1984c). Subsequently, aqueous ozone penetrates into the cyst and damages the plasma membranes, additional penetration of ozone eventually affects the nucleus, ribosomes, and other ultrastructural components.

## **1.9 Ozone generation**

### **1.9.1 Selecting an ozone generation system**

Ozone can be generated using air or oxygen. The concentrations achievable with oxygen are higher than using air. Making a choice between using air or oxygen for the production of ozone requires a full cost benefit analysis. The approximate concentrations of ozone produced in air and oxygen with technology currently on the market are UV irradiation and the gas which it utilizes is air ranging from 0.001 to 1.0% and oxygen gas is not typically used. Corona discharge utilizes air ranging from 2 to 4% and oxygen ranging from 6 to 12%. Oxygen is not typically used as a source of gas for UV systems due to the expense of oxygen preparation equipment and the small concentration of ozone produced. There are three systems used to generate ozone: corona discharge, UV and electrolysis.

### **1.9.2 Corona discharge**

A corona discharge is created when a high voltage is applied across two electrodes separated by a dielectric and a discharge gap (EPA Guidance Manual, 1999). Electrons flowing through the discharge gap are used to bombard oxygen atoms in the feed gas, causing some of the oxygen molecules to break into atoms which then combine with molecular oxygen to form ozone.

The electrodes are usually arranged as two concentric reaction cylinders around each other with the discharge gap between them. The outer electrode is usually jacketed in cooling water. The dielectric is usually made of glass or ceramic and together with a metallic coating this makes the inner electrode. The outer electrode is usually stainless steel.

Up to 20kV is required to ensure an adequate corona discharge rate. Frequencies exceeding 50 Hz improve the efficiency. Most ozone generators are equipped with solid state medium frequency converters to produce frequencies around 500 Hz ensuring higher ozone concentrations, higher production and to reduce power consumption (EPA, 1999).

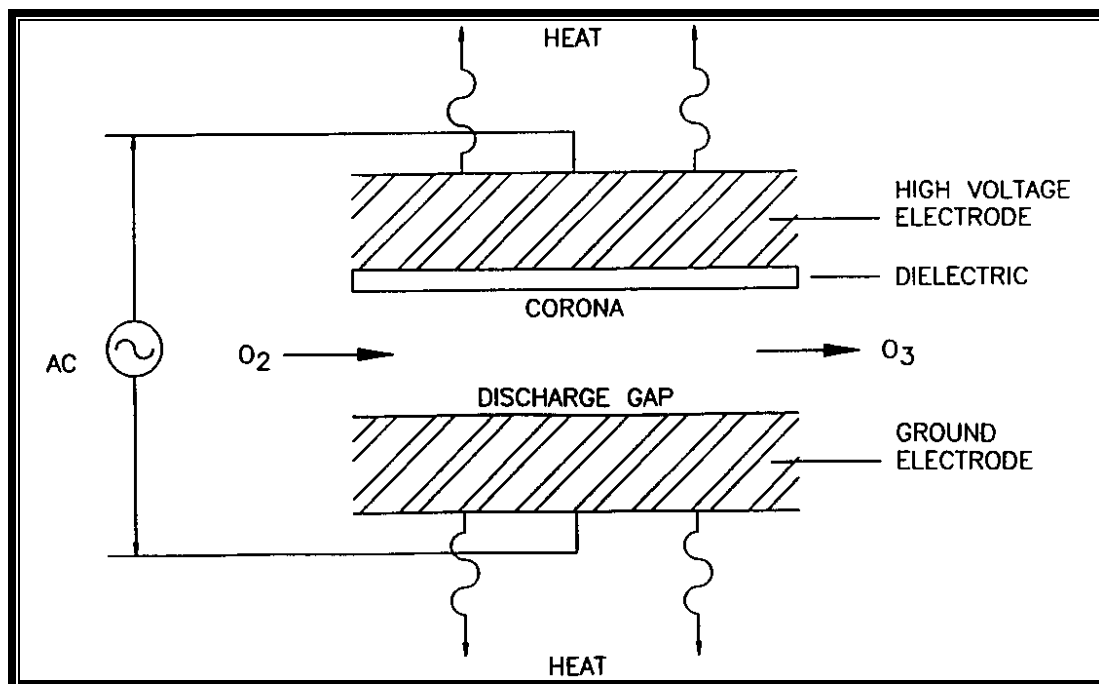
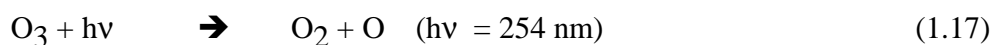


Figure 1.3. Basic Ozone Generators (Bajinath *et al.*, 2001).

### 1.9.3 Generation of ozone with UV

Ultraviolet lamps have been used for decades to generate ozone. This lamp emits UV light at wavelengths of 187 nm and 254 nm. 187 nm light causes reactions which lead to the production of ozone and the 254 nm light causes photo-dissociation of ozone as shown in equation 1.17 below. The net result is that only a small amount of ozone is produced.



This is a much less expensive system than corona discharge, although lower concentrations of ozone are formed. Air is passed through a quartz sleeve with UV light shone through the quartz into the air. Quartz is used as it is transparent to UV whereas glass and plastic absorb UV (EPA, 1999).

Production of ozone with UV has the advantage of not requiring air to be dried. UV units are also easy to control as the rate of ozone production is linearly related to the power

applied to the lamp. Low production of ozone limits this application to small systems. It is however a far cheaper system as it requires no air preparation and the UV equipment is less expensive than corona discharge.

Low production of ozone in UV is due to the wavelengths of light generated by low pressure mercury lamps which are used as the UV light source. Future developments in UV lamps may lead to improved efficiency.

#### **1.9.4 Electrolysis**

The electrolysis process consists of electrodes placed in water dosed with an acidic electrolyte. Ozone is produced at the anode by the oxidation of water and oxygen is reduced at the cathode to produce water. To date practical problems related to power consumption have limited this technology. It is possible in the near future that this process will develop to the stage of being economically feasible.

#### **1.10 Ozone Generators**

The voltage required to produce ozone by corona discharge is proportional to the pressure of the source gas in the generator and the width of the discharge gap. Theoretically, the highest yield (ozone produced per unit area of dielectric) would result from a high voltage, a high frequency, a large dielectric constant, and a thin dielectric (EPA Guidance Manual, 1999). However, there are practical limitations to these conditions. As the voltage increases, the electrodes and dielectric materials are more subject to failure due to thermal corrosion. Operating at higher frequencies produces higher concentrations of ozone and more heat requiring increased cooling to prevent ozone decomposition. Thin dielectrics are more susceptible to puncturing during maintenance. The design of any commercial generator required a balance of ozone yield with operational reliability and reduced maintenance.

## **1.11 Determination of Ozone concentrations**

Literature survey shows that many previous attempts were made to measure gaseous ozone. All have various shortcomings and demonstrate the past degree of difficulty in developing methods that have been used in monitoring gaseous ozone as summarized below (Bajinath and Jonnalagadda, 2001).

### **1.11.1 Iodometric methods**

Iodometric procedures have been used for all of the ozone concentration ranges encountered in water treatment plants (Gordon *et al.*, 1992). This includes measurement of ozone directly from the generator and of ozone as stripped from aqueous solution. For the iodometric method, the ozone containing gas is passed into an aqueous solution containing excess potassium iodide. Any oxidizing materials act as interferences with iodometry (Gordon *et al.*, 1992). Nitrogen oxides (that may be present when ozone is generated in air) also act as interferences to iodometric methods. The effects of nitrogen oxides may be eliminated by passing the ozone containing gas through absorbents such as potassium permanganate that are specific for nitrogen oxide gases. However, no iodometric method is recommended for the determination of ozone in solution because of the unreliability of the method (Gordon *et al.*, 1989).

### **1.11.2 Chemiluminescence**

Chemiluminescence methods can be used for the determination of low concentration of ozone in the gas phase (Gordon *et al.*, 1992). One of the most commonly used methods is ethylene chemiluminescence. Gas-phase ozone can be measured using the chemiluminescence reaction between ethylene and ozone. This method is specific to ozone and is suitable for measurement of ozone in the ambient air. The ethylene chemiluminescence procedure was adopted in 1985 by the EPA as its reference method for determining ozone in the ambient atmosphere (McKee *et al.*, 1975). Chemiluminescence instruments are approved by the EPA for monitoring ambient ozone concentrations of 0 to

0.5 or 0 to 1.0 ppm by volume. With regular calibration, this type of instrument is capable of providing reliable analysis of any ozone in the ambient air from an ozonation plant. An alternative to ethylene chemiluminescence is rhodamine B/gallic acid chemiluminescence, which avoids the handling of ethylene (Gordon *et al.*, 1992). This alternative method is considerably more complex than the more common ethylene chemiluminescence instruments. The sensitivity of this method tends to drift and a procedure has been developed by which corrections are made for the sensitivity on a frequent basis (Van Dijk *et al.*, 1977). Given the wide availability of ethylene chemiluminescence monitors and their approval by EPA, ethylene monitors should be considered before rhodamine B/gallic acid monitors.

### **1.11.3 Gas-Phase Titration**

Two gas-phase titration methods have been studied as possible calibration methods for ambient ozone analyzer and monitors (Gordon *et al.*, 1992). These procedures are based on titration with nitric oxide and back titration of excess nitric oxide (Rehme *et al.*, 1980). These gas phase titration procedures, evaluated by EPA, were compared with UV absorption and iodometry as calibration methods for ethylene chemiluminescent ambient air ozone analyzers. As a result of these comparisons, UV absorption has been specified as the method of calibration for ambient ozone analyzers. Therefore, gas-phase titration methods are not recommended for use at ozonation facilities (Gordon *et al.*, 1992).

In this study Potassium Iodide Method was followed because of its simplicity, rapidness, and sensitivity and it is also very easy to use.

## **1.12 Primary Use and Points of Application of Ozone in water treatment**

In potable water treatment ozone is primarily used as an oxidant to improve the removal of undesirable reductants. Ozone is also used as a disinfectant but usually another disinfectant is subsequently required to establish a stable residual for distribution systems. Chlorine, chloramine or chlorine dioxide, are typically used (EPA Guidance Manual, 1999).

### 1.12.1 Oxidation of Taste and Odour Compounds

Several studies using natural waters have shown ozone to be quite effective at removing Geosmin and 2-methylisoborneol (2-MIB) (HDR Engineering, Inc, 2002). These aliphatic molecules are produced by microbial activity in eutrophic waters and are responsible for many of the earthy, musty odours found in some drinking water supplies (HDR Engineering, Inc, 2002). The OH radical is much more powerful oxidant than the O<sub>3</sub> molecule, especially towards these organisms; Geosmin and MIB. They can have a detrimental effect on the aesthetics of water at threshold concentrations of 4 ng/l for Geosmin and 8.5 ng/l for 2-MIB. Ozone on its own or followed by GAC treatment can be very effective in the removal of tastes and odors caused by these organisms. Ozone is used to oxidize/destroy taste and odour-causing compounds because many of these compounds are very resistant to oxidation. Suffet *et al.* (1986) confirmed that ozone is an effective oxidant for use in taste and odor treatment. They found ozone doses of 2.5 to 2.7 mg/L and 10 minutes of contact time (ozone residual 0.2 mg/L) significantly reduced taste and odours in the specific waters they tested. Most early U.S. water plants (i.e., 1940-1986) installed ozonation specifically for taste and odour removal.

### 1.12.2 Control of algae

The algae are a nuisance organism in water treatment plants, tending to impair the efficiency of treatment (Plummer, 1999). However ozone is effective in the destruction of this organism in water treatment systems.

### 1.12.3 Removal of Blue-Green Algae (Cyanobacteria) Toxins.

Toxic cyanobacteria in regions, which have had blooms of these organisms in their water supply, pose a significant health risk (Hoeger *et al.*, 2002). Break-point chlorination is typically used as a pre-oxidant in water treatment for the destruction of algal toxins. The use of chlorine in a pre-oxidation step has the disadvantage of causing high THM and other chlorinated byproducts. Ozone has been found to be particularly effective in destroying the peptide hepatotoxin Microcystin-LR which is produced by *Microcystis* cyanobacteria

which frequent eutrophic waters. It also apparently destroys cylindrospermopsin, a toxin from *Cylindrospermopsis* (Hoeger *et al.*, 2002).

#### **1.12.4 Colour removal**

The colour is mainly caused by light absorption on conjugated bonds. Colour removal is achieved by oxidation of the responsible organic molecules. These bonds are easily oxidized by ozone resulting in the disappearance of color (Langlais *et al.*, 1991).

#### **1.12.5 Microflocculation**

The microflocculation is caused by particle destabilization and is dependant on the carbonate content of the water and the organic content. Improved filter run lengths, and reduced coagulation demand are the benefits of microflocculation. Reduction in the required doses of coagulant and flocculant has been observed in Queensland coastal waters. A further subsequent benefit of microflocculation is a reduced production of sludge, reducing the load on clarifiers and potentially opening the door to direct filtration.

#### **1.12.6 Improved biodegradability**

The oxidation of organics in water by ozone will convert many non-biodegradable organic molecules into a more biodegradable form. If followed by some type of biological process this could result in a decrease in the total organic carbon (TOC) of the water (Bezbarua and Reckhow, 2004). Care must be taken to remove the new biodegradable organics. Ozone biodegradation will result in an increase in BOD without having changed the TOC of the water. If provision for biodegradation (e.g. biological activated carbon, or biofiltration), ozonation is a benefit to the removal of organics. Without this provision ozonation can be a liability with respect to promoting biological regrowth within the distribution system.

#### **1.12.7 Iron and Manganese Removal**

Ozone oxidizes iron and manganese, converting ferrous  $2^+$  iron into the ferric  $3^+$  state and  $2^+$  manganese to the  $4^+$  state. The oxidized forms will precipitate as ferric hydroxide and manganese hydroxide (AWWA, 1990).

Iron and manganese can impart undesirable colour to water, causing aesthetic problems for water suppliers. Iron and manganese form in impoundments when anoxic conditions occur. During seasonal turnover these cations become mixed into throughout the reservoir, and eventually to the consumers' taps this in turn results in consumer complaints and a general dissatisfaction with the water utility.

### **1.13 Other Potential Uses of Ozone**

The most important applications of ozone in wastewater treatment are disinfection and sludge bulking control. Tertiary treatment of wastewater is closely related and similar to potable water treatment. Other uses of ozone include cooling water treatment, swimming pool disinfection, advanced oxidation, biological growth enhancement and industrial applications (Guzel-Seydim *et al.*, 2004).

#### **1.13.1 Wastewater disinfection**

Although ozone has been a popular and successful disinfectant for drinking water, it has not been widely used for wastewater disinfection due to operation and maintenance problems of first generation systems, as well as the high ozone demand of many effluents (Robson *et al.*, 1991; Xu *et al.*, 2002). Several smaller plants in the US, Canada, Japan, Korea and Europe (Paraskeva *et al.*, 2002; Larocque, 1999) are currently using ozone for wastewater disinfection. The kinetics of ozone reactions has received the attention of many researchers. The kinetics is complex because of the different reaction rates with different chemicals in solution, speciation of the ozone and its decomposition products, and the interaction of these with the microorganisms. It is well accepted that ozone is effective against all organisms likely to be encountered in wastewaters, including viruses and protozoan cysts. Even organisms resistant to chlorination, such as poliovirus Type 3, as well as *Cryptosporidium* and *Giardia* protozoa, can be inactivated by ozone at residual concentrations of 1 mg/L or even less, and sufficient contact time (several minutes) (Lazarova *et al.*, 1998 ; Water Environmental Federation, 1996).

### **1.13.2 Sludge bulking control with ozone**

Filamentous bacteria cause foaming and sludge bulking which occur in most activated sludge plants around the world, severely affecting solids separation. Ozone is used to destroy the exposed ends of filamentous bacteria in flocs which assist in settlement. The advantage of ozone over disinfectants with a residual is that the ozone will destroy filamentous bacteria projecting from the floc without penetrating which would destroy all bacteria within the floc. High doses of residual disinfectants such as chlorine can penetrate the floc destroying bacteria inside the floc.

### **1.13.3 Ozone in the water reclamation process**

The aim of water reclamation is to reuse water from treated sewage effluent for another purpose. Some applications such as irrigation or industrial reuse require a single disinfectant to ensure that human contact with the water is safe from pathogenic contamination, whereas uses such as garden watering and drinking water require multiple barriers to infection. The benefit of ozone in multiple barrier water reuse systems is that ozone works to disinfect water by a considerably different route to chlorine disinfectants.

### **1.13.4 Biological Activated Carbon (BAC)**

The combination of ozonation and granular activated carbon (GAC) is commonly referred to as the biological activated carbon (BAC). GAC has been used extensively for the removal of dissolved organics from drinking water. This process is used in swimming pool water treatment, potable water purification and in wastewater treatment (Scranton Gillette Communications, Inc. 2008).

### **1.13.5 Cooling Water Treatment**

Ozone is used as an effective disinfectant for high recirculation cooling water systems such as air conditioning. High water temperatures can quickly deactivate ozone and temperatures below 30°C are recommended. Cooling towers can strip ozone from the water during atmospheric exposure.

### **1.13.6 Swimming Pool and Spa Water Treatment**

Swimming pool disinfection by ozone has become a very important application of ozone (Tamir, 1989). The objectives of swimming pool disinfection are primarily to remove pathogenic organisms, secondarily to reduce odours, colour and irritation of swimmers. The tertiary aim is to reduce aggressivity and corrosivity of the water. These objectives relate to all activities where the body comes in contact with the water such as spas and water slides. When ozone is used prior to chlorination many of the impurities are partially oxidized which reduces the chlorine demand. Chlorine is then only required for the residual disinfection which considerably reduces the problems with chlorination.

### **1.13.7 Use of ozone in the fish industry**

One very interesting application of ozone that has been reported is the use of ozonated ice for the preservation of fish. The ozone in the frozen water appears to be unusually stable. Improvement of the microbial standard of inlet water and stringent microbiological restrictions on effluent water are in many cases needed to control diseases in the fish farming industry (Liltved *et al.*, 1995).

## **1.14 Industrial Applications of ozone**

### **1.14.1 Pulp and Paper Treatment**

Ozone is a very powerful oxidizing agent and the biggest challenge in using it to bleach wood pulp is to get selectivity so that the desirable cellulose is not degraded. Ozone reacts with the carbon carbon double bonds in lignin, including those within aromatic rings. Ozone is used in pulp and paper plants around the world as a means of reducing the requirement for chlorine bleaching and hence reducing the toxicity of effluents.

### **1.14.2 Cyanide and thiocyanates oxidation by Ozone**

Ozone could also be used to oxidize cyanides and thiocyanates, both occurring in high concentrations in coke oven wastewaters. The use of ozone for the treatment of cyanidation effluents emerges as a promising alternative that can now be seriously considered, (Ordenez *et al.*, 2005). These substances are more economically removed by biological treatment, but at concentrations of hundreds mg/L these substances become toxic to the biological process. Ozone is a good candidate for removing these substances (Nava, 2003).

### **1.14.3 Mining Service Water Treatment**

A pilot plant investigation into mine service water treatment with ozonation was carried out on 4 ML/d scale at Kloof Gold Mine. Although this was not a potable treatment situation, the aim was still to achieve disinfection as underground workers drink the water. It was found that ozonation was highly suitable and economical in a situation where high nitrite and ammonia levels were encountered due to the use of explosives (Jooste *et al.*, 1994).

#### **1.14.4 Advanced Oxidation Processes (AOP)**

The combination of drugs in order to obtain an effect greater than that of any compound taken alone has been practiced for generations (Dahi, 1976). In a similar manner, combination of two or more disinfection techniques has also been applied to water treatment. Hybrid techniques employ the combination of various oxidation techniques, which can result in the generation of sufficient hydroxyl radicals and their oxidizing potential for water purification. These processes are known as the advanced oxidation processes. Advanced oxidation systems generally combine ozone, ultra violet irradiation, hydrogen peroxide, higher pH values, ultrasound, and titanium dioxide e.g.  $O_3$  and  $h\nu$ ;  $O_3 + H_2O_2$ ;  $O_3$  at  $pH > 8.5$ ;  $O_3 + TiO_2$ ; and  $O_3 +$  ultrasound. Advanced oxidation has been found very effective at destroying a range of organic contaminants in water including petrochemicals and pesticides (Jyoti *et al.*, 2003).

#### **1.14.5 Basic of ozone applications for post-harvest treatment of fresh produce**

In a lengthy self-affirmation and extensive petition process, an expert advisory panel asserted the determination that ozone qualified for listing as a generally recognized safe material. Ozone-based treatment of fresh vegetables and fruits had been used within the post-harvest handling industry for decades. Relatively few produce handlers and processors have used ozone for water disinfection, surface sanitation, cold room air treatment, and other post-harvest applications such as final rinses of whole, trimmed-in-the-field, peeled, or minimally processed produce. Ozone has been approved for use under good manufacturing practices, meaning “exposure of foods to sufficient ozone to accomplish its intended purpose.” This translates to the minimum exposure of fruits and vegetables to that dose of ozone necessary to provide the target antimicrobial benefits on specific edible horticultural commodities.

#### **1.15 Physical and Chemical Properties of Ozone**

Ozone is a pale blue gas at ordinary temperatures; it naturally occurs in troposphere and it is formed from nitrogen oxides and organic oxides emitted as a product of combustion

engines or from passing an electric current through air. Table 1.2 summarises the oxidation potentials of various normally used oxidizing agents and shows that  $O_3$  has much high oxidizing capability compared to  $MnO_4^-$ ,  $ClO_2$ ,  $HOCl$  and  $Cl_2$ .

**Table 1.2. Oxidizing agents and their oxidation potential** (Manley *et al.*, 1967)

Oxidising agent	Oxidation potential (mV)
Flourine	3.06
Ozone	2.07
Permanganate	1.67
Chlorine dioxide	1.50
Hypochlorous acid	1.49
Chlorine gas	1.36

### 1.16 Ozone Disinfection Considerations

The following table presents a summary of the considerations for the use of ozone as a disinfectant.

**Table 1.3 Summary of Ozone Disinfection Considerations (EPA, 1999)**

Considerations	Description
Generation	Because of its instability, ozone should be generated at the point of use. Ozone can be generated from oxygen present in air or high purity oxygen. The feed gas source should be clean and dry, with a maximum dewpoint of $-60^{\circ}\text{C}$ . Ozone generation consumes power at a rate of 8 to 17 kWhr/kg $\text{O}_3$ . Onsite generation saves a lot of storage space.
Primary uses	Primary uses include primary disinfection and chemical oxidation. As an oxidizing agent, ozone can be used to increase the biodegradability of organic compounds destroys taste, and odor control, and reduce levels of chlorination DBP precursors. Ozone should not be used for secondary disinfection because it is highly reactive and does not maintain an appreciable residual level for the length of time desired in the distribution system.
Inactivation efficiency	Ozone is one of the most potent and effective germicide used in water treatment. It is effective against bacteria, viruses, and protozoan cysts. Inactivation efficiency for bacteria and viruses is not affected by pH; at pH levels between 6 and 9.
Points of application	For primary disinfection, ozone addition should be prior to biofiltration/filtration and after sedimentation. For oxidation, ozone addition can be prior to coagulation /sedimentation or filtration depending on the constituents to be oxidized.
Safety considerations	Ozone is a toxic gas and the ozone production and application facilities should be designed to generate, apply, and control this gas, so as to protect plant personnel. Ambient ozone levels in plant facilities should be monitored continuously.
Byproduct formation	Ozone itself does not form halogenated DBPs however if bromide ion is present in the raw water or if chlorine is added as a secondary disinfectant, halogenated byproducts including bromate ion may be formed. Other ozonation byproducts include organic acids and aldehydes.

## **2.1 Experimental material**

### **2.1.1 Preparation of equipment**

#### **Autoclave**

All solutions, media and glassware were sterilized using the Speedy autoclave (Model HL 340).

#### **Colony Counter**

The colonies on culture dishes were counted employing Suntex colony counter (Model 560) after incubation.

#### **Glassware**

All glassware were cleaned using detergent and rinsed in deionised double distilled water (ddH<sub>2</sub>O). The glassware used for disinfection experiments was sterilized at 121°C for 15 minutes.

#### **Incubator**

Heraeus incubator was used to incubate *E. coli* at 37±0.5°C, *P. aeruginosa* at 37±0.5°C and *B. subtilis* at 37±0.5°C with a high level of humidity. KIC refrigerator was used to store microorganisms, petri dishes, and solutions at approximately 5°C.

#### **Deionized water**

Deionized distilled water was obtained from Millipore (Modulab, water systems Model LABDI 20 2200) and was used for all disinfection experiments in the laboratory.

#### **Ozone Generator**

A Prosep Systems equipment used for the generation of ozone utilises the corona discharge to convert oxygen to ozone. The gas emerging from the instrument contained a mixture of ozone and oxygen.

#### **Petri Dishes**

Petri dishes (90 mm) were used to prepare the culture dishes for incubation of *E. coli*, *P. aeruginosa* and *B. subtilis* using aseptic procedures.

## pH Meter

The pH measurements were made using the Crison pH meter (Model Micro pH 2000).

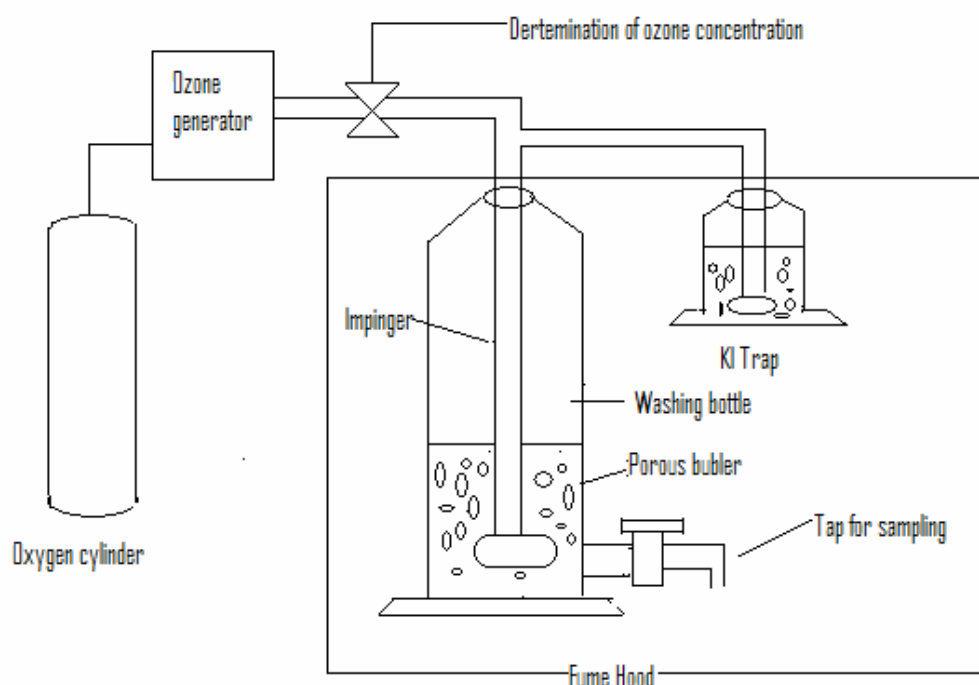
## Pipettes

Sterile disposable pipettes were used for sampling and preparing dilution series.

## 2.2 Preparation of the bacteria culture

All the strains of Gram-negative bacteria, *Escherichia coli*, and *Pseudomonas aeruginosa*, and a Gram-positive bacterium, *Bacillus subtilis* were obtained from the School of Biochemistry, Genetics, Microbiology and Plant Pathology at Westville campus, University of KwaZulu-Natal. The bacterial isolate was inoculated into the nutrient broth using a sterilized loop and was allowed to grow for 24 hours at 37°C.

## 2.3 Instrumental setup



**Figure 2.1** Apparatus set-up used for disinfection experiments

The apparatus consists of 1 L gas washing bottle with a tap for sampling at different time intervals. The oxygen stream containing ozone is bubbled into the bacteria suspension through an impinger. The impinger consists of one inlet and one outlet. The amount of ozone in the system is regulated in the generator by adjusting the voltage of the current or the flow rate of the oxygen into the instrument. The flow rate of the incoming oxygen stream ranged from 2 L/min to 9 L/min and the voltage applied ranged from 40 V to 80 V.

4.0 ml of 24 hr culture was transferred into 400 ml sterilized (autoclaved) distilled water and oxygen stream containing different concentrations of ozone gas was bubbled for 6 to 30 minute duration depending on the experimental requirement. The samples were normally collected at 1 minute intervals into sterile test tubes for further analysis of either ozone or microbial count.

## **2.4 Experiments for the calibration of the ozone generator**

### **2.4.1 Reagents**

Potassium iodide (KI) (Merck), sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) (Merck), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) (Aldrich), sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) (Aldrich), starch and all other reagents used for the study were of Analar grade, and all solutions prepared using ddH<sub>2</sub>O.

### **2.4.2 Stock Solutions**

2% KI solution was prepared by dissolving 20 g of KI in 1000 ml ddH<sub>2</sub>O.

1 M  $\text{H}_2\text{SO}_4$  was prepared by diluting 13.60 ml of 18.38 M  $\text{H}_2\text{SO}_4$  to 250 ml ddH<sub>2</sub>O and after 24 h, the solution was standardized using primary standard, sodium tetraborate (0.510 M).

0.10 M Standard  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  was prepared by dissolving 24.818 g  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$

in 1000 ml ddH<sub>2</sub>O.

0.01667 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was prepared by dissolving 4.904 g anhydrous K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> into 1000 ml ddH<sub>2</sub>O.

Starch indicator solution: To 0.125 g of starch approximately 0.5-1 ml cold water was added and ground in a mortar to a thin paste. The mixture was then poured into 25 ml of boiling distilled water, while stirring. The solution was kept in the refrigerator when not in use.

## **2.5 Methods**

### **2.5.1 Standardization of sodium thiosulfate solution by Dichromate Method**

To 80 ml of distilled water, 1 ml of 18.38 M H<sub>2</sub>SO<sub>4</sub>, 10.00 ml  $1.667 \times 10^{-2}$  M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, and 1.0 g KI were added with constant stirring. The resulting solution was titrated immediately with 0.10 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> as titrant until the yellow colour of the iodine was almost discharged. 1 ml of starch indicator solution was added and the titration was continued until the blue colour had just disappeared.

### **2.5.2 Calibration of the ozone generator.**

The gaseous mixture consisting of ozone and oxygen was bubbled for 2 minutes into 200 ml 2% KI solution. This procedure was repeated using different voltages on the ozone generator, i.e., different ozone concentrations. All experiments were carried out at a flow rate of 2 L/min. After the absorption of the ozone, 20 ml of 1.0 M H<sub>2</sub>SO<sub>4</sub> was added to reduce the pH below 2. The resulting yellow solution (20 ml) was then titrated with 0.05 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (standardized using dichromate) until the yellow colour of the liberated iodine almost was discharged. At this stage 2 ml of starch indicator solution was added and titration continued carefully but rapidly to the end point, at which the blue colour just disappeared (Standard Methods for the examination of Water and Wastewater, 1975).

### 2.5.2.1 Blank Test

To 200 ml 2% KI solution, 20 ml 1.0 M  $\text{H}_2\text{SO}_4$  was added. To 20 ml of this solution, 2 ml of starch indicator was added (Baijnath *et al.*, 2001).

- 1) If a blue colour appeared, the solution was titrated with 0.005 M  $\text{Na}_2\text{S}_2\text{O}_3$  titrant until the blue colour just disappeared.
- 2) If no blue color appeared, the solution was titrated with 0.005 M iodine solution until a blue colour appeared. The solution was then back-titrated with 0.005 M  $\text{Na}_2\text{S}_2\text{O}_3$  to the disappearance of the blue colour.

In case one, the results were subtracted from the original volume required for the sample titration. In case two, the results were added to the original volume required for the sample titration. In most experiments no blue colour appeared with the blank.

## 2.6 Enumeration of the bacteria culture

Using sterile micropipettes, 1.0 ml of the ozonated sample was sequentially diluted into 9.0 ml of diluents. 0.1 ml of each ozonated suspension was spread evenly over the nutrient agar-plate in triplicate. After incubation at 37°C for 24 hours the total viable count (CFU/ml) of the isolate was determined by the spread plate technique as described by Balows *et al.*, 1992.

## 2.7 Monitoring the microbial inactivation

### 2.7.1 Reagents

Nutrient agar (Merck), nutrient broth (Merck), dipotassium hydrogen orthophosphate anhydrous ( $\text{K}_2\text{HPO}_4$ ) (Aldrich), and potassium dihydrogen orthophosphate anhydrous ( $\text{KH}_2\text{PO}_4$ ) (Aldrich) were used. All solutions were prepared using dd $\text{H}_2\text{O}$ .

### 2.7.2 Stock Solutions

*Nutrient Agar:* This medium was prepared by dissolving 31 g of nutrient agar powder in 1 L of dd H<sub>2</sub>O and boiling for 1 minute to dissolve completely. It was then sterilized at 121°C for 15 minutes. The final pH was 6.8±0.2 at 25°C.

*Nutrient Broth:* This medium was prepared by dissolving 4.89 g of nutrient broth powder in 350 ml of dd H<sub>2</sub>O. The solution was sterilized at 121°C for 15 minutes. The final pH was 6.8±0.2 at 25°C.

1.0 M K<sub>2</sub>HPO<sub>4</sub>: This solution was prepared by dissolving 174.18 g K<sub>2</sub>HPO<sub>4</sub> in 1 L of dd H<sub>2</sub>O. The solution was sterilized at 121°C for 15 minutes.

1.0 M KH<sub>2</sub>PO<sub>4</sub>: This solution was prepared by dissolving 136.09 g KH<sub>2</sub>PO<sub>4</sub> in 1 L of ddH<sub>2</sub>O. The solution was sterilized at 121°C for 15 minutes.

### 2.7.3 Method

All glassware were washed with deionized distilled water and autoclaved at 121°C for 15 minutes. The inactivation experiments were conducted at two temperatures, 5°C and 25°C and at various pH values of 5.0, 6.0, 7.0, 8.0, and 9.0 in deionised distilled water. The pH was controlled with 0.1 M phosphate buffer solution. At each pH, the inactivation experiments were performed in duplicate to determine the reproducibility of the data. All solutions were prepared using ddH<sub>2</sub>O.

## 2.8 Determination of Chemical Oxygen Demand (COD) in Water Samples

For the natural water experiments, water samples from the Msunduzi River from Enkanyezini, Pietermaritzburg in KwaZulu-Natal were used. The flowing water samples were withdrawn along the river banks.

### 2.8.1 Reagents

All reagents used were of AnalaR grade. Iron(II) ammonium sulfate (FAS) ( $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ ) (Merck), potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) (Merck), mercury(II) sulfate ( $\text{HgSO}_4$ ) (Merck), silver sulfate ( $\text{Ag}_2\text{SO}_4$ ) (Merck), Ferroin indicator (1, 10-phenanthroline iron (II) complex) and sulfuric acid were used.

### 2.8.2 Stock Solutions

0.025 M Iron(II) ammonium sulfate:

9.804 g of FAS was dissolved in 1000 ml of double distilled water.

0.02083 M Potassium dichromate solution:

6.128 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  was dissolved in 1000 ml of double distilled water.

20% Mercury(II) sulfate ( $\text{HgSO}_4$ ) in 10 volume per cent sulfuric acid:

20.00 g  $\text{HgSO}_4$  dissolved in 10 ml of concentrated  $\text{H}_2\text{SO}_4$  and made up to 100.0 ml with dd  $\text{H}_2\text{O}$ .

1% Silver sulfate ( $\text{Ag}_2\text{SO}_4$ ) in concentrated sulfuric acid:

1.00 g  $\text{Ag}_2\text{SO}_4$  dissolved in 100 ml of concentrated sulfuric acid.

0.1 % Sulfuric acid:

1 ml of conc. Sulfuric acid (18.38 M) was diluted to 1000 ml of dd $\text{H}_2\text{O}$ . The standardized sulfuric acid stock solution was diluted to 1000 ml using dd $\text{H}_2\text{O}$ .

### 2.8.3 Method

Two reflux condensers were set up side by side, on heating mantles. Two labeled round-bottomed flasks were placed on cork rings and 10.0 ml of water sample was pipetted into one flask and 10.0 ml of ddH<sub>2</sub>O into the other. Into each flask 5.0 ml of 0.02083M potassium dichromate solution was pipetted followed by 1.0 ml of mercury(II) sulfate reagent. Each flask was swirled carefully to ensure thorough mixing. 16.0 ml of 1% silver sulfate in concentrated sulfuric acid was measured out in a measuring cylinder and added in small portions to the sample in the flask. The flask was carefully swirled after each addition. The flask containing ddH<sub>2</sub>O was used as the control. Few glass beads were added to each flask in order to minimize bumping. Each flask was attached to its condenser and secured on the heating equipment. The flasks were then refluxed for 90 minutes. The water-cooling was switched on at all times and the top of the condenser was open to prevent build-up of pressure. After refluxing, the flasks were allowed to cool for 10 minutes and then cooled to room temperature under running water. The inner tube of each condenser was washed with 1% sulfuric acid, collecting the washings in the appropriate round-bottomed flask. The flasks were swirled to ensure thorough mixing. About five drops of Ferroin indicator were added to each flask and the solutions were titrated with 0.025 M FAS until the solution just became pink. The COD value was calculated using the following equation:  $\text{COD (mg dm}^{-3}\text{)} = (T_1 - T_2) \times 0.2 \times 100$ , where  $T_1$  is the titration value for ddH<sub>2</sub>O (ml), and  $T_2$  is the titration value for the water sample (ml).

## **2.9 Determination of Biological Oxygen Demand (BOD) in Water Samples**

### **2.9.1 Reagents**

All reagents were of AnalaR grade. Sodium hydroxide (Merck), sodium iodide (Merck), sodium azide (Merck), Mn(II) sulfate (Merck), sodium thiosulfate (Merck), mercury(II) iodide (Merck) and starch indicator were used.

### **2.9.2 Stock Solutions**

Alkaline iodide solution: 40 g of sodium hydroxide was dissolved in 56 ml of ddH<sub>2</sub>O, then 90 g of sodium iodide was added and the solution was kept hot until the iodide dissolved. The solution was cooled, diluted to 100 ml ddH<sub>2</sub>O, and filtered before use.

2.5 % of Sodium azide solution: 2.5 g of sodium azide was dissolved in 100 ml of ddH<sub>2</sub>O.

Alkaline iodide-azide solution: 100 ml of alkaline iodide solution was mixed with 30 mL of 2.5 % of sodium azide solution.

Manganese(II) sulfate solution: 50 g of manganese(II) sulfate, MnSO<sub>4</sub>·5H<sub>2</sub>O, was dissolved in ddH<sub>2</sub>O, filtered and made up to 100 ml using ddH<sub>2</sub>O.

Sodium thiosulphate: 6.3012 g of sodium thiosulfate pentahydrate Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O, was dissolved in 1 litre of copper-free water, 10 mg of mercury(II) iodide was added to stabilize the solution. The solution was stored in a brown bottle.

Sodium thiosulfate working solution: 0.025 M sodium thiosulfate was diluted to 0.0125 M sodium thiosulfate and stored in a brown bottle as well.

### 2.9.3 Method

Glass bottles (380 ml) were cleaned, rinsed thoroughly, drained and autoclaved at 121°C for 15 minutes. The air was prevented for being drawn into the samples during the incubation at 20°C by placing parafilm over the stopper to reduce evaporation of the water and light was excluded to prevent the possibility of photosynthetic production of dissolved oxygen.

25 BOD bottles were used for samples and the values of BOD were obtained for 5 samples for each day. The 5 samples for each day consisted of the following:

- Bottle A: River water
- Bottle B: River water spiked with 1 ml *E. coli*
- Bottle C: River water spiked with 1 ml *P. aeruginosa*
- Bottle D: River water spiked with 1ml *E. coli* and ozonized
- Bottle E: River water spiked with 1 ml *P. aeruginosa* and ozonized

The BOD values of the river water samples were obtained as follows:

On each day 1.0 ml manganese(II) sulfate solution was added to the samples followed by 1.0 ml alkali-iodide-azide reagent and sealed carefully with a stopper to exclude air bubbles and mixed by inverting the bottle a few times. When the precipitate had settled sufficiently to leave clear supernatant above the manganese hydroxide floc, 1.0 ml conc. sulfuric acid was added. It was then restoppered and mixed by inverting several times until dissolution was complete. 50 ml of sample was titrated with 0.0125 M sodium thiosulfate solution to a pale straw colour. Three drops of starch solution were added and the titration continued to first disappearance of blue colour.

## 3.0 Results and Discussion

A number of inactivation experiments on *E. coli*, *P. aeruginosa* and *B. subtilis* were performed in the laboratory under different conditions. Colony forming unit (CFU) is a measure of viable bacterial cells and expressed as CFU/ml, colony forming units per milliliter, which represents the degree of contamination in water samples. Most of the experiments were conducted with initial microbial density  $\approx 10^7$ - $10^8$  CFU/ml and at temperature =  $25 \pm 2$  °C. All the inactivation experiments were performed in duplicate.

The concentration of ozone in the oxygen stream is the essential parameter in the inactivation studies. The capability of a corona discharge of the ozone generator depends upon the voltage settings of the instrument, while the concentration of the ozone depends on various conditions. The most important factors are the flow rate of the air or oxygen and the operating voltage setting of the generator. If compressed air is used in place of oxygen, there is a possibility for formation of nitrogen oxides along with ozone. Hence to avoid such contamination, only pure compressed oxygen was used for all the experiments.

### 3.1 Calibration of the ozone generator

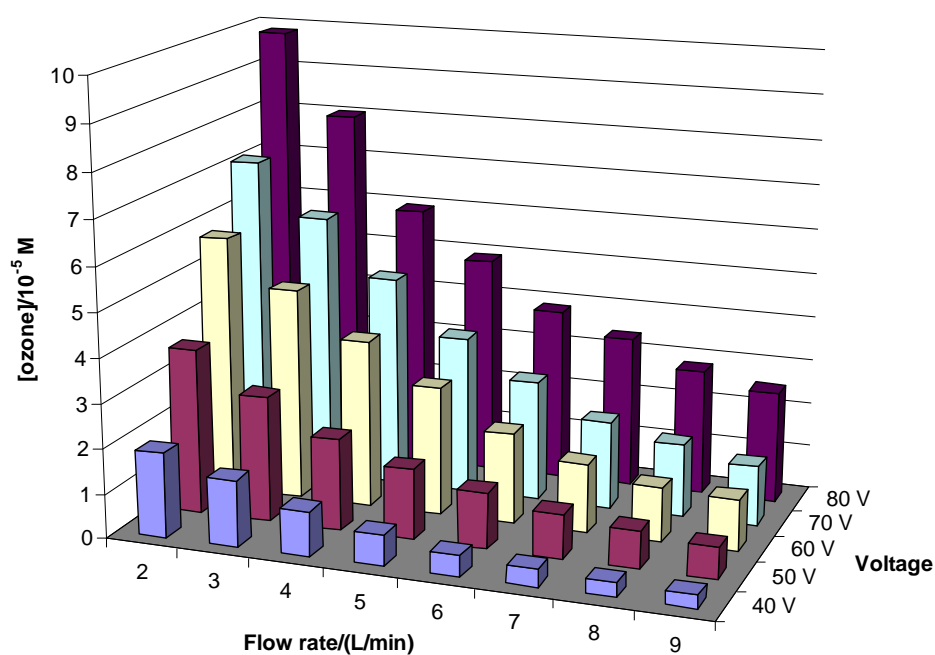
To establish the actual concentrations of ozone in the oxygen stream after ozonation, the ozoniser instrument was calibrated for various voltage settings and as function of the oxygen flow rates. The mean values of the results obtained for triplicate experiments are summarized, the concentrations expressed as moles/L in Table 3.1. In most of the discussion  $[O_3]$  is described as moles/L. An examination of the tabulated results indicates that, for a given voltage, an increase in oxygen flow rate results in a decrease of ozone concentration per unit volume of oxygen. For a given flow rate, an increase in voltage enhanced the ozone concentration and the increased flow rates for any fixed voltage decreased the ozone levels in the sample.

**Table 3.1 Relation between ozone concentration ( $10^{-5}$  M) and oxygen flow rate (L/min) at different voltages (V)**

Voltage →	40 V	50 V	60 V	70 V	80 V
-----------	------	------	------	------	------

Flow rate, L/min	[Ozone]/10 <sup>-5</sup> M				
2	1.888	3.710	5.794	7.169	9.842
3	1.477	2.810	4.750	6.015	8.023
4	0.998	2.050	3.769	4.750	5.938
5	0.704	1.581	2.888	3.529	4.913
6	0.465	1.258	2.039	2.706	3.865
7	0.392	0.981	1.531	1.973	3.402
8	0.325	0.838	1.218	1.642	2.821
9	0.289	0.694	1.156	1.352	2.506

n = 3, coefficient of variance < 4%



**Figure 3.1 3-D plot of ozone concentrations as function of flow rates and instrument set potentials**

The profiles of the ozone concentrations as function of both varying voltage and the flow rates are illustrated in Figure 3.1. At a fixed voltage of 40V, as the oxygen flux was

increased from 2 L/min to 9 L/min, the concentration of ozone in the gas stream was decreased from 1.89 to  $0.29 \times 10^{-5}$  M

Figure 3.1 illustrates that at a fixed flow rate (2 L/min), as the voltage of ozone generator was increased from a voltage of 40 V to 80 V, the ozone concentration also increased from 1.89 to  $9.84 \times 10^{-5}$  M respectively. A positive and linear relationship is observed between voltage change and ozone concentration in the oxygen stream.

A lower ozone concentration was observed at higher flow rates. Higher flow rate tends to give less residence time for ozone in the aqueous mixtures containing the samples. Ozone transfer from the gas phase to aqueous phase will be efficient with smaller bubble size and lower flow rate, which will give more residence time for ozone in water. Smaller bubbles are preferable because of high surface to volume ratio- more ozone in direct contact with water which can be achieved by controlling the pore size of sintered bubbler at the tip of the impinger (Rasplicka, 1993).

### 3.11 Inactivation of *Escherichia coli*

*Escherichia coli* was selected as the test organism in the current studies, due to its wide use and accepted relevance as an indicator of pathogenic contamination in drinking water as well as wastewater (Elmund *et al.*, 1999).

#### **Table 3.2 Control experiments for inactivation of *E. coli* without O<sub>3</sub>**

*E. coli* =  $10^8$  CFU/ml; Ozone = Nil; Flow rate 2 L/min. Temperature =  $25 \pm 2$  °C

Time/min	Log CFU/ml
0	8.3605
3	8.3675
6	8.3461
9	8.3581

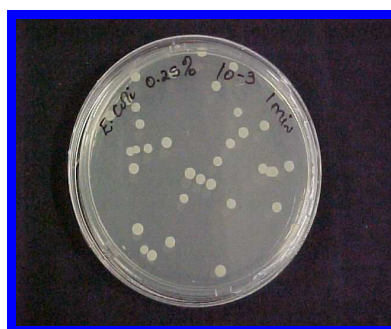
No significant observable change in the *E. coli* count during the experiment proves that in the absence of ozone, inactivation due to oxygen bubbled through the solution is negligible.

### 3.12 Effect of ozone concentration on inactivation of *Escherichia coli* population

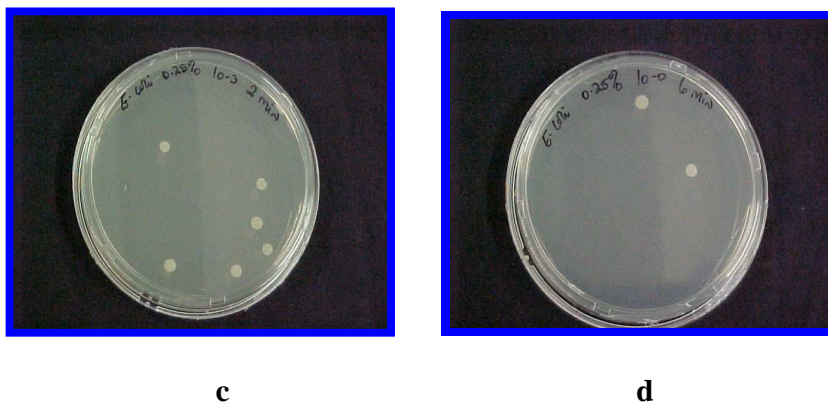
Typical example of the effect of ozone concentration of  $1.89 \times 10^{-5}$  M with 2 L/min flow rate at 40 V on *Escherichia coli* population of  $10^7$  CFU/ml grown on a nutrient agar plate is shown in Figure 3.2 a, b, c and d overleaf.



a



b



**Figure 3.2. Effect of ozone concentration on *Escherichia coli* population**  
 $[O_3] = 1.89 \times 10^{-5} \text{ M}$ ; *E. coli* =  $10^8 \text{ CFU/ml}$ . Flow rate = 2 L/min; Temperature =  $25 \pm 2 \text{ }^\circ\text{C}$ . **a:** 0 min, **b:** 1 min, **c:** 2 min, **d:** 6 min. Dilution factor = **a:** 1000, **b:** 1000, **c:** 1000, **d:** stock.

*E. coli* inactivation experiments were conducted with the microbe concentration of  $10^8 \text{ CFU/ml}$  and the ozone concentrations in large excess greater than  $10^{16}$  ozone molecules/ml. The depletion of microbe population was monitored as function ozonation time. The inactivation experiments were repeated with different ozone concentrations in the oxygen bubbled through the aqueous solution containing microbes.

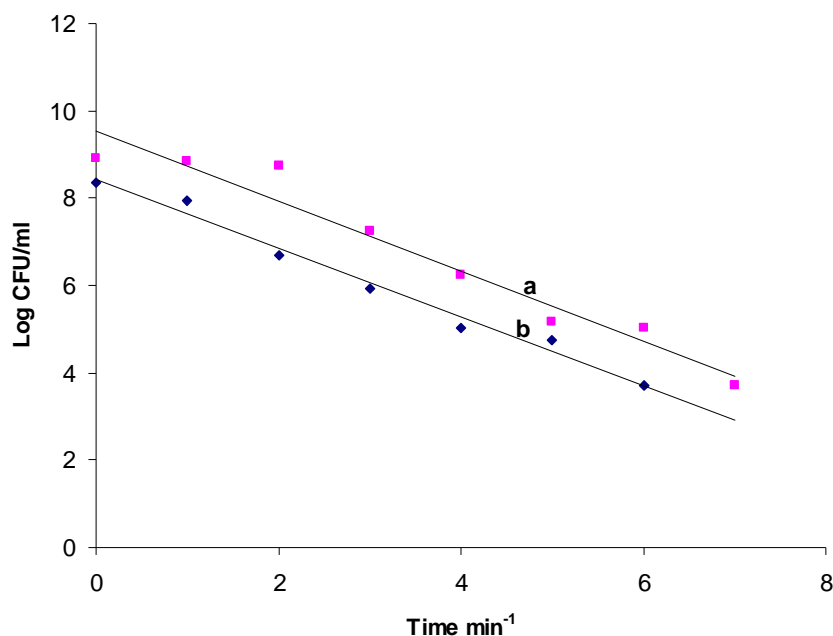
Experiments were conducted two different initial concentration of *E. coli* under otherwise identical conditions. The results obtained are summarized in Table 3.3 overleaf.

**Table 3.3 Log CFU/ml at two dilutions of *E. coli* as a function of time**

Ozone =  $1.89 \times 10^{-5}$  M. Flow rate 2 L/min; Temperature =  $25 \pm 2$  °C.

Time/ min	$2.318 \times 10^8$	$8.108 \times 10^8$
	CFU/ml	CFU/ml
	Log CFU/ml	Log CFU/ml
0	8.3652	8.9089
1	7.9282	8.8552
2	6.6978	8.7499
3	5.9408	7.259
4	5.0302	6.2531
5	4.7391	5.1516
6	3.6972	5.0392
7		3.6968

n = 2 and relative variance coefficient < 5%

**Figure 3.3 Log CFU/ml versus time plots**

Flow rate = 2 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M and  $[E. coli]/10^8$  CFU/ml = Curve a: 8.108 and b: 2.318.

**Table 3.4 Pseudo first-order rate constants (k') for two dilutions of *E. coli***

[ <i>E. coli</i> ]/ 10 <sup>8</sup> CFU/ml	k'/min <sup>-1</sup>
2.318	1.81 ± 0.08
8.108	1.85 ± 0.08

n = 2 and relative variance coefficient < 5%

The *pseudo* first-order rate constants were found to be similar as presented in Table 3.4 and illustrated in Figure 3.3 for both dilutions of the *E. coli*, implying that the rate of inactivation of the microbes by ozone is independent of the initial concentration of the bacteria and the inactivation follows first-order kinetics with respect to *E. coli*.

### 3.13 Kinetics of survival of *Escherichia coli* at different ozone concentrations

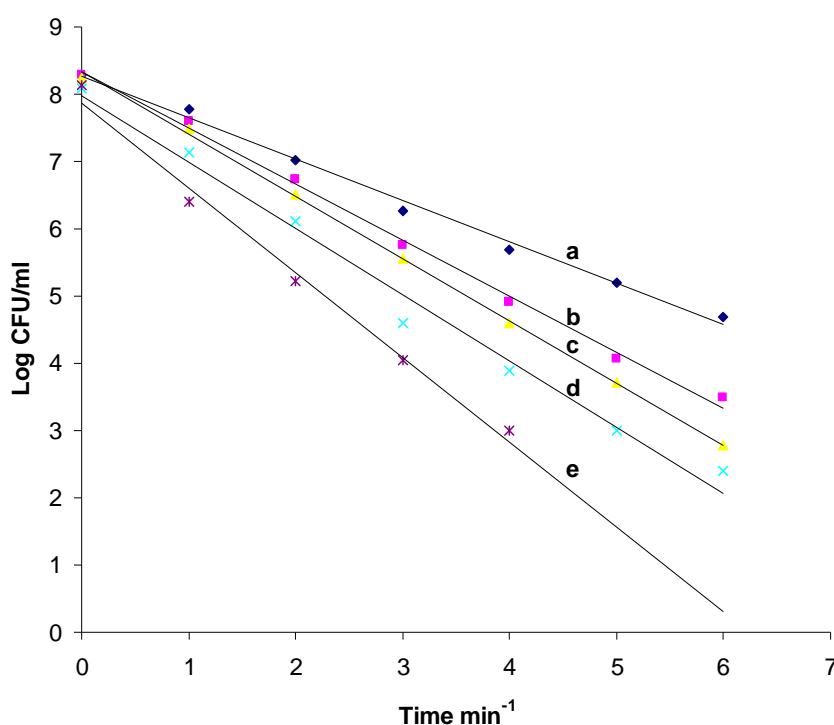
A series of experiments in duplicate were conducted with fixed initial *E. coli* counts and different flow rates, and varying the voltage settings of the ozoniser instrument, resulting in generation of different initial concentrations of ozone in the oxygen flow bubbled into the aqueous solutions containing microbe, *E. coli*.

**Table 3.5 Kinetics of inactivation of *E. Coli***

*E. coli* = 10<sup>8</sup> CFU/ml; Flow rate = 2 L/min; Temperature = 25 ± 2 °C.

Time/min	[Ozone]/10 <sup>-5</sup> M				
	1.89	3.71	5.79	7.17	9.84
	Log CFU/ml				
0	8.2792	8.2881	8.2345	8.0881	8.1303
1	7.7753	7.5902	7.4960	7.1266	6.3890
2	7.0192	6.7260	6.5090	6.1010	5.2160
3	6.2771	5.7610	5.5600	4.6021	4.0512
4	5.6909	4.9031	4.5920	3.8865	3.0000
5	5.2000	4.0607	3.7080	2.9890	
6	4.6989	3.4920	2.7780	2.4000	

n = 2 and relative variance coefficient < 5%



F

**figure 3.4 Effect of ozone concentration on *E. coli* inactivation**

Flow rate = 2 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3]/10^{-5}$  M = Curve **a**: 1.89, **b**: 3.71, **c**: 5.79; **d**: 7.17 and **e**: 9.84.

Figure 3.4 shows that the *E. coli* population (measured as log CFU/ml) decreased exponentially with time. The larger rate constant indicates faster inactivation of the microbe.

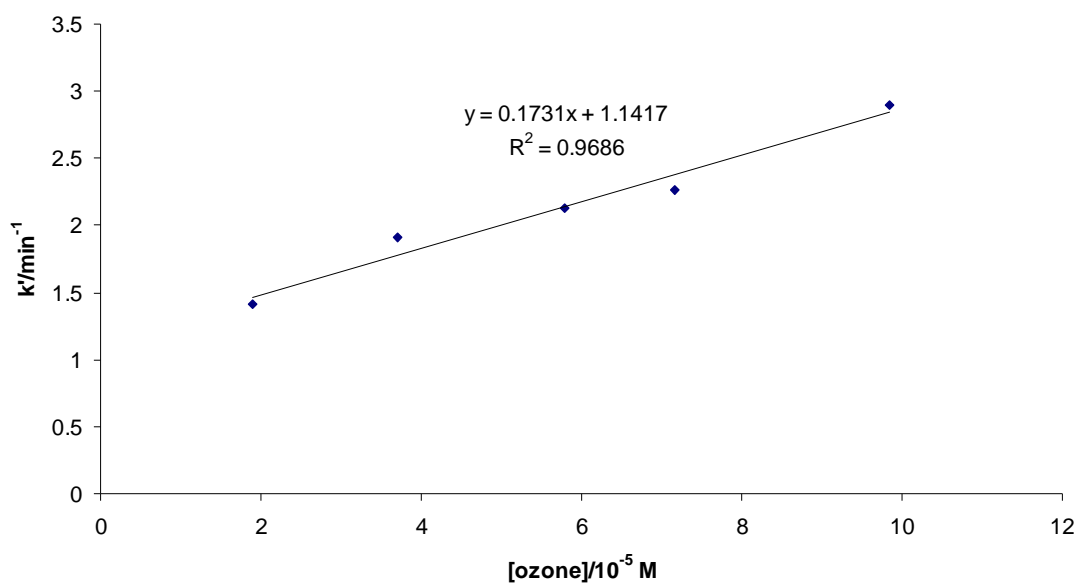
**Table 3.6 Pseudo first-order inactivation rate constants for *E. coli***

Flow rate = 2 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	$[O_3]/$ $10^{-5}$ M	$O_3 / 10^{19}$ molecules/L	$k'/$ $\text{min}^{-1}$	Half life/ min
40	1.8875	1.136	1.416	0.489
50	3.7104	2.234	1.914	0.362
60	5.7938	3.488	2.127	0.326
70	7.1688	4.316	2.266	0.306
80	9.8417	5.925	2.901	0.239

$n = 2$  and relative variance coefficient < 5%

As the voltage settings changed from 40 V to 80 V, the ozone concentration increased from  $1.89$  to  $9.84 \times 10^{-5}$  M and the corresponding rate constants also increased from  $1.416$  to  $2.901 \text{ min}^{-1}$  respectively. The higher the ozone concentration the faster is the rate of inactivation. An observation of the kinetic data in table 3.6 shows that the *E. coli* population exponentially decreased with ozonation as function of time. Ozone has proved to be capable against vegetative cells of *E. coli*. A typical experiment with ozone of  $1.89 \times 10^{-5}$  M produced a reduction of 4 log (from  $10^8$  CFU to  $10^4$  CFU) in 6 minutes in populations of approximately  $1 \times 10^8$  cells/ml, with a dose of ozone  $9.84 \times 10^{-5}$  M the reduction was much higher approximately 5 log in 4 minutes. *E. coli* inactivation followed *pseudo* first-order kinetics with respect to microbe. The calculated slopes of the plots which are the *pseudo* first-order rate constants for different initial concentrations of ozone are summarized in Table 3.6. The *pseudo* first-order rate constants were plotted against the corresponding initial concentration of ozone (Figure 3.5), which gave a linear plot indicating order with respect to ozone is possibly first-order. The second-order rate constant for the overall reaction is represented by the slope =  $0.1731 \text{ M}^{-1} \text{ min}^{-1}$ , therefore, the second-order rate constant may be expressed as  $k = 1.731 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ . The first two columns in table 3.6 show the ozone concentrations expressed in moles per litre and molecules per litre.



**figure 3.5 Plot of *pseudo* first-order rate constant,  $k'$  versus [ozone]**  
**Flow rate = 2 L/min**

**F**

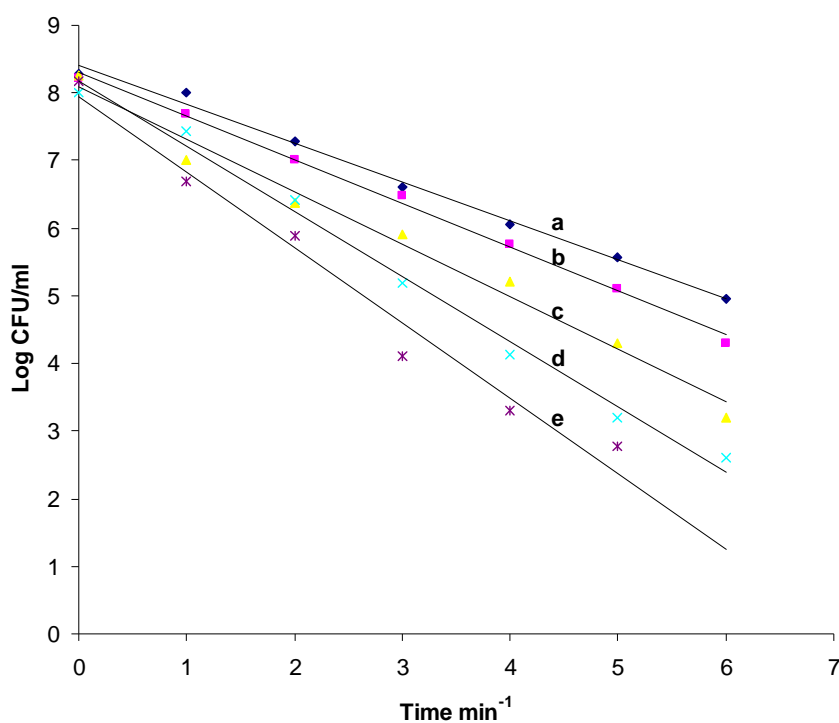
The kinetics of inactivation of *E. coli* was repeated at different flow rates and with varied initial concentrations of ozone in the oxygen bubbled into the reaction mixture. The results obtained with 3 L/ min rate are summarized in Table 3.7.

Figure 3.6 shows a plot of log microbial count *versus* time at different initial ozone concentrations. These plots are straight lines, confirming first-order rate dependence with respect to *E. coli*. The exponential decay of the *E. coli*, confirms the *pseudo* first-order reaction at a flow rate of 3 L/min. The rate constants determined using the slopes are summarized in Table 3.8.

**Table 3.7 Kinetics of inactivation of *E. Coli***

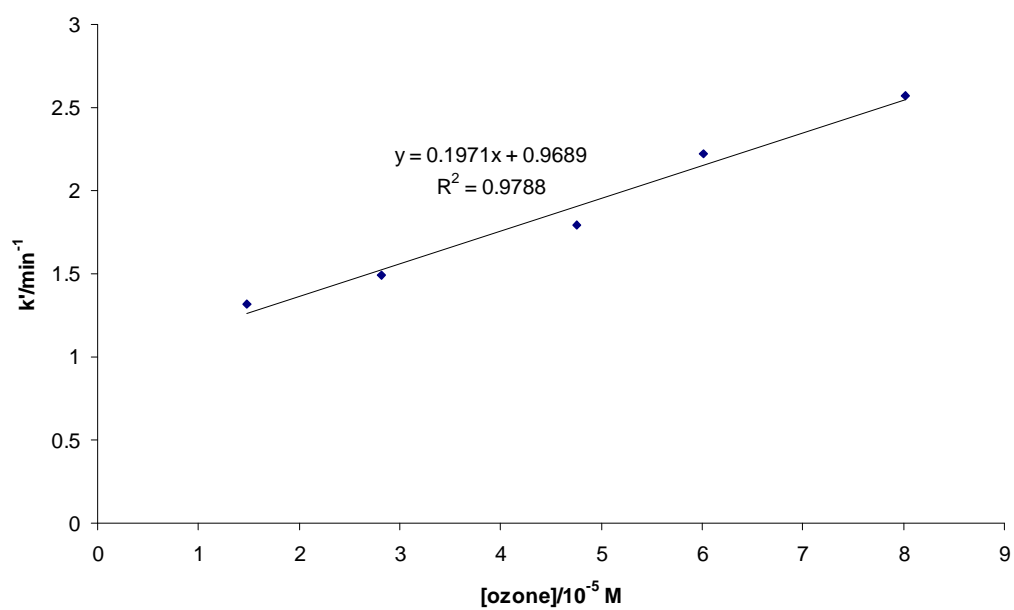
*E. coli* =  $10^8$  CFU/ml; Flow rate = 3 L/min. Temperature =  $25 \pm 2$  °C.

Time/min	[Ozone]/ $10^{-5}$ M				
	1.48	2.81	4.75	6.01	8.02
	Log CFU/ml				
0	8.2798	8.2173	8.2527	8.01284	8.1843
1	7.9989	7.6919	7.0170	7.4419	6.6989
2	7.2788	7.0000	6.3780	6.4211	5.8900
3	6.6031	6.4771	5.9100	5.1853	4.1120
4	6.0553	5.7610	5.2040	4.1240	3.2930
5	5.5761	5.0998	4.2976	3.2050	2.7790
6	4.9542	4.3010	3.2041	2.6010	
n = 2 and relative variance coefficient < 5%					



**Figure 3.6 Effect of [ozone] on *E. coli* inactivation**

Flow rate = 3 L/min. *E. coli* =  $10^8$  CFU/ml;  $[\text{O}_3]/10^{-5}$  M = Curve **a**: 1.48, **b**: 2.81, **c**: 4.75; **d**: 6.01 and **e**: 8.02.



**Figure 3.7 Plot of pseudo first-order rate constant,  $k'$  versus [ozone]**

**Table 3.8 Kinetic data for first-order reaction at 3 L/min**Flow rate = 3 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k'/ min <sup>-1</sup>	Half life/ min
40	1.4771	1.3198	0.525
50	2.8104	1.4946	0.464
60	4.7500	1.7897	0.387
70	6.0146	2.2212	0.312
80	8.0229	2.5676	0.270
n = 2 and relative variance coefficient < 5%			

The *pseudo* first-order rate constants were plotted against the corresponding initial concentration of ozone (Figure 3.7), which gave a straight line suggesting that order with respect to ozone also is first-order. The second-order rate constant for the overall reaction is slope =  $0.1971 \text{ L mol}^{-1} \text{ min}^{-1}$  or  $k = 1.971 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ .

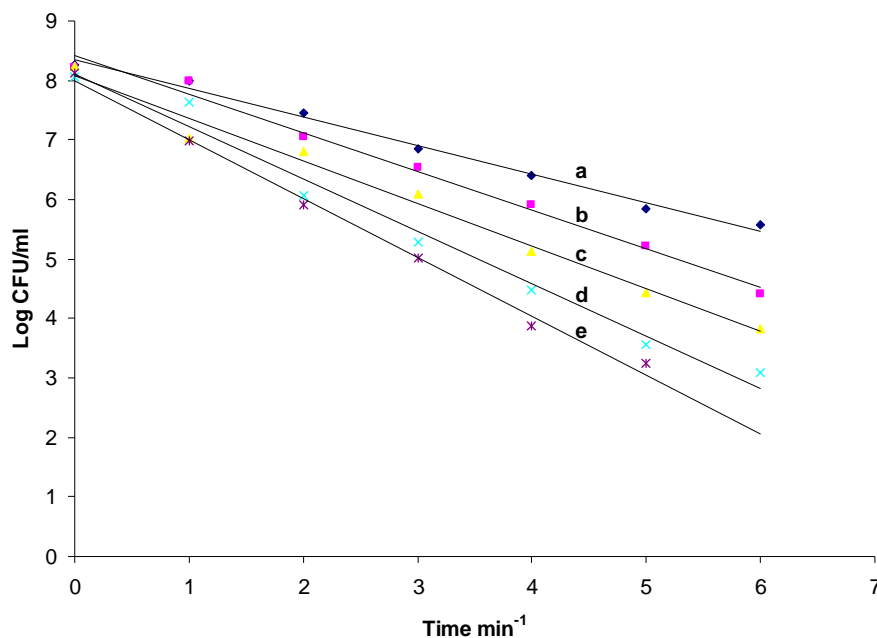
The results of the microbial count of *E. coli* monitored as function of time at 4 L/min flow rate and with varied initial concentration of ozone are shown in Table 3.9.

**Table 3.9 Kinetics of inactivation of *E. Coli***

*E. coli* =  $10^8$  CFU/ml; Flow rate of 4 L/min. Temperature =  $25 \pm 2$  °C.

Time/min	[Ozone]/ $10^{-5}$ M				
	0.99	2.05	3.77	4.75	5.94
Log CFU/ml					
0	8.2711	8.2173	8.2430	8.0607	8.1185
1	7.9992	7.9979	7.0215	7.6345	6.9894
2	7.4472	7.04139	6.8031	6.0760	5.9180
3	6.8451	6.5300	6.0920	5.2820	5.0090
4	6.3979	5.9123	5.1230	4.4771	3.8830
5	5.8335	5.2200	4.4430	3.5610	3.2390
6	5.5682	4.4040	3.8240	3.0990	

n = 2 and relative variance coefficient < 5%



**Figure 3.8 Effect of ozone concentration on inactivation of *E. coli***

Flow rate = 4 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3]/10^{-5}$  M = Curve **a**: 0.99, **b**: 2.05, **c**: 3.7688; **d**: 4.75 and **e**: 5.94.

Figure 3.8 shows the exponential decay of the *E. coli* in a first order reaction at a flow rate of 4 L/min with the rate determined by  $k$ . These plots are straight lines, confirming a *pseudo* first-order reaction. The rate constant  $k'$  determined from the gradients of the graphs are tabulated below.

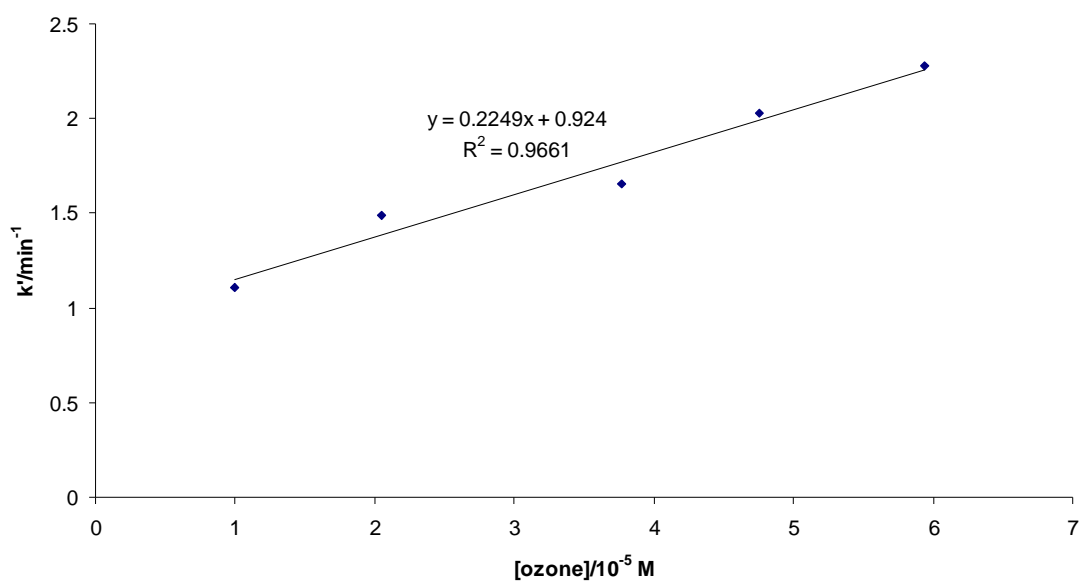
**Table 3.10 Kinetic data for first-order reaction at 4 L/min**

Flow rate = 4 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	$k'$ / min <sup>-1</sup>	Half life/ min
40	0.9979	1.1096	0.625
50	2.0500	1.4907	0.465
60	3.7688	1.6526	0.419
70	4.7500	2.0259	0.342
80	5.9375	2.2784	0.304

$n = 2$  and relative variance coefficient < 5%

The *pseudo* first-order rate constants obtained from the log CFU versus time plots (Table 3.10) were plotted against the corresponding ozone concentrations (Figure 3.9), which gave a linear curve confirming the inactivation of microbe follows first order kinetics. The second-order rate constant obtained for the overall reaction is  $2.249 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ .



**Figure 3.9** Plot of *pseudo* first-order rate constant,  $k'$  versus [ozone]

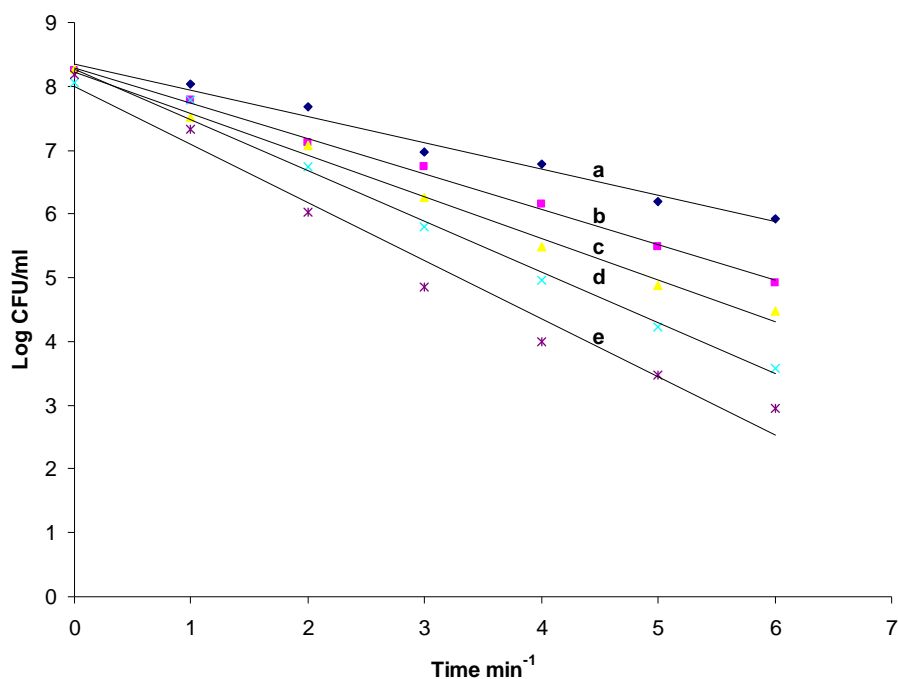
**Table 3.11** Kinetics of inactivation of *E. Coli*

*E. coli* =  $10^8$  CFU/ml; Flow rate of 5 L/min. Temperature =  $25 \pm 2$  °C.

Time/min	[Ozone]/10 <sup>-5</sup> M				
	0.70	1.58	2.89	3.53	4.91
	Log CFU/ml				
0	8.2519	8.2383	8.2723	8.0563	8.1892
1	8.0376	7.7900	7.5051	7.7918	7.3232
2	7.6822	7.1139	7.0670	6.7380	6.0330
3	6.9672	6.7310	6.2560	5.8070	4.8530
4	6.7761	6.1450	5.4750	4.9590	3.9990
5	6.1989	5.4850	4.8780	4.2220	3.4770
6	5.9221	4.9110	4.4710	3.5880	2.9510
n = 2 and relative variance coefficient < 5%					

The kinetic data obtained for the inactivation of microbe, *E. coli* monitored at 5 L/min flow rate and with different initial ozone concentrations are summarized in Table 3.11 Figure

3.10 illustrates the log CFU/ml versus time plots which are straight lines, confirming the first- order dependence of microbial inactivation with respect to *E. coli* at 5 L/min flow rate.



**Figure 3.10 Effect of ozone on *E. coli* inactivation**

Flow rate = 5 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3]/10^{-5}$  M = Curve **a**: 0.70, **b**: 1.58, **c**: 2.89; **d**: 3.53 and **e**: 4.91.

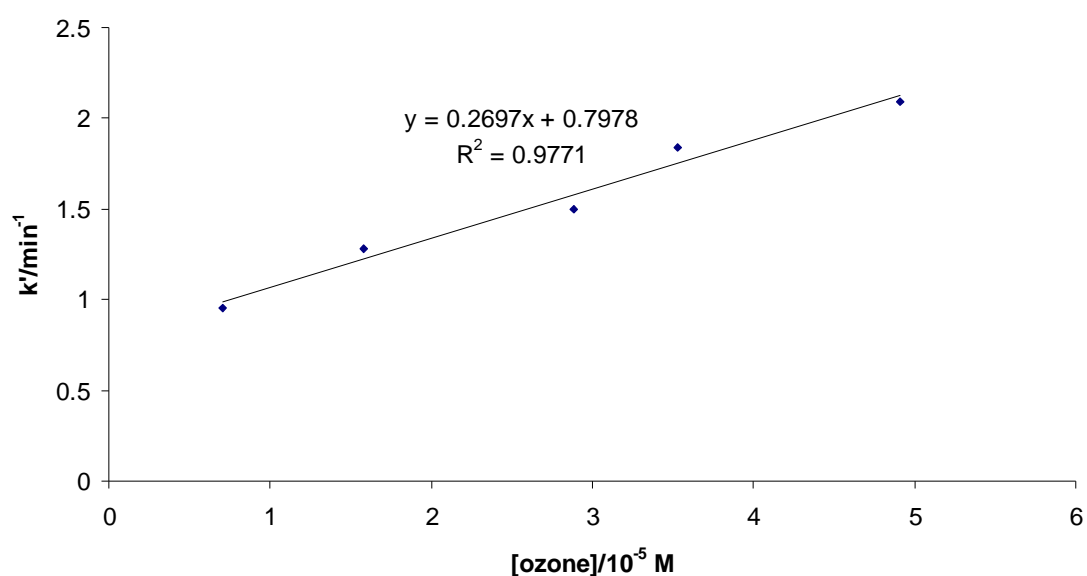
These plots in fig. 3.10 are straight lines, confirming reaction follows *pseudo* first-order kinetics. The respective slopes are presented in Table 3.12.

**Table 3.12 Kinetic data for the first-order reaction at 5 L/min**

Flow rate = 5 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k'/ min <sup>-1</sup>	Half life/ min
40	0.7042	0.9518	0.728
50	1.5813	1.2798	0.541
60	2.8875	1.5011	0.461
70	3.5292	1.8362	0.377
80	4.9125	2.0925	0.331

n = 2 and relative variance coefficient < 5%



**Figure 3.11** Plot of *pseudo* first-order rate constant,  $k'$  versus [ozone]

The *pseudo* first-order rate constants from the log CFU/ml *versus* time plots (Table 3.12) were plotted against the corresponding ozone concentrations (Figure 3.11), which gave a straight line indicating that again with 5L/min flow rate, the inactivation of *E. coli* follows first-order kinetics. The second-order rate constant obtained for the overall reaction is  $2.697 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ .

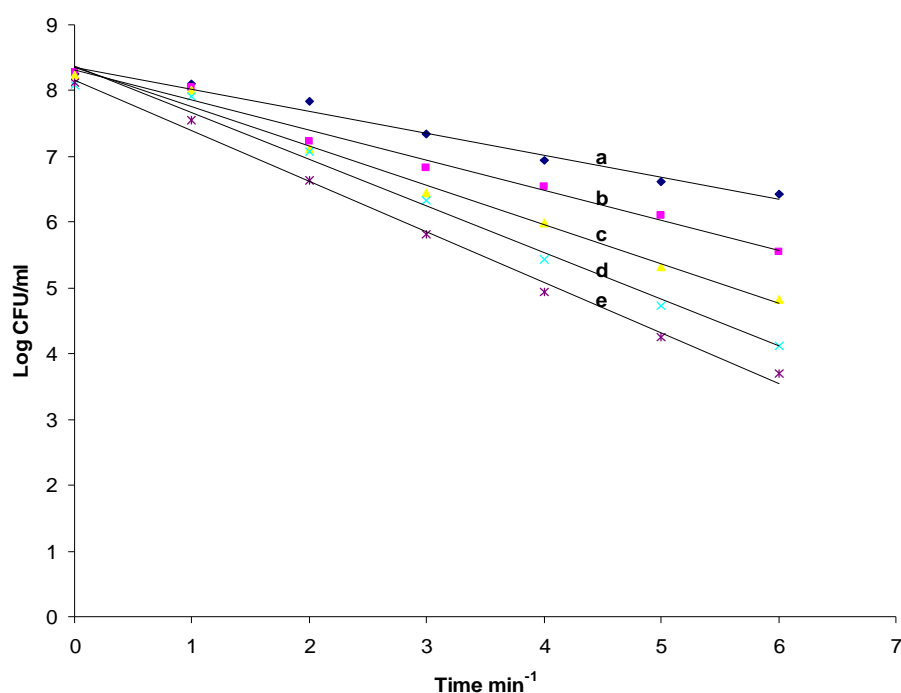
The results of the kinetic data monitoring the inactivation of microbe, *E. coli* monitored as function of time at 6 L/min flow rate and with varied initial concentration of ozone are shown in Table 3.13.

**Table 3.13** Kinetics of inactivation of *E. Coli*

*E. coli* =  $10^8$  CFU/ml; Flow rate of 6 L/min. Temperature =  $25 \pm 2$  °C.

Time/min	[Ozone]/10 <sup>-5</sup> M				
	0.47	1.26	2.04	2.71	3.86
0	8.253	8.2721	8.2430	8.0881	8.1304
1	8.0971	8.0472	8.0124	7.9183	7.5482
2	7.8321	7.2304	7.1132	7.0718	6.6330
3	7.3412	6.8310	6.4410	6.3320	5.8120
4	6.9345	6.5320	5.9850	5.4393	4.9320
5	6.6075	6.1110	5.3220	4.7290	4.2550
6	6.4253	5.5400	4.8240	4.1230	3.7010

n = 2 and relative variance coefficient < 5%



**Figure 3.12 Effect of ozone on *E. coli* inactivation**

Flow rate = 6 L/min. *E. coli* = 10<sup>8</sup> CFU/ml; [O<sub>3</sub>]/10<sup>-5</sup> M = Curve **a**: 0.46, **b**: 1.26, **c**: 2.04; **d**: 2.71 and **e**: 3.86.

Figure 3.12 shows the log microbial count against time plots at different ozone concentrations, which were good straight lines, confirming the order with respect to the

microbe is first-order at 6 L/min flow rate too. The *pseudo* first-order rate constants obtained from the plots are summarized in Table 3.14.

**Table 3.14 Kinetic data for the first-order reaction at 6 L/min**

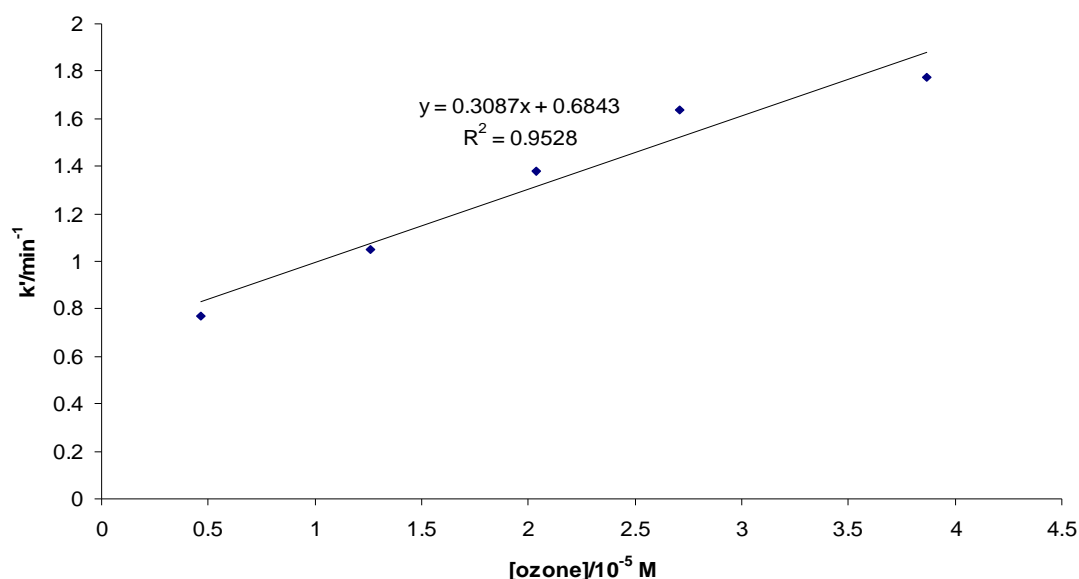
Flow rate = 6 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k'/ min <sup>-1</sup>	Half life/ min
40	0.4646	0.7699	0.900
50	1.2583	1.0502	0.659
60	2.0396	1.379	0.502
70	2.7063	1.6372	0.423
80	3.8646	1.7747	0.390

n = 2 and relative variance coefficient < 5%

At fixed voltage and flow rate, fixed initial concentration of ozone was generated in the oxygen flow, but at fixed voltage, as the flow rate was varied from 2L/min – 6L/min the rate of disinfection decreased. When comparing flow rate of 2L/min with the flow rate of 6L/min, it was observed that ozone of  $1.89 \times 10^{-5}$  M at 6L/min produced a reduction of 2 log in 6 minutes in population of  $1 \times 10^8$  cells/ml and at 2L/min a reduction of 4 log in 6 minutes was achieved for the same population. With a dose of  $9.84 \times 10^{-5}$  M ozone at 6L/min the reduction was 5 log in 6 minutes while at 2L/min 5 log inactivation was achieved in 4 minutes.

Korol *et al*, (1995) when they were comparing action between ozone and chlorine on microorganisms observed that ozone of 0.35 mg/L produced a reduction of >5 log in population of approximately  $1 \times 10^6$  cells/ml of *Escherichia coli* and *Pseudomonas aeruginosa*. With a dose of 0.50 mg/L chlorine, the reduction was much smaller for the same microorganisms, while the effect of 2 mg/L chlorine was similar to the ozone treatment.

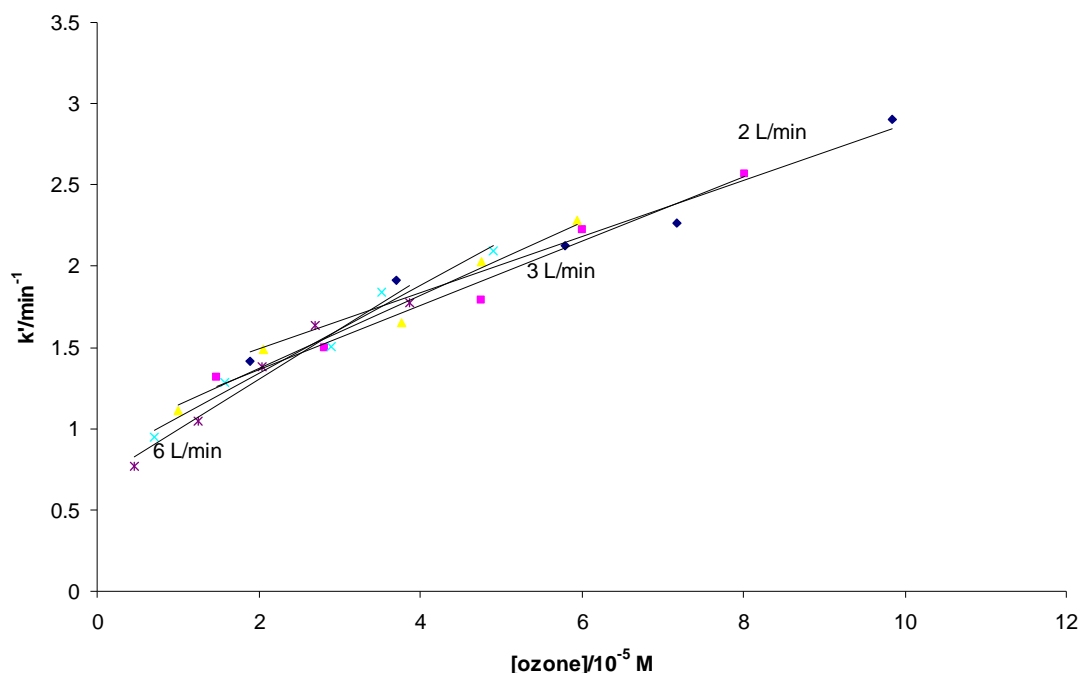


**Figure 3.13** Plot of *pseudo* first-order rate constant,  $k'$  versus [ozone]

The plots of *pseudo* first-order rate constants against ozone concentration for all flow rates gave the straight line further confirm that the order with respect to ozone is the first order. The *pseudo* first-order rate constants,  $k'$  from the log CFU/ml *versus* time plots (Table 3.14) were plotted against the corresponding ozone concentrations. An observation of Figure 3.13 shows that with 6L/min flow rate is a linear curve suggesting the inactivation of *E. coli* follows first-order kinetics. The second-order rate constant obtained for the overall reaction is  $3.087 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ .

**Table 3.15** Ozone concentration and half lives at different flow rates

Flow rate, 2L/min		3 L / min		4 L / min		5 L / min		6 L / min	
[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k/min <sup>-1</sup>	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k/min <sup>-1</sup>	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k/min <sup>-1</sup>	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k/min <sup>-1</sup>	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	k/min <sup>-1</sup>
1.888	1.416	1.477	1.3198	0.998	1.1096	0.704	0.9518	0.465	0.7699
3.71	1.914	2.81	1.4946	2.05	1.4907	1.58	1.2798	1.258	1.0502
5.794	2.127	4.75	1.7897	3.77	1.6526	2.89	1.5011	2.04	1.379
7.169	2.266	6.015	2.2212	4.75	2.0259	3.53	1.8362	2.706	1.6372
9.842	2.901	8.023	2.5676	5.94	2.2784	4.91	2.0925	3.865	1.7747



**Figure 3.14** Plots of  $k'$  versus ozone concentration at different flow rates

At fixed flow rate, the increase in concentration of ozone in gas enhanced the rate of inactivation of *E. coli*, as indicated by the high *pseudo* first-order rate constant,  $k'$  value and shorter half life,  $t_{1/2}$  (0.24 min) at high voltage (80 V) and low flow rate (2 L /min). Slowest reaction was with 40 V and 6 L/min flow rate, having half life of 0.90 min as presented in Table 3.15. Interestingly at the fixed voltage, the rate of inactivation decreased with the increase in flow rate from 2L/min to 6L/min, which is due the decrease in  $[O_3]$  and residence time of ozone in reactor.

Further, the value of mean second order rate constant for the five flow rates is estimated to be  $(4.9 \pm 1.1) \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ . A slight increase in overall second-order rate constant value was observed with increasing flow rates, which is possibly due to relatively low concentrations of ozone with increased flow rates, for otherwise similar voltage conditions as illustrated in Figure 3.14. When gaseous ozone is bubbled through aqueous solution, a steady state exists between dissolved ozone and gaseous ozone. While dissolved ozone is more effective in reacting with microbe, the gaseous ozone is less interactive due less residence time and much is wasted. Thus, one possibility is that all the ozone may not be

proportionally participating in the inactivation processes due to its solubility limitations in water and the interaction constraints of gaseous ozone with microbe.

In this study, the voltage of 40 V and the flow rate of 2 L/min were chosen for rest of the experiments which gave the ozone concentration of  $1.89 \times 10^{-5}$  M, which was relatively a slow reaction allowing the investigation of various variables on the inactivation kinetics. The bacterial population used was of approximately  $10^6$ - $10^8$  CFU/ml.

### **3.14 Effect of temperature on the inactivation of *Escherichia coli***

Aquatic organisms are dependent on certain temperature ranges for optimal health. Temperature affects many other parameters in water, including the amount of dissolved oxygen available, the types of plants and animals present, and the susceptibility of organisms to parasites, pollution and disease (Lower Colorado River Authority, 1996). Causes of temperature changes in water include weather conditions, shade and discharges into the water from urban sources or groundwater inflows. The trends are season dependent. Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature even at very low temperatures. The temperature range in which most bacteria grow is between 5°C and 60°C (Wagner, Jr., 1998).

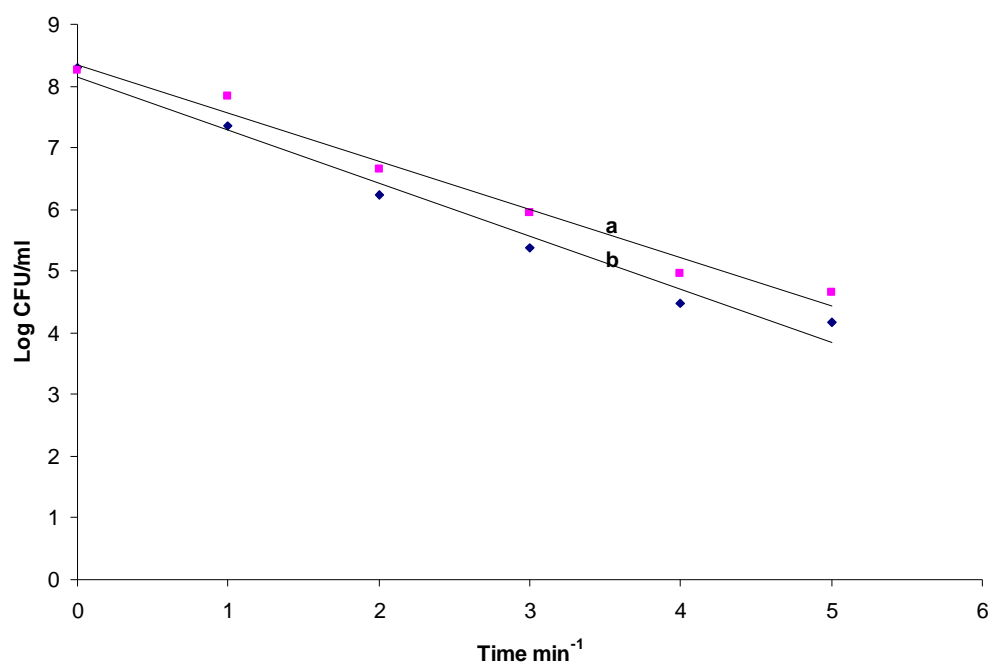
Ozone is stable at lower temperatures and decomposes at higher temperatures (Handbook of Ozone Technology and Applications, 1982). The experiments to investigate if temperature affects the disinfection rates were conducted at the temperatures between 8°C and 25°C, since *E. coli* minimum growth temperature is approximately 4°C and the maximum is approximately 50°C (Martin and Brewer, 2000).

**Table 3.16 Log CFU/ml at two temperatures as a function of time**

*E. coli* =  $10^8$  CFU/ml; Flow rate of 2 L/min; [Ozone] =  $1.89 \times 10^{-5}$  M.

Time/ min	Temperature	
	8°C	25°C
	Log CFU/ml	
0	8.306	8.248
1	7.350	7.846
2	6.231	6.657
3	5.386	5.951
4	4.472	4.967
5	4.175	4.652

n = 2 and relative variance coefficient < 5%

**Figure 3.15 Log CFU/ml at two temperatures as a function of time**

Flow rate = 2 L/min. *E. coli* =  $10^8$  CFU/ml; [O<sub>3</sub>] =  $1.89 \times 10^{-5}$  M. Temperature = Curve a: 25°C and b: 8°C.

**Table 3.17 Kinetic data for ozonation of *Escherichia coli* at two temperatures**

Temp./°C	k'/min <sup>-1</sup>	Half life /min
8	1.983	0.35
25	1.798	0.38
n = 2 and relative variance coefficient < 5%		

Temperature was observed to have marginal effect on the rate of disinfection of the test microorganism, *E. coli*. As presented in Table 3.17 as the rate constants were found to be almost similar for both temperatures, with very small difference.

Earlier reported results with the *E. coli* also showed that the disinfection rate was found to be independent of temperature (Kinman, 1975), where it was reported that “although increasing the temperature from 0 to 30°C can significantly reduce the solubility of ozone and increase the decomposition rate, temperature has no effect on the disinfection rate of bacteria”.

### **3.15 Effect of pH on *Escherichia coli* inactivation**

A pH test measures the alkalinity or acidity concentration in water. A pH of 7 is neutral, below 7 is acidic, and above 7 is basic or alkaline. Acid rain, caused by the auto exhaust pollutants or coal-fired power plants, causes the drop in the pH of water (Lower Colorado River Authority, 1996). Pollution from accidental spills, agricultural runoff and sewer overflows can also change the pH. Buffering capacity is water’s ability to resist changes in pH, and is critical to the survival of aquatic life. While young fish and insect larvae are sensitive to a low pH (acid), extreme values on either end of the scale can be lethal to most organisms. Expected levels for natural water are from 6.5 to 9.0 respectively.

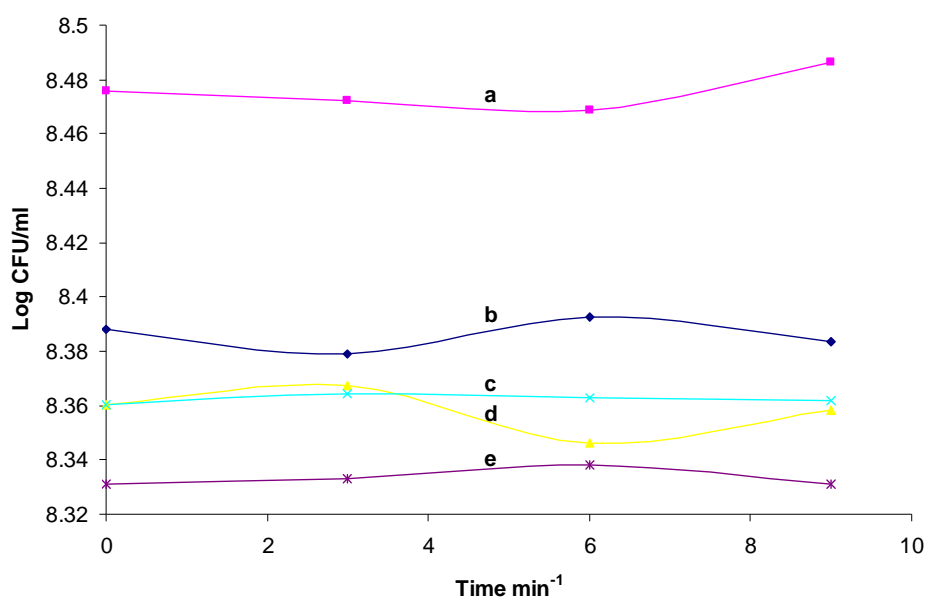
Since the expected values of pH in natural water range from 6.5 to 9.0, it is important to conduct experiments in the laboratory using the pH controlled deionised distilled water, to investigate the effectiveness of ozone as a function of pH variation.

Bacteria can be classified by pH ranges in which they grow. The internal pH of the cell remains close to neutral, an organism's tolerance to fluctuations in pH reflects the capacity of the membrane pumps to maintain that pH. Most bacteria grow between pH 5.0 and pH 8.0. The *E. coli* inactivation experiments were conducted using the pH 5.0 and pH 9.0 since *E. coli* organism's minimum growth pH is 4.4, and maximum pH is 9.0. (Martin *et al.*, 2000).

**Table 3.18 Control experiments without O<sub>3</sub> at different pH of *E. coli***

*E. coli* = 10<sup>8</sup> CFU/ml; Flow rate of 2 L/min. Temperature = 25 ± 2 °C.

Time/ min	pH				
	4.93	5.93	6.96	7.93	9.16
	Log CFU/ml				
0	8.3879	8.4759	8.3605	8.3605	8.3312
3	8.3789	8.4723	8.3675	8.3645	8.3329
6	8.3924	8.4685	8.3461	8.3629	8.3381
9	8.3835	8.4866	8.3581	8.362	8.3312
n = 2 and relative variance coefficient < 5%					



F

**figure 3.16 Control experiments at different pH of *E. coli***

Flow rate = 2 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve **a**: 5.93, **b**: 4.93, **c**: 7.93; **d**: 6.96 and **e**: 9.16.

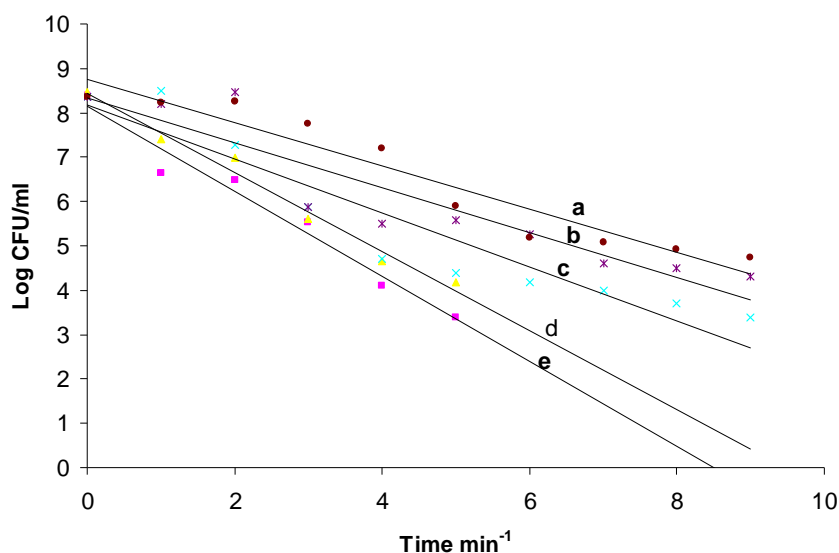
The control runs did not receive any ozone dose. The results shows that the base or acid addition in the investigated pH range plays no role in the inactivation of the *Escherichia coli*, as the number of bacterial cells were constant after a period of time being exposed in acidic or basic medium, the results are presented in Table 3.18 and illustrated in Figure 3.16 above.

**Table 3.19 Log CFU/ml at various pH values as a function of time**

*E. coli* =  $10^8$  CFU/ml; Ozone =  $1.89 \times 10^{-5}$  M. Flow rate = 2 L/min Temp. =  $25 \pm 2$  °C.

Time/ min	pH				
	4.93	5.93	6.96	7.93	9.16
	Log CFU/ml				
0	8.388	8.476	8.361	8.361	8.351
1	6.652	7.401	8.502	8.193	8.232
2	6.476	6.982	7.272	8.456	8.260
3	5.525	5.600	5.874	5.886	7.739
4	4.096	4.652	4.698	5.502	7.206
5	3.397	4.175	4.391	5.575	5.895
6			4.175	5.271	5.186
7			3.999	4.614	5.069
8			3.698	4.494	4.909
9			3.397	4.300	4.739

n = 2 and relative variance coefficient < 5%

**Figure 3.17 Log CFU/ml at various pH as a function of time**

Flow rate = 2 L/min. *E. coli* =  $10^8$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve **a**: 9.16, **b**: 7.93, **c**: 6.93; **d**: 5.93 and **e**: 4.93.

Table 3.19 presents the variation of pH from 4.93-9.16 for *E. coli* inactivation and Figure 3.17 shows the plot of log CFU/ml against time. The plot is straight, confirming a first-order reaction, and its slopes are presented in Table 3.20.

**Table 3.20 Kinetic data for ozonation of *Escherichia coli* at different pH**

*E. coli* =  $10^8$  CFU/ml; Ozone =  $1.89 \times 10^{-5}$  M. Flow rate = 2 L/min Temp. =  $25 \pm 2$  °C.

pH	$k'/\text{min}^{-1}$	Half life/min
4.93	2.209	0.31
5.93	2.049	0.34
6.96	1.396	0.49
7.93	1.164	0.59
9.16	1.126	0.62
n = 2 and relative variance coefficient < 5%		

The *pseudo* first-order rate constants were determined and are presented in Table 3.20 as the pH was increased from 4.93 to 9.16, the rate constants decreased by approximately 56% indicating a decrease in inactivation rate. This is also supported by the magnitude of  $t_{1/2}$  values. For inactivation at pH 9.16, it took approximately double the time relative to the time required at pH 4.93.

Ozone has shown to be efficient against *E. coli* inactivation, particularly at lower pH values where molecular ozone is the predominant species. As represented in Table 3.20, inactivation at pH 4.93 – 5.93 was more effective than at pH 6.96 – 9.16. It was observed that ozone of  $1.89 \times 10^{-5}$  M at pH 4.93 produced a reduction of 5 log in population of approximately  $2 \times 10^8$  cells/ml in 5 minutes; a same dose of ozone at pH 9.16, the reduction was smaller approximately 4 log in population of approximately  $2 \times 10^8$  cells/ml in 9 minutes.

The action of ozone on pathogenic organisms has been debated, with the debate fueled by a wide variation in published values for rate constants of organisms of interest in water treatment. In one camp are those who believe that direct action of ozone on

microorganisms is the dominant mechanisms of inactivation. In the second, indirect action of ozone through generation of hydroxyl radicals is assumed to be the dominant mechanism in inactivation (Bartrand, 2004).

von Gunten (2003b) reported that the predominant mechanism for inactivation is direct action of ozone, with hydroxyl radicals playing a minor role that was based on comparison of inactivation rates in the presence of dissolved ozone with inactivation rates for organisms exposed to known concentration of hydroxyl radicals.

Studies with *E. coli* and *G. muris* have suggested that these organisms undergo greater inactivation when ozone residuals persist, rather than when ozone is rapidly decomposed (Gehr *et al.*, 2003).

Morris (1975) reported that the ozone disinfection efficiency is not affected by pH, although because of hydroxyl free radicals and rapid decay, efficiency is the same. More ozone should be applied at high pH. Analysis Of Variance (ANOVA) also indicated that the pH did not significantly affect the rate of disinfection by ozone, the amount of ozone that could be dissolved in the medium, however was affected by the pH (Magbanua Jr. *et al.*, 2006).

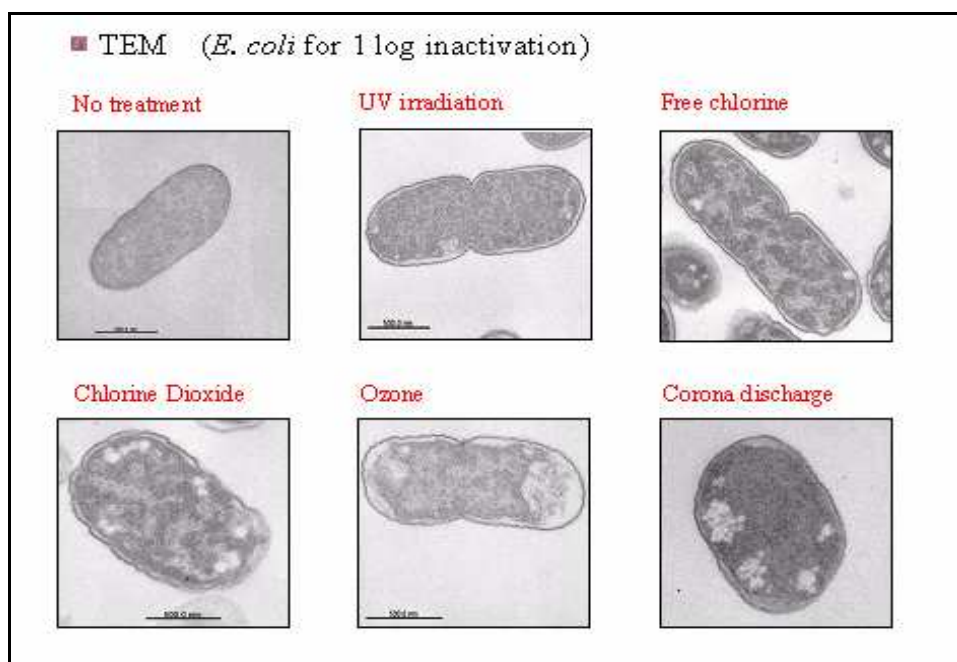
It is well accepted that ozone is effective against all organisms likely to be encountered in wastewaters, including viruses and protozoan cysts. Even organisms resistant to chlorination, such as poliovirus Type 3, as well as *Cryptosporidium* and *Giardia* protozoa, can be inactivated by ozone at residual concentrations of 1 mg/L or even less, and sufficient contact time (several minutes) (Lazarova *et al.*, 1998 - WEF Wastewater disinfection, 1996).

Initial studies in *E. coli* have suggested that ozone primarily attacks the double bonds of unsaturated lipids in the membrane, leading to alteration of permeability and eventual cell death (Scott *et al.*, 1963). Hamelin *et al.* (1978) suggested that ozone produced single

strand breaks in DNA which, if unrepaired, caused extensive breakdown of DNA in *E. coli* ultimately resulting in the loss of viability. Besides these lesions, ozone might also induce base damage and protein cross-linking in DNA molecules (Hamelin *et al.*, 1977).

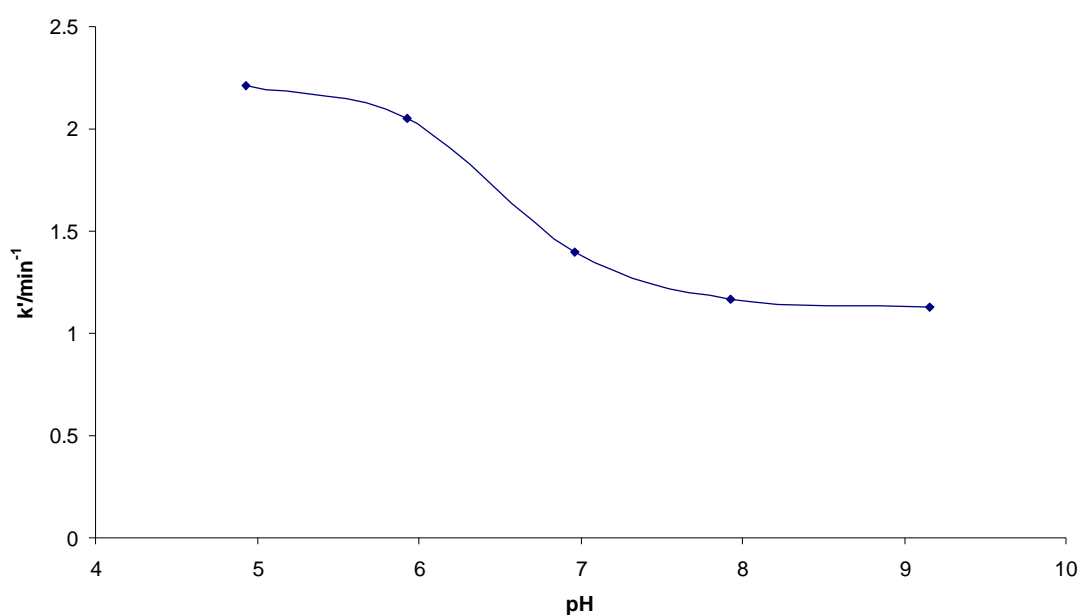
Perrich *et al.* (1975) concluded that cell lysis was not the main mechanism for inactivation of *E. coli* and that the cells remained morphologically intact after inactivation. Ishizaki *et al.* (1987) determined that ozone affected plasmid DNA harbored in *E. coli* cells by converting the closed circular DNA to open circular DNA. This observation indicated that ozone was able to diffuse through the cell membrane and react with cell constituents. The authors proposed that damage to chromosomal DNA might be one of the reasons for inactivation of *E. coli* by ozone.

Representative micrographs obtained for the transmission electron microscope (TEM) analysis of *E. coli* for 1 log inactivation are shown in Figure 3.18 for different disinfection agents.



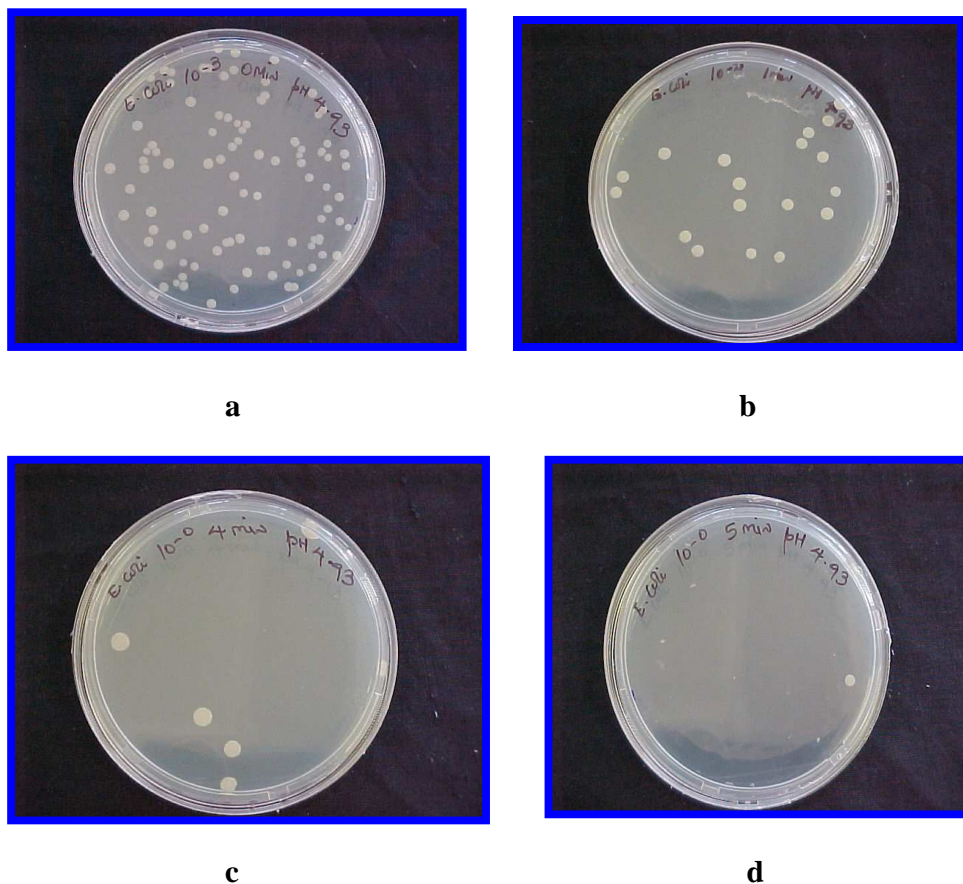
**Figure 3.18** Transmission electron micrographs of *E. coli* cells after exposure to different disinfectants (Yoon-jeyong, 2008).

As illustrated in Figure 3.18 the mechanism of disinfection by chemical disinfectants such as chlorine, chlorine dioxide, corona discharge and ozone differ considerably from disinfection by physical disinfection such as with UV light. Chemical disinfectants inactivate microorganisms by destroying or damaging cellular structures, interfering with metabolism, and hindering biosynthesis and growth. UV light inactivates microorganisms by damaging their nucleic acid, thereby preventing the microorganism from replicating and the microorganism that cannot replicate cannot infect a host.



**Figure 3.19 Plot of pH against  $k'$  for *E. coli* inactivation.**

As illustrated in figure 3.19, the increase in pH has generally decreased the value inactivation rate constant. The  $k'$  values are higher at pH region 5 to 7, further decreasing at higher pH values. Ozone is more potent under acidic conditions suggesting molecular ozone may be reactive species in the inactivation of microbe.



**Figure 3.20** *E. coli* colonies growing on a nutrient agar plate at pH 4.93

Ozone =  $1.89 \times 10^{-5}$  M; *E. coli* =  $10^8$  CFU/ml. Flow rate = 2 L/min Temp. =  $25 \pm 2$  °C. **a**: 0 min, **b**: 1 min, **c**: 4 min and **d**: 5 min. Dilution factor = a: 1000, b: 100, c: stock, d: stock.

Typical example of the effect of ozone concentration of  $1.89 \times 10^{-5}$  M with 2 L/min flow rate on *Escherichia coli* population of  $10^8$  CFU/ml growing on a nutrient agar plate poised at pH 4.93 are illustrated in Figure 3.20 a, b, c and d. The rest of the figures are available on appendix B1.

### 3.20 Inactivation of *Pseudomonas aeruginosa*

A limited number of inactivation experiments on *Pseudomonas aeruginosa* were undertaken to further compare the trends found with *Escherichia coli*, since both strains are gram negative.

**Table 3.21 Control experiments for inactivation of *P. aeruginosa* without O<sub>3</sub>.**

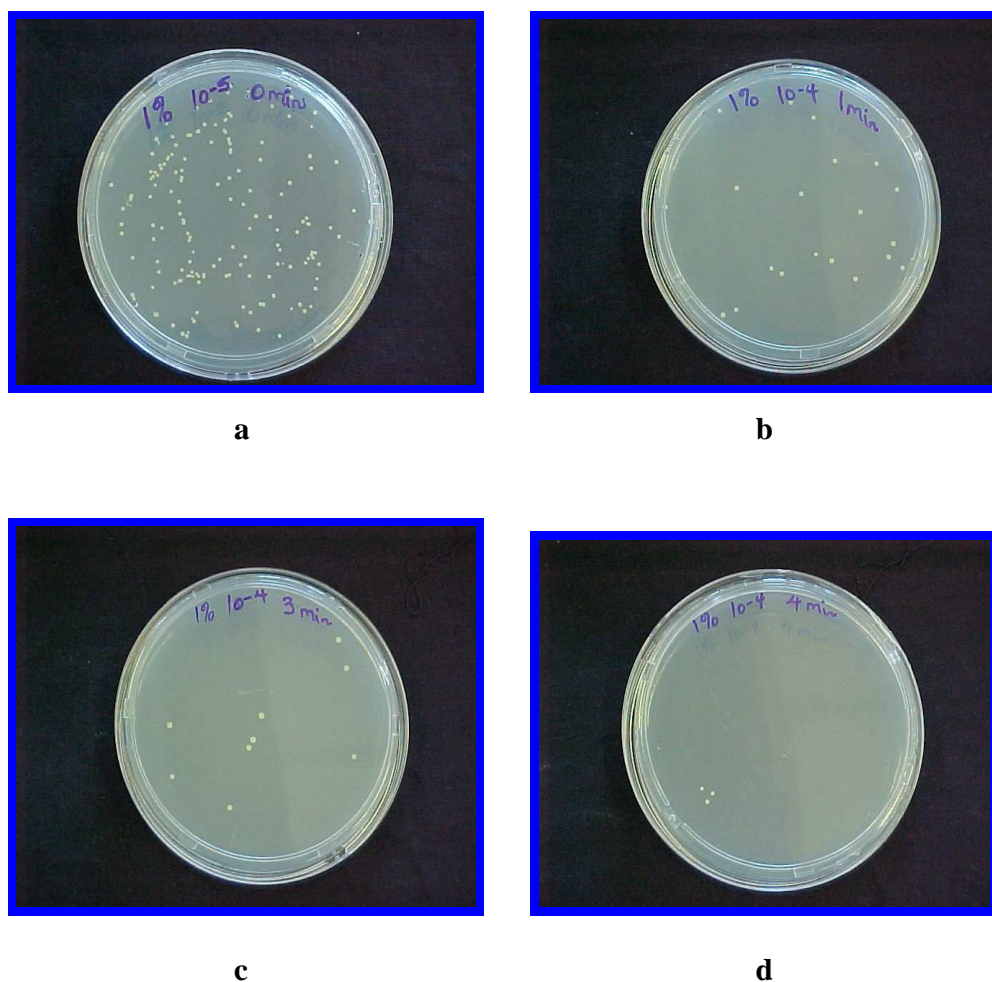
*P. aeruginosa* = 10<sup>7</sup> CFU/ml; Ozone = Nil; Flow rate 2L/min. Temperature = 25 ± 2 °C

Time/min	Log CFU/ml
0	7.8753
3	7.8678
6	7.8632
9	7.8761

No observable change in the *P. aeruginosa* count during the experiment proves that in absence of ozone, inactivation due to oxygen bubbled through the solution is negligible.

### 3.21 Effect of ozone concentration on inactivation of *P. aeruginosa* population

Typical example of the effect of ozone concentration of 1.89 x 10<sup>-5</sup> M and the flow rate; 2 L/min on *Pseudomonas aeruginosa* population of 10<sup>11</sup> CFU/ml growing on a nutrient agar plates is shown in Figure 3.21 a, b, c and d overleaf.



**Figure 3.21 Effect of ozone concentration on *Pseudomonas aeruginosa* population**  
 [Ozone] =  $1.89 \times 10^{-5}$  M; *P. aeruginosa* =  $10^{11}$  CFU/ml. Flow rate = 2 L/min.  
 Temperature =  $25 \pm 2$  °C. **a:** 0 minute, **b:** 1 minutes, **c:** 3 minutes, **d:** 4 minutes. Dilution factor = a: 100000, b: 10000, c: 10000, d: 10000.

### 3.22 Effect of the population of *P. aeruginosa* cultured in nutrient broth on it's inactivation with ozone

Experiments were conducted in duplicate with two different dilutions of microbe and excess of ozone in the oxygen stream bubbled through the microbial solution. (Table 3.22)

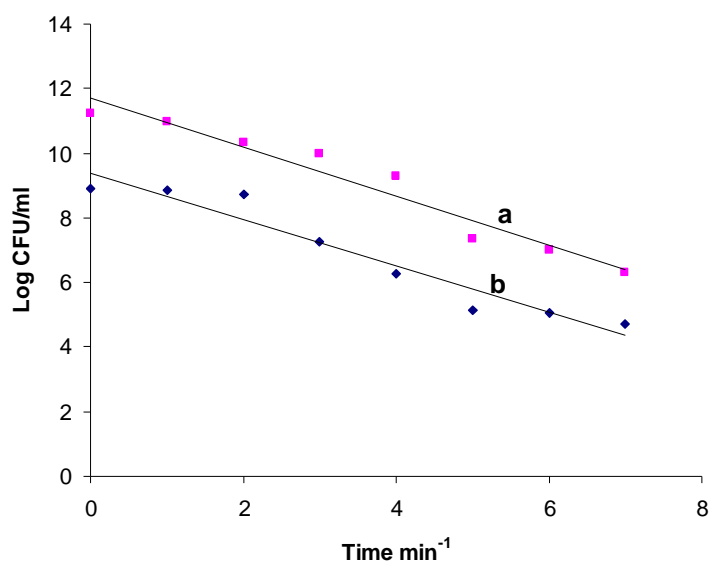
**Table 3.22 Log CFU/ml at two dilutions of stock culture as a function of time**

*P. aeruginosa* =  $10^8$  -  $10^{11}$  CFU/ml; [Ozone] of  $1.89 \times 10^{-5}$  M.

Flow rate: 2 L/min. Temperature =  $25 \pm 2$  °C.

Time/min	$8.107 \times 10^8$	$1.658 \times 10^{11}$
	Log CFU/ml	Log CFU/ml
0	8.9089	11.2196
1	8.8552	10.9574
2	8.7499	10.3418
3	7.259	9.9988
4	6.2531	9.2967
5	5.1516	7.3342
6	5.0392	7.0131
7	4.6968	6.2939

n = 2 and relative variance coefficient < 0.6%

**Figure 3.22 Log CFU/ml at two dilutions of stock culture as a function of time**

Flow rate = 2 L/min. [O<sub>3</sub>] =  $1.89 \times 10^{-5}$  M. [*P. aeruginosa*] =  $10^8$  -  $10^{11}$  CFU/ml = Curve **a**: 1.658, **b**: 8.107.

**Table 3.23 Kinetic data for ozonation of *P. aeruginosa* at two dilutions of culture**

[ <i>Ps. aeruginosa</i> ]/ CFU/ml	k/min <sup>-1</sup>
8.107 x 10 <sup>8</sup>	1.66 ± 0.08
1.658 x 10 <sup>11</sup>	1.75 ± 0.08

n = 2 and relative variance coefficient < 6%

The rate constants for both dilutions on the *P. aeruginosa* were similar as presented in Table 3.23; confirming that rate constant is independent of the microbial count and the reaction has first-order dependence on the microbial concentration. These results were similar to the inferences drawn from experiments on *E. coli*. The results obtained are illustrated in Figure 3.22.

### 3.23 Effect of ozone concentration on inactivation *P. aeruginosa* population

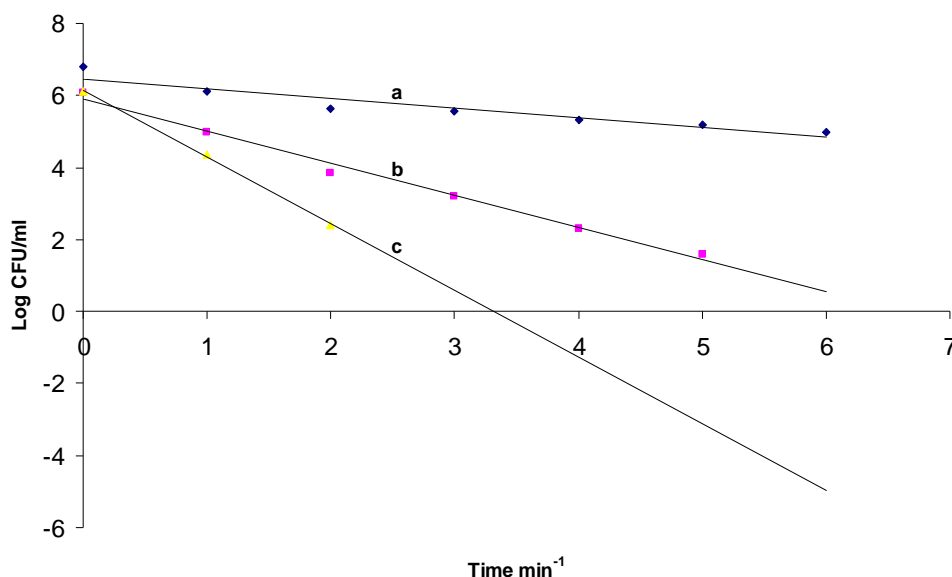
*Pseudomonas aeruginosa* inactivation experiments were conducted with the microbe concentration of 10<sup>6</sup> CFU/ml and ozone concentration in large excess. The depletion of microbe population was monitored as function ozonation time at different initial ozone concentrations.

**Table 3.24 Inactivation of *Pseudomonas aeruginosa* at different Ozone concentrations**

*P. aeruginosa* = 10<sup>6</sup> CFU/ml; Flow rate of 2 L/min. Temperature = 25 ± 2 °C.

	[Ozone]/10 <sup>-5</sup> M		
	1.89	5.79	9.84
Time/min	Log CFU/ml		
0	6.796	6.079	6.115
1	6.097	4.975	4.364
2	5.641	3.852	2.412
3	5.559	3.196	
4	5.33	2.298	
5	5.176	1.578	
6	4.966		

n = 2 and relative variance coefficient < 5%



**Figure 3.23 Effect of [ozone] on *P. aeruginosa* inactivation**

Flow rate = 2 L/min. *P. aeruginosa* =  $10^6$  CFU/ml;  $[O_3]/10^{-5}$  M = Curve **a**: 1.89, **b**: 5.79, **c**: 9.84.

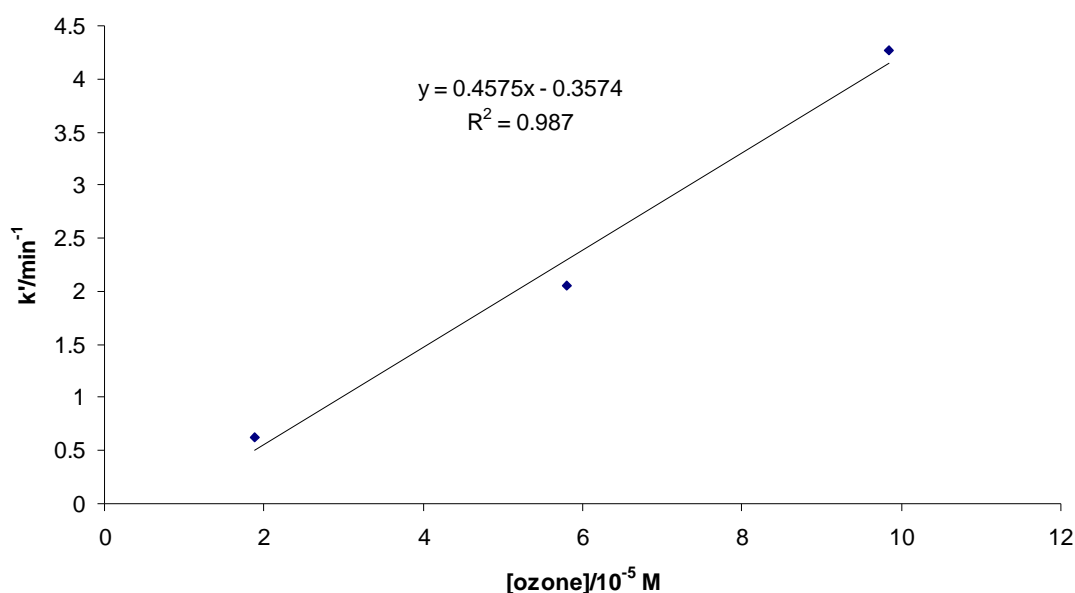
Figure 3.23 shows that the bacteria population (measured as log CFU/ml) decrease exponentially with time. The larger the rate constant, the more rapid is the decay. The plot is straight, confirming a first-order reaction, and the pseudo first-order rate constants ( $k'$ ) estimated using the slopes of the curves are presented in Table 3.25. Table 3.25 also shows the voltage settings of the instrument and the ozone concentrations in the oxygen flow, expressed as mg/L, moles/L and molecules/L in addition to the half lives for the corresponding  $k'$  values.

As was observed with *E. coli*, with the increase in ozone concentration from  $1.89 - 9.84 \times 10^{-5}$  M, the rate of inactivation increased. Ozone has proved to be efficient against vegetative cells of *Pseudomonas aeruginosa*. It was observed that the ozone concentration of  $1.89 \times 10^{-5}$  M produced a reduction of approximately 1 log in 6 minutes in populations of approximately  $1 \times 10^6$  cells/ml, with a dose of  $9.84 \times 10^{-5}$  M ozone, the reduction was found to be 4 log in 2 minutes for almost the same population.

**Table 3.25 Pseudo first-order inactivation rate constants for *P. aeruginosa***Flow rate: 2 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	[O <sub>3</sub> ]/ 10 <sup>-5</sup> M	O <sub>3</sub> / 10 <sup>19</sup> molecules/L	k'/ min <sup>-1</sup>	Half life/ min
40	1.8875	1.136	0.629	1.102
60	5.7938	3.488	2.052	0.338
80	9.8417	5.925	4.264	0.163

n = 2; relative variance coefficient &lt; 6%

**Figure 3.24 Plot of pseudo first-order rate constant against ozone concentration for inactivation of *E. coli* where *E. coli* concentration was maintained constant**

The rate of inactivation of *Pseudomonas aeruginosa* increases as the concentration of ozone is increased. The higher is the voltage of the generator applied, the higher is the ozone concentration and the higher is the rate of inactivation.

The *pseudo* first-order rate constants were plotted against the corresponding initial concentration of ozone (Figure 3.24), which gave a linear plot confirming that order with respect to ozone is first-order. The second-order rate constant for the overall reaction is

represented by the slope =  $0.4575 \text{ L mole}^{-1} \text{ min}^{-1}$ . Therefore, the second-order rate constant may be expressed as  $k = 4.575 \times 10^4 \text{ M}^{-1} \text{ min}^{-1}$ .

### 3.24 Effect of temperature on the inactivation of *P. aeruginosa*

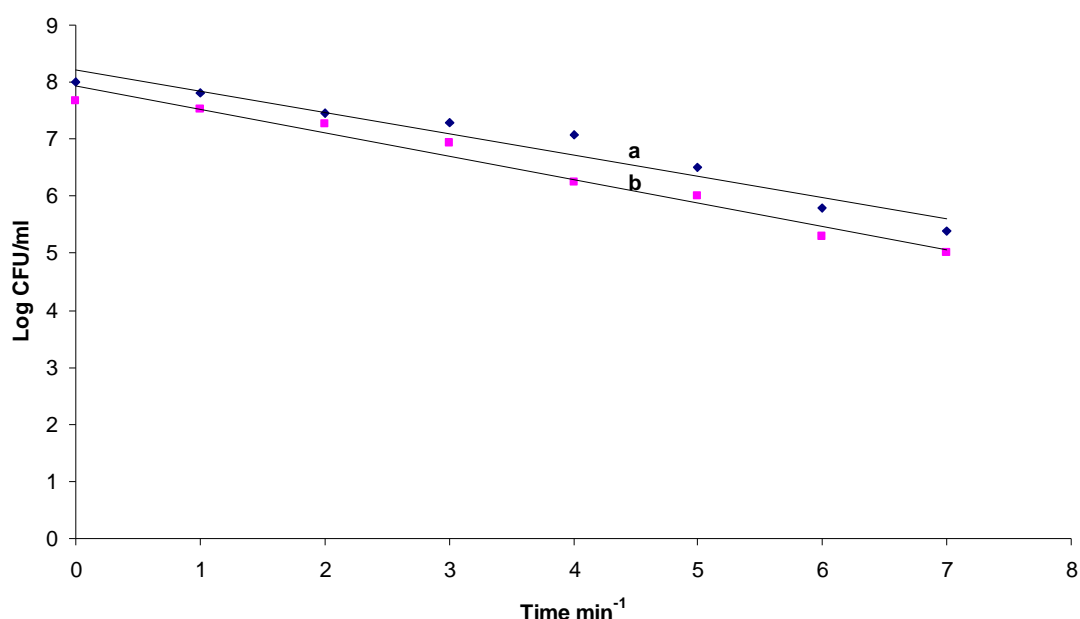
*Pseudomonads* can grow at temperatures between 0 – 20°C, optima growth is 15°C. They are frequently found in naturally cold waters and soils. The following experiments are conducted at temperature between 8°C and 25°C.

**Table 3.26 Log CFU/ml at two temperatures as a function of time**

*P. aeruginosa* =  $10^7$  CFU/ml; Flow rate of 2 L/min. Voltage = 40 V

Time	Temperature	
	8°C	25°C
	Log CFU/ml	
0	7.9988	7.6635
1	7.8049	7.5359
2	7.4538	7.26
3	7.2917	6.9251
4	7.078	6.2418
5	6.5108	5.9988
6	5.7948	5.2991
7	5.3968	4.9989

n = 2 and relative variance coefficient < 6%



**Figure 3.25 Log CFU/ml at two temperatures as a function of time**

Flow rate = 2 L/min. *P. aeruginosa* =  $10^7$  CFU/ ml;  $[O_3] = 1.89 \times 10^{-5}$  M. Temperature = Curve a: 8°C, b: 25°C.

**Table 3.27 Effect of temperature on ozone initiated inactivation of *P. aeruginosa***

*P. aeruginosa* =  $10^7$  CFU/ ml; Flow rate of 2 L/min. Voltage = 40 V

Temp./°C	k'/min <sup>-1</sup>	Half life /min
8	0.941	0.73
25	0.858	0.81
n = 2 and relative variance coefficient < 6%		

Figure 3.25 illustrate that varying the temperature from 8°C - 25°C at a fixed flow rate of 2 L/min and fixed voltage of 40 V does not affects the inactivation rate. The ozonation time is almost the same in both temperatures and the rate constants differed marginally by 8.8 %. The observation was similar to the results found on *Escherichia coli*. The results are presented in Table 3.26 and the rate constants in Table 3.27.

### 3.25 Effect of pH on *P. aeruginosa* inactivation

The experiments in duplicate were conducted in the pH range 4.91 – 9.28, the range in which most bacteria can withstand. The blank experiments in pH controlled distilled water

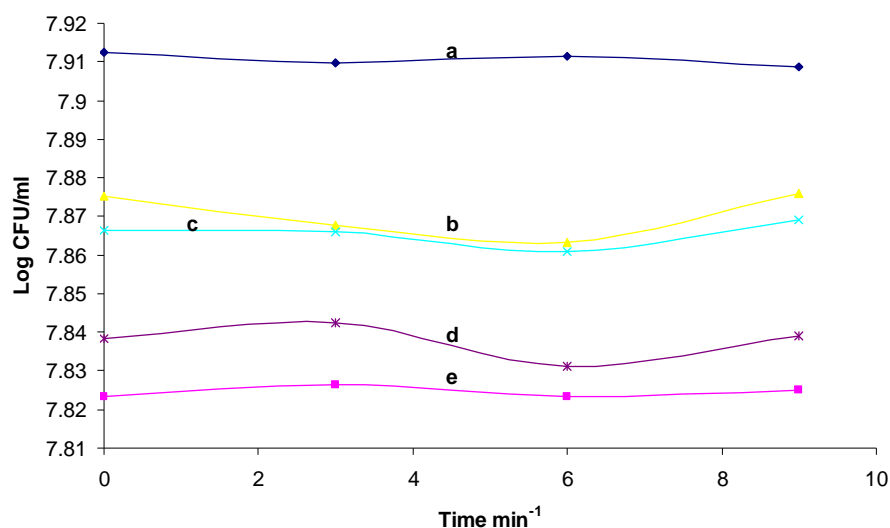
were also performed on *Pseudomonas aeruginosa* in absence of ozone with oxygen flow (2 L/min) under otherwise similar conditions and no inactivation of the microbe was observed, similar to previous observations on *E. coli*.

**Table 3.28 Control experiments at different pH of *Pseudomonas aeruginosa***

*P. aeruginosa* =  $10^7$  CFU/ml; Flow rate of 2 L/min. Temperature =  $25 \pm 2$  °C.

Time/ min	pH				
	4.91	5.95	6.96	7.96	9.28
	Log CFU/ml				
0	7.9126	7.8232	7.8753	7.8665	7.8384
3	7.9099	7.8265	7.8678	7.866	7.8423
6	7.9113	7.8232	7.8632	7.861	7.8313
9	7.9086	7.8249	7.8761	7.8691	7.8392

n = 2 and relative variance coefficient < 6%



**Figure 3.26 Control experiments at different pH of *Pseudomonas aeruginosa***

Flow rate = 2 L/min. *P. aeruginosa* =  $10^7$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve a: 4.91, b: 6.96, c: 7.96; d: 9.28 and e: 5.95.

Figure 3.26, illustrates and Table 3.28 presents the control runs that did not receive any ozone dose. The results shows that the base or acid plays no role in the inactivation of the

*P. aeruginosa* as the number of bacterial cells are constant after a period of time being exposed in acidic or basic medium as the same effect was observed on *Escherichia coli*.

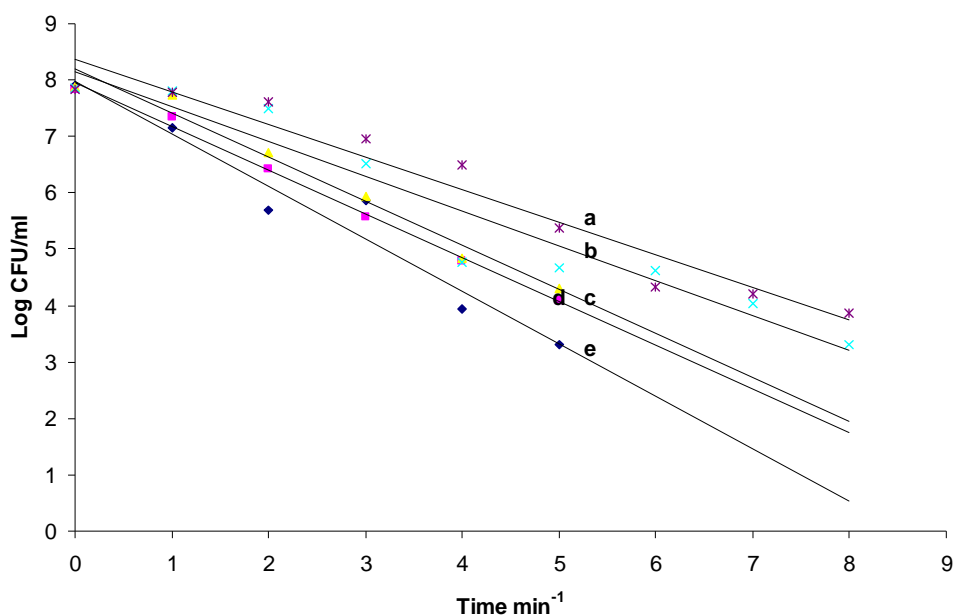
**Table 3.29 Log CFU/ml at various pH as a function of time**

*P. aeruginosa* =  $10^7$  CFU/ml; [Ozone] =  $1.89 \times 10^{-5}$  M. Flow rate = 2 L/min  
Temperature =  $25 \pm 2$  °C.

Time/ min	pH				
	4.91	5.95	6.96	7.96	9.28
	Log CFU/ml				
0	7.9126	7.8232	7.8753	7.8665	7.8384
1	7.1614	7.3509	7.7459	7.7981	7.7805
2	5.6978	6.4179	6.7049	7.4866	7.6142
3	5.8591	5.5786	5.9408	6.5107	6.9649
4	3.9408	4.7947	4.8439	4.7584	6.4936
5	3.3131	4.0957	4.2998	4.6639	5.3741
6				4.6272	4.3261
7				4.0499	4.2096
8				3.2979	3.8738

n = 2 and relative variance coefficient < 6%

Figure 3.27 shows the plot of log CFU/ml against time. The plot is straight line, confirming a first-order reaction, and the pseudo first-order rate constants calculated using the respective slopes are presented in Table 3.30.



**Figure 3.27 Log CFU/ml at various pH values as a function of time**

Flow rate = 2 L/min. *P. aeruginosa* =  $10^7$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve **a**: 9.28, **b**: 7.96, **c**: 6.96; **d**: 5.95 and **e**: 4.91.

It was observed that with ozone of  $1.89 \times 10^{-5}$  M at pH 4.91 produced a reduction of 4 log in 5 minutes in a population of approximately  $8 \times 10^8$  cells/ml, with the same dose at pH 9.28 the reduction was 4 log in 8 minutes in almost the same population. Table 3.29 summarizes the results obtained.

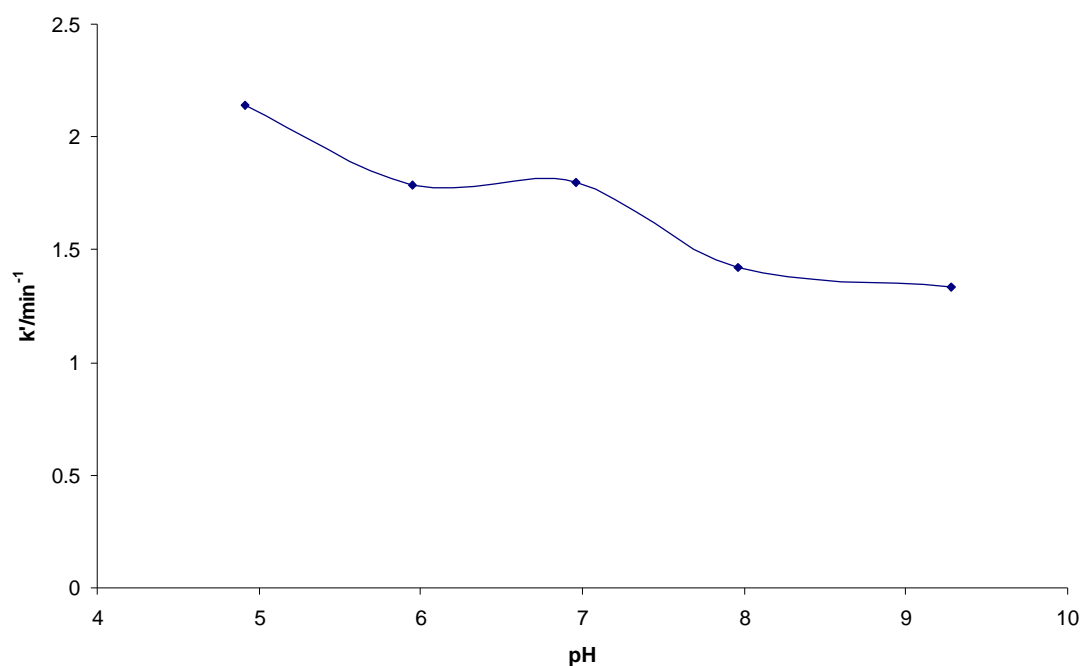
**Table 3.30 Kinetic data for ozonation of *Pseudomonas aeruginosa* at different pH**

Flow rate: 2 L/min; Temperature =  $25 \pm 2$  °C.

pH	$k'/\text{min}^{-1}$	Half life/min
4.91	2.138	0.32
5.95	1.786	0.39
6.96	1.799	0.38
7.96	1.423	0.49
9.28	1.333	0.52

$n = 2$  and relative variance coefficient < 6%

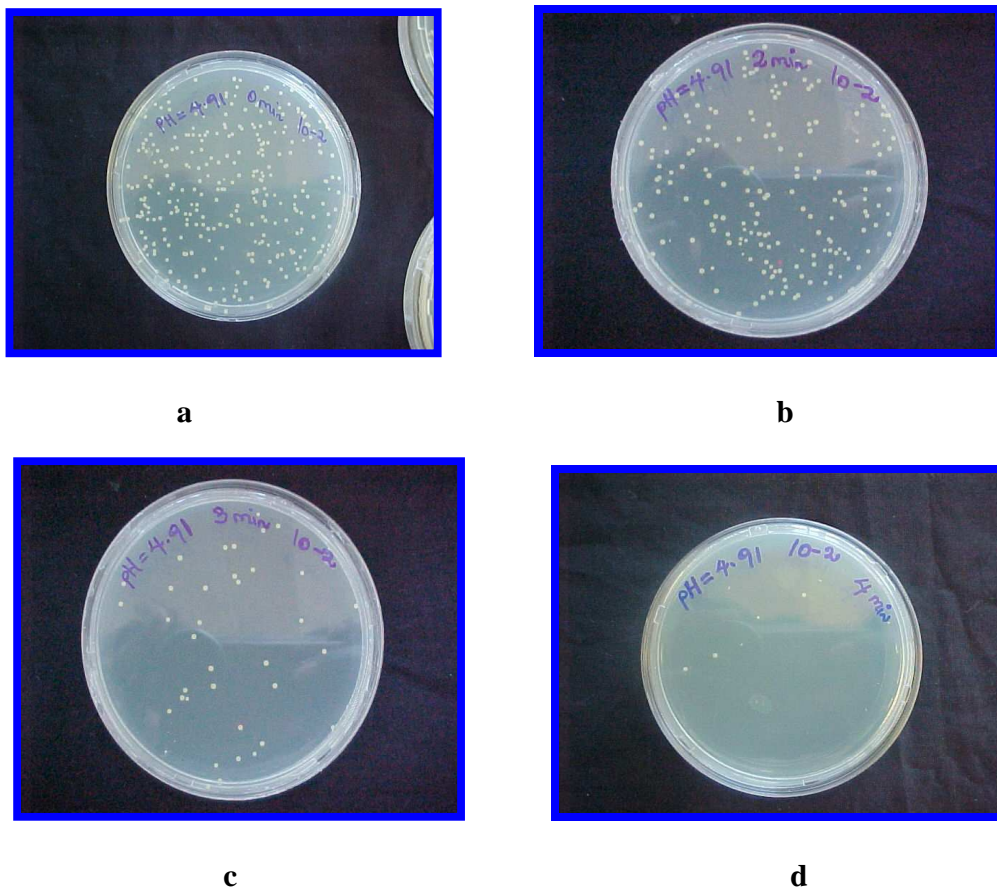
The rate constants were also determined and are presented in Table 3.30 and the rate constants decreased with the increasing pH and led to the decrease in inactivation rate. The magnitude of half life also supports the results since the inactivation took approximately 4 minutes at pH 4.91 while it took approximately 7 minutes at pH 9.28.



**Figure 3.28 Plot of pH against  $k'$  for *P. aeruginosa* inactivation**

As illustrated in Figure 3.28, the increase in pH has generally decreased the value of the inactivation rate constant. The  $k'$  values remained stable in the pH region 6 to 7, further decreasing at higher pH values. Ozone is more potent under acidic conditions suggesting molecular ozone may be reactive species in the inactivation of microbe.

Typical example of the effect of ozone on *Pseudomonas aeruginosa* colonies growing on a nutrient agar plate poised at pH 4.91 is shown in Figure 3.29 a, b, c and d overleaf. The rest of the figures are available on appendix C1.



**Figure 3.29** Typical example of the effect of ozone on *Pseudomonas aeruginosa* colonies growing on a nutrient agar plate poised at pH 4.91

[Ozone] =  $1.89 \times 10^{-5}$  M. *P. aeruginosa* =  $10^7$  CFU/ml; Flow rate = 2 L/min. Temperature =  $25 \pm 2$  °C. **a:** 0 min, **b:** 2 min, **c:** 3 min, **d:** 4 min. Dilution factor = **a:** 100, **b:** 100, **c:** 100, **d:** 100.

### 3.30 Inactivation experiments on *Bacillus subtilis* endospore

Limited experiments were performed to investigate the scope of ozone in the inactivation of *Bacillus subtilis* endospore which is gram positive bacterial strain. In this experiment, it is aimed to compare the ozone destruction of a sporeformer, a Gram-positive bacterium *B. subtilis* and Gram-negative bacteria, *E. coli* and *P. aeruginosa*.

### 3.31 Effect of ozone concentration on inactivation of *Escherichia coli* population

**Table 3.31 Control experiments for inactivation of *B. subtilis* without O<sub>3</sub>**

*B. subtilis* = 10<sup>7</sup> CFU/ml; Ozone = Nil; Flow rate 2L/min. Temperature = 25 ± 2 °C

Time/min	Log CFU/ml
0	7.0869
15	7.0957
30	7.0779

Table 3.31 presents that, no observable change in the *B. subtilis* count during the experiment proves that in absence of ozone, inactivation due to oxygen bubbled through the solution is negligible.

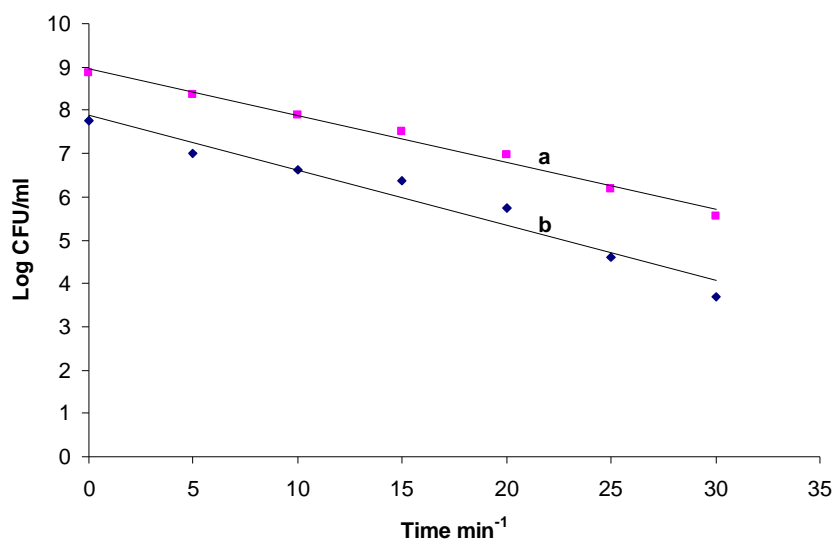
### 3.32 Effect of the population of *Bacillus subtilis* endospore cultured in nutrient broth in ozone inactivation

Experiments were undertaken to investigate influence of the initial population of bacteria on its ozone initiated inactivation, using two dilutions of stock culture of *B. subtilis*. Table 3.32 summarizes the log of *B. subtilis* count as function of time for the two runs.

**Table 3.32** Log CFU/ml at two dilutions of *B. subtilis* as a function of time

Ozone =  $1.89 \times 10^{-5}$  M. Flow rate 2 L/min; Temperature =  $25 \pm 2$  °C.

Time /min	$5.735 \times 10^7$	$7.479 \times 10^8$
	CFU/ml	CFU/ml
	Log CFU/ml	Log CFU/ml
0	7.7585	8.8739
5	6.9988	8.3523
10	6.6142	7.893
15	6.3767	7.5012
20	5.7431	6.9843
25	4.6008	6.1918
30	3.6977	5.5581
n = 2 and relative variance coefficient < 6%		

**Figure 3.30** Log CFU/ml at two dilutions of stock culture as a function of time  
Flow rate = 2 L/min. [*B. subtilis*] =  $10^7$ - $10^8$  CFU/ml = Curve a: 7.479, b: 5.735.

**Table 3.33 Pseudo first-order rate constants (k') for two dilutions of *B. subtilis***

[ <i>B. subtilis</i> ]/ CFU/ml	k/min <sup>-1</sup>
5.735 x 10 <sup>7</sup>	0.293 ± 0.008
7.479 x 10 <sup>8</sup>	0.294 ± 0.008

n = 2 and relative variance coefficient < 6%

The *pseudo* first-order rate constants were found to be almost constant as presented in Table 3.32 and 3.33. As presented in Table 3.32 and illustrated in Figure (3.30) the rate constants for both dilutions of the *B. subtilis* were similar, implying that the rate of inactivation of the microbes by ozone is independent of the initial concentration of the bacteria and the inactivation follows first-order kinetics with respect to *B. subtilis*. When investigating the effect of initial microbial density on disinfection efficiency, the data were analyzed and it was found that this phenomenon was valid for both Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive spore former bacteria (*B. subtilis*).

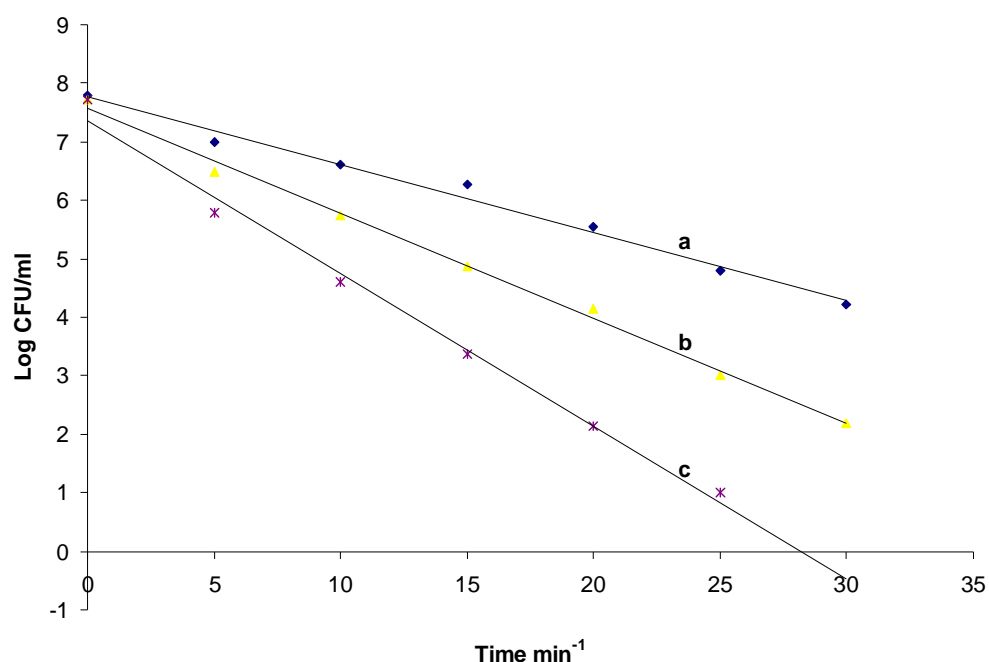
### 3.33 Effect of ozone concentration on the inactivation of *B. subtilis*

**Table 3.34 Kinetics of inactivation of *B. subtilis***

*B. subtilis* = 10<sup>7</sup> CFU/ml; Flow rate of 2 L/min. Temperature = 25 ± 2 °C.

	[Ozone]/10 <sup>-5</sup> M		
	1.89	5.79	9.84
Time/min	Log CFU/ml		
0	7.7856	7.7185	7.7124
5	6.9988	6.4988	5.783
10	6.6142	5.7425	4.6142
15	6.2767	4.8672	3.3767
20	5.5431	4.1431	2.1431
25	4.8008	3.0208	1.0062
30	4.2124	2.1782	

n = 2 and relative variance coefficient < 6%



**Figure 3.31 Effect of [ozone] on *B. subtilis* inactivation**

Flow rate = 2 L/min. *B. subtilis* =  $10^7$  CFU/ml;  $[O_3]/10^{-5}$  M = Curve a: 1.89, b: 5.79, c: 9.84.

Figure 3.31 shows that the bacteria population (measured as log CFU/ml) decrease exponentially with time. The larger the rate constant, the more rapid is the decay. The plot is straight, confirming a first-order reaction. As the ozone concentration is increased from  $(1.89- 9.84) \times 10^{-5}$  M, the microbial population decreases significantly as presented in Table 3.34 above.

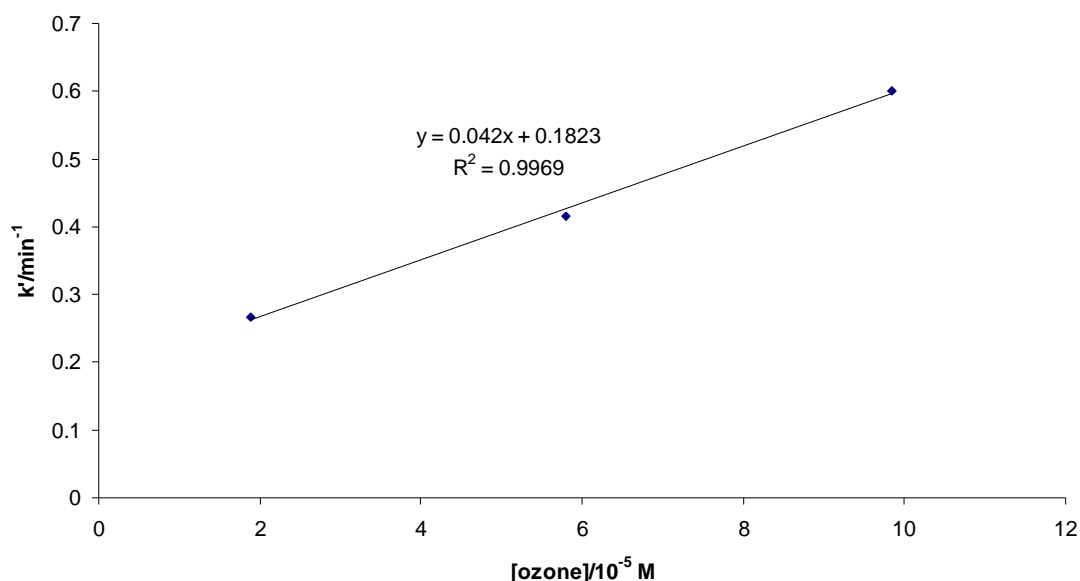
**Table 3.35 Pseudo first-order inactivation rate constants for *Bacillus subtilis***

Flow rate: 2 L/min; Temperature =  $25 \pm 2$  °C.

Voltage/ V	$[O_3]/$ $10^{-5}$ M	$O_3 / 10^{19}$ molecules	$k'/$ $\text{min}^{-1}$	Half life/ min
40	1.8875	1.136	0.267	2.594
60	5.7938	3.488	0.415	1.672
80	9.8417	5.925	0.601	1.153

n = 2 and relative variance coefficient < 6%

The pseudo first-order rate constants ( $k'$ ) estimated using the slopes of the curves are presented in Table 3.35. Table 3.35 also shows the voltage settings of the instrument and the ozone concentrations in the oxygen flow (expressed as moles/L and molecules/L) in addition to the half lives for the corresponding  $k'$  values.



**Figure 3.32** Plot of *pseudo* first-order rate constant against ozone concentration for inactivation of *B. subtilis*

The *pseudo* first-order rate constants were plotted against the corresponding initial concentrations of ozone (Figure 3.32), which gave a linear plot confirming that order with respect to ozone is first-order. The second-order rate constant for the overall reaction is represented by the slope =  $0.042 \text{ L mol}^{-1} \text{ min}^{-1}$ . Therefore the second-order rate constant may be expressed as  $k = 4.2 \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$ .

As was observed on the vegetative cells of two Gram-negative strains namely; *E. coli* and *P. aeruginosa*, the rate of inactivation of Gram-positive endospore *B. subtilis* increases as the concentration of ozone is increased. The higher the voltage of the generator applied, the higher the ozone concentration and the rate of inactivation.

Table 3.36 summarizes the second order rate constants obtained for the three microbes used in this study as test organisms.

**Table 3.36 Second order rate constants for three microbes**

Microbe	$k'/M^{-1} \text{ min}^{-1}$
<i>E. coli</i>	$4.9 \times 10^4$
<i>P. aeruginosa</i>	$4.575 \times 10^4$
<i>B. subtilis</i>	$4.20 \times 10^3$

The rate constants tabulated in Table 3.36 shows that the rate of inactivation was higher in gram negative microbes, *E. coli* and *P. aeruginosa* since the values are  $4.9 \times 10^4$  and  $4.575 \times 10^4 M^{-1} \text{ min}^{-1}$  respectively. The rate of inactivation on gram positive *B. subtilis* was found to be lower since it possesses an endospore and has the value of  $4.20 \times 10^3 M^{-1} \text{ min}^{-1}$ .

### 3.34 Effect of temperature on the *Bacillus subtilis* endospore

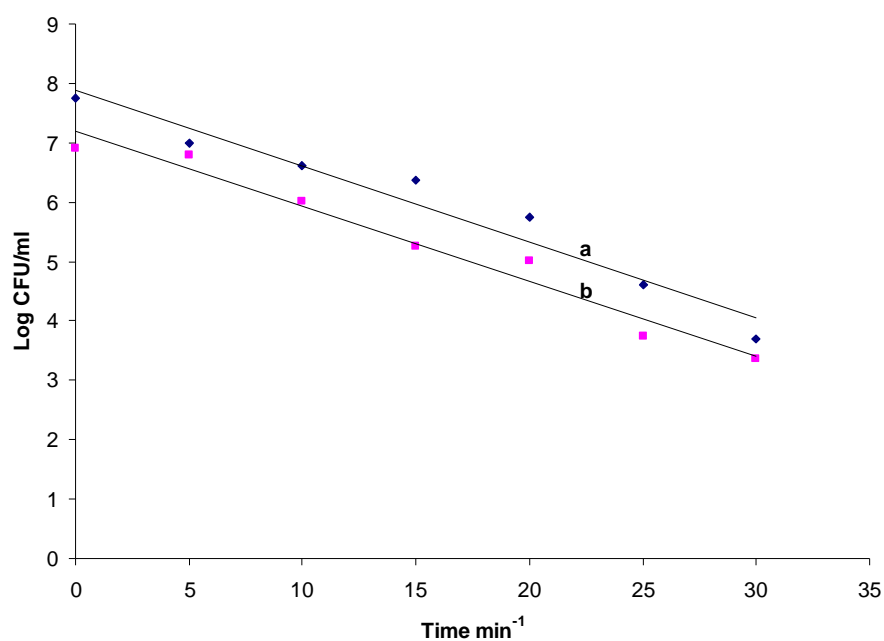
The genus bacillus encompasses a great diversity of strains. Some species are strictly aerobic, others are facultative anaerobes. The bacilli also exhibit variation in temperatures of growth; some thermophiles grow from a minimum temperature of 45°C to 75°C or higher, and some psychrophiles grow at temperature from -5°C to 25°C. The following experiments on *B. subtilis* are conducted between 8°C and 25°C.

**Table 3.37 Log CFU/ml at two temperatures as a function of time**

*B. subtilis* =  $10^7$  CFU/ml; Flow rate 2 L/min. ozone =  $1.89 \times 10^{-5}$  M. Voltage = 40 V

Temp. →	25°C	8°C
Time/min	Log CFU/ml	
0	7.7585	6.9152
5	6.9988	6.7947
10	6.6142	6.023
15	6.3767	5.2504
20	5.7431	5.0161
25	4.6008	3.7352
30	3.6977	3.3744

n = 2 and relative variance coefficient < 6%

**Figure 3.33 Log CFU/ml at two temperatures as a function of time**

Flow rate = 2 L/min. *B. subtilis* =  $10^7$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. Temperature = Curve a: 25°C, b: 8°C.

**Table 3.38 Kinetic data for ozonation of *Bacillus subtilis* at two temperatures**

Temp.	k/min <sup>-1</sup>	Half life/min
8°C	0.292	2.373
25°C	0.294	2.357
n = 2 and relative variance coefficient < 5%		

Figure 3.33 illustrate that temperature does not affect the rate of inactivation as this is observed on the experiments conducted on *Escherichia coli* and *Pseudomonas aeruginosa* and the rate constants and magnitude of  $t_{1/2}$  presented in Table 3.38 which are only marginally different. Results obtained are summarized in Table 3.37.

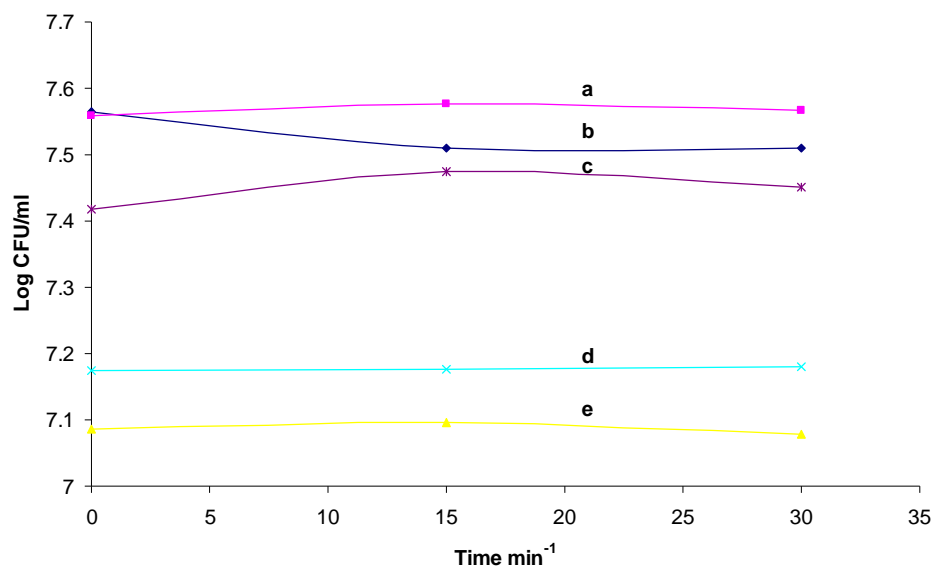
### 3.35 Effect of pH on *Bacillus subtilis* endospore

The experiments in duplicate were conducted in the pH range 4.93 – 9.16. The blank experiments in pH controlled distilled water were also performed on *B. subtilis* in absence of ozone with oxygen flow (2 L/min) under otherwise similar conditions and no inactivation of the microbe was observed as illustrated in Figure 3.34 and presented in Table 3.39, similar to previous observations on *E. coli* and *P. aeruginosa*.

**Table 3.39 Control experiments at different pH of *Bacillus subtilis***

*B. subtilis* = 10<sup>7</sup> CFU/ml; Flow rate of 2 L/min. Temperature = 25 ± 2 °C.

Time/ min	pH				
	4.93	5.93	6.95	7.93	9.16
	Log CFU/ml				
0	7.5639	7.5596	7.0869	7.1749	7.4179
15	7.5107	7.5757	7.0957	7.1768	7.4749
30	7.5107	7.5669	7.0779	7.1811	7.4509
n = 2 and relative variance coefficient < 6%					



**Figure 3.34 Control experiments at different pH of *Bacillus subtilis* endospore**  
 Flow rate = 2 L/min. *B. subtilis* =  $10^7$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve **a**: 5.93, **b**: 4.93, **c**: 9.16; **d**: 7.93 and **e**: 6.95.

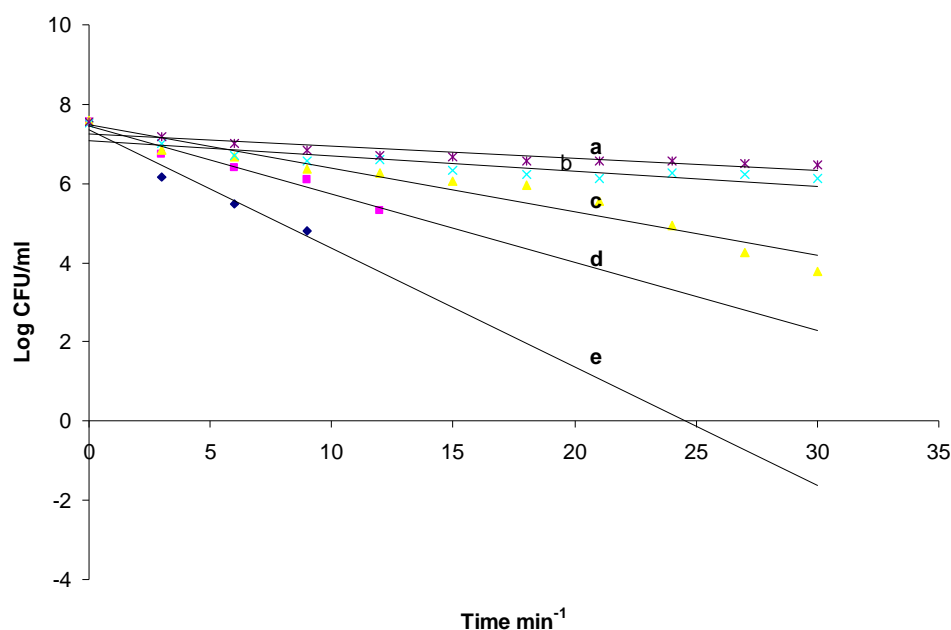
Table 3.39 above presents the results obtained for control runs that did not receive any ozone dose, the results show that the base or acid plays no role in the inactivation of the *Bacillus subtilis* as the number of bacterial cells are constant after a period of time being exposed in acidic or basic medium as the same effect was observed on *Escherichia coli* and *Pseudomonas aeruginosa*. Figure 3.34 further illustrate the results obtained.

**Table 3.40 Log CFU/ml at various pH as a function of time**

*B. Subtilis* =  $10^7$  CFU/ml; [Ozone]  $1.89 \times 10^{-5}$  M. Temperature =  $25 \pm 2$  °C.

Time/ min	pH				
	4.93	5.93	6.95	7.93	9.16
Log CFU/ml					
0	7.56	7.5596	7.5869	7.5179	7.5596
3	6.1769	6.7408	6.8439	6.9978	7.1683
6	5.4866	6.4116	6.6584	6.7156	6.9969
9	4.7947	6.0869	6.3519	6.5699	6.8329
12		5.2998	6.2714	6.594	6.6889
15			6.0565	6.3246	6.6552
18			5.9626	6.2303	6.5814
21			5.561	6.1159	6.5697
24			4.9469	6.2683	6.5581
27			4.2718	6.2131	6.5059
30			3.7947	6.1291	6.4536

n = 2 and relative variance coefficient < 6%



**Figure 3.35 Log CFU/ml at various pH as a function of time**

Flow rate = 2 L/min. *B. subtilis* =  $10^7$  CFU/ml;  $[O_3] = 1.89 \times 10^{-5}$  M. pH = Curve **a**: 9.16, **b**: 7.93, **c**: 6.95; **d**: 5.93 and **e**: 4.93.

It was observed that ozone of  $1.89 \times 10^{-5}$  M at pH 4.93 produced a reduction of 3 log in 9 minutes in population of approximately  $3 \times 10^7$  cells/ml, while the same dose of ozone at pH 9.16 produced a reduction of 1 log in 30 minutes of the same population. The *B. subtilis* inactivation needed longer ozonation duration as compared to *E. coli* and *P. aeruginosa*, because it possesses an endospore. The results obtained are summarised in Table 3.40 and illustrated in Figure 3.35 above.

When comparing the inactivation kinetics of the three strains, the *B. subtilis* was found to be more resistant than the vegetative bacteria namely, *P. aeruginosa* and *E. coli* because it has ability to form a tough, protective endospore. Similar results were reported by Bablon *et al.*, 1991.

Cho *et al.* (2003) in their study reported that in pH controlled distilled water, *Bacillus subtilis* endospore population ranged approximately from  $10^4$  to  $10^6$  CFU/ml inactivation by ozone of approximately 1.0 to 4.0 mg/L at pH 8.2 was approximately 25% more

effective than that at pH 5.6 because of the hydroxyl radicals presence. This study found that molecular ozone (lower pH) is more effective than the hydroxyl radicals (higher pH).

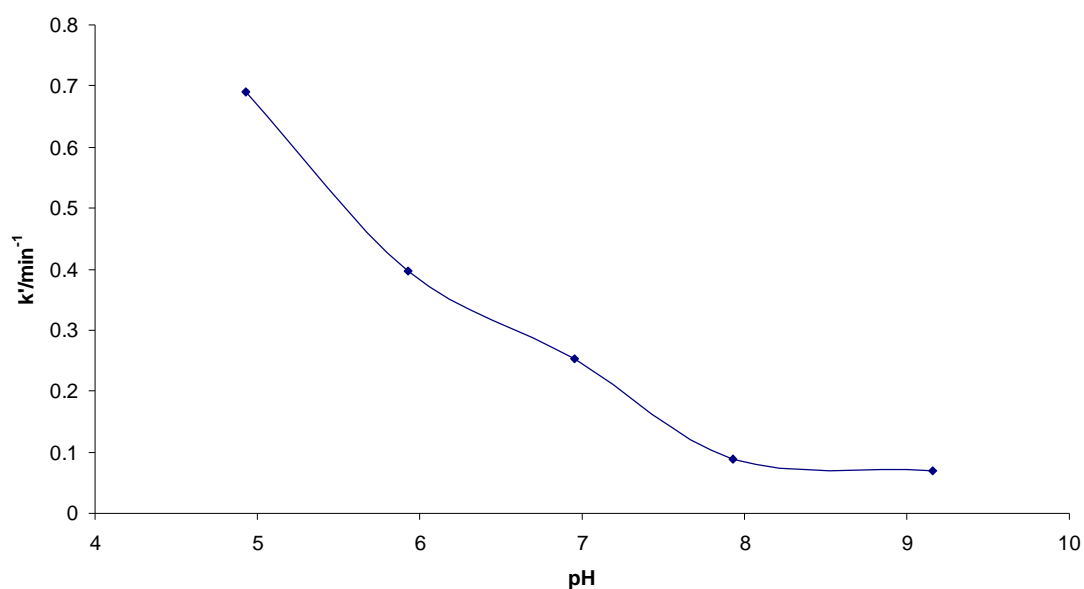
**Table 3.41 Kinetics of ozonation of *Bacillus subtilis* at different pH conditions**

Flow rate: 2 L/min; Temperature =  $25 \pm 2$  °C.

pH	$k'$ /min <sup>-1</sup>	Half life/min
4.93	0.6897	1.0047
5.93	0.3972	1.7447
6.95	0.2531	2.7380
7.93	0.0887	7.8128
9.16	0.0707	9.8019

n = 2 relative variance coefficient < 6%

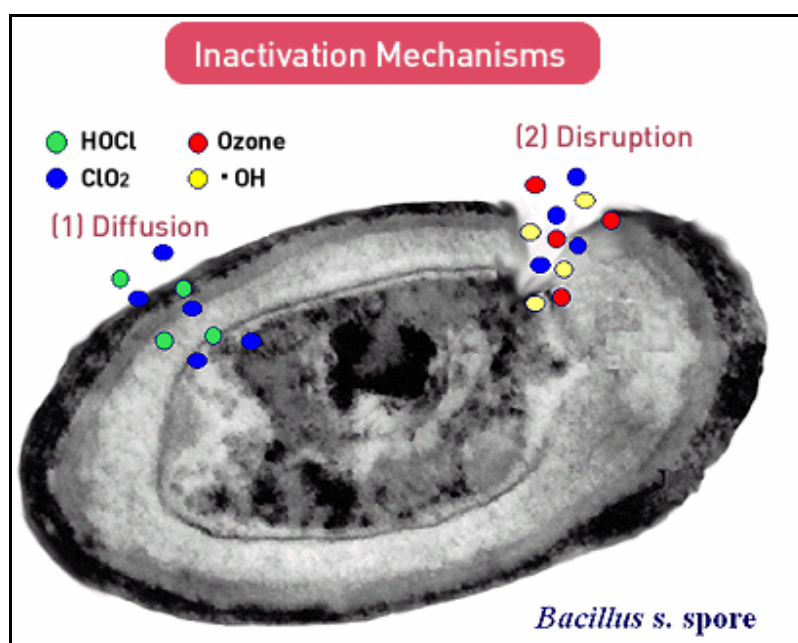
The rate constants were also determined and are presented in Table 3.41 as the pH increased from 4.93 to 9.16, the rate constants decreased significantly. Thus the rate of inactivation decreased as reflected by the increased half life for the reaction.



**Figure 3.36 Plot of  $k'$  against pH for *B. subtilis* inactivation**

As illustrated in Figure 3.36, the increase in pH has generally decreased the value of inactivation rate constant. The  $k'$  values are higher at pH region 5 to 7, decreasing with increasing pH values. Ozone is more potent under acidic conditions suggesting that molecular ozone may be the reactive species in the inactivation mechanism of microbe.

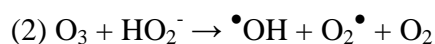
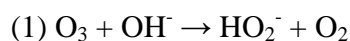
The ability to oxidize biological molecules and the ability to diffuse through the cell walls are requirements of an effective disinfectant. The following Figure 3.37 illustrates the inactivation mechanisms by different disinfectants on the *Bacillus subtilis* endospore.



**Figure.3.37 Inactivation mechanisms of *Bacillus subtilis* spore**  
(Yoon-jeyong, 2008)

Though the four disinfectants indicated on the Figure 3.37 are all effective against the *B. subtilis* endospore, the mechanisms they use to attack differs significantly, and that leads to other disinfectants to be more favoured than the other. For instance the inactivation of *B. subtilis* endospore by ozone, chlorine chloride and hydroxyl radicals is attributed to an oxidation or rupture of the cell wall with consequent cellular disintegration, while hypochlorite and chlorine dioxide first diffuse into the cell before they interfere with cellular activity and more duration time is required before the disinfection is achieved.

The kinetics of inactivation of *Bacillus subtilis*, *P. aeruginosa* and *E. coli* were much faster in acidic medium than in basic medium. The reason for improved inactivation of these strains at moderately lower is possibly due to the reaction of ozone in aqueous solution with microbes by two major pathways. One is the direct attachment by molecular ozone, which is predominant at low pH, and indirect reaction by the radical species formed when ozone decomposes at high pH. At higher pH ozone decomposes partly and the formation of  $\text{OH}^\bullet$  radicals increase as hydroxide ions acts as initiators for the decay of ozone.



The radicals produced during reaction (2) can introduce other reactions with ozone, causing more  $\text{OH}^\bullet$  radicals to be formed. However this study shows that the free radicals formed by the decomposition of ozone are less effective for microbial inactivation than molecular ozone. A possible explanation could be that there are hydroxyl radical scavengers present at higher pH such as bicarbonate ions ( $\text{HCO}_3^-$ ) which maybe contained by microbial cells that quench the free radical reaction, or perhaps it could be the catalase enzyme that is contained by the *E. coli*, *P. aeruginosa* and *B. subtilis* that controls the free radicals produced by aerobic respiration (WHO, 2004). This also suggests that the factors that accelerate ozone decomposition are undesirable for inactivation because the ozone residual dissipates faster and therefore reduces the contact time credit, requiring a corresponding increase in the ozone applied, whereas in acid medium the molecular ozone predominate and therefore the inactivation is more effective.

Ozone is known to act by two paths, i.e., direct attack by molecular ozone and indirectly through the hydroxyl radical (EPA, 1999).

Rate Law:

$$\text{Rate of inactivation} = \{k_1[\text{O}_3] + k_2 [\bullet\text{OH}]\}[\text{M}],$$

where  $[M]$  represents the microbe and  $k_1$  and  $k_2$  are the rate constants for inactivation of the microbe by the molecular ozone and the hydroxyl radical respectively. With ozone concentration in excess, the reaction may be expressed as,

$$\text{Rate} = k'[M],$$

where  $k'$  represents the pseudo first-order rate constant and  $k' = \{k_1[O_3] + k_2[{}^{\bullet}OH]\}$ . The concentrations of molecular ozone and  ${}^{\bullet}OH$  radical are a function of pH. To simplify the proposed reaction mechanism, one needs to consider the reactivity of dissolved oxygen in water and the gaseous ozone towards the microbe are not be the same. Possibly the dissolved ozone will be more effective in disinfection than the gaseous ozone due its longer residence time. The distribution of ozone between aqueous phase and gaseous phase is clearly the function of overall ozone concentration and steady state will be maintained between them. As the pseudo first-order constant,  $k'$  is the resultant of two path ways and the reactivity of ozone is a function of its solubility in water and pH.

Generally all three microbial strains showed similar behaviour, i.e., a first-order rate dependence on microbe and the ozone concentrations. The three microbial strains have different extent of resistance to ozone mainly because ozone susceptibility depends upon the structure of the bacteria. The cell envelopes of bacteria are composed of intricate multilayers. Covering the bacterial cytoplasm to form the innermost layer of the envelope is the cytoplasmic membrane, made of phospholipids and proteins; a polymeric layer built with a giant peptidoglycan molecule provides bacteria with a stable architecture (Russell, 1995).

The gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* were more susceptible to ozone due to the fact that the gram negative bacteria possess a thin peptidoglycan lamella on which is superimposed an outer membrane made of lipoproteins and polysaccharides therefore the contact time is smaller because of the weaker resistance. By contrast, gram positive endospore organism like *Bacillus subtilis*, the peptidoglycan shell is thick and rigid thereby increasing the contact time because of the strong resistance. Bacterial spores are invariably the most resistant of all types of bacteria. They have an additional spore coat, which makes them more resistant to the action of some agents.

Nevertheless, this study confirms that ozone has a high germicidal effectiveness against the three strains.

### 3.40 Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD)

The dissolved oxygen (DO) test measures the amount of oxygen dissolved in the water. Oxygen is essential for both plants and animals, but high levels in water can be harmful to fish and other aquatic organisms. Non-point source pollution can decrease the amount of dissolved oxygen in water. The decomposition of leaf litter, grass clippings, sewage, and runoff from feedlots decreases the DO readings (Lower Colorado River Authority, 1996). Decreased dissolved oxygen can be harmful to fish and other aquatic organisms. Dissolved oxygen is measured in milligrams per litre (mg/L). Expected levels range from 4.0 to 12.0 mg/L respectively.

Biochemical oxygen demand refers to the amount of oxygen that microorganisms require to decompose the organic matter in water. Chemical oxygen demand is the measure of the amount of oxygen required to oxidise the organic matter content of a sample that is susceptible to oxidation by a strong oxidant such as potassium dichromate.

Five water matrices were considered for the study of BOD

- (i) Raw river water
- (ii) River water spiked with *Escherichia coli*.
- (iii) River water spiked with *Pseudomonas aeruginosa*.
- (iv) River water spiked with *Escherichia coli* and ozonized.
- (v) River water spiked with *Pseudomonas aeruginosa* and ozonized.

Tables 3.42 and 3.43 summarize the values for the amount of dissolved oxygen and the calculated BOD<sub>5</sub> values respectively estimated from duplicate runs.

#### Table 3.42 Amount of dissolved oxygen in incubated water as a function of time

River water = ca.300 ml; Ozone =  $1.89 \times 10^{-5}$  M; Flow rate = 2 L/min; Ozonation duration = 15 minutes. Temperature =  $25 \pm 2$  °C.

Samples	Dissolved oxygen/ mg/L				
	Day 1	Day 2	Day 3	Day 4	Day 5
(i) River water- Control	10.22	9.98	9.00	8.64	5.96
(ii) River water spiked with <i>E. coli</i>	9.88	4.16	0.20	0.12	0.12
(iii) River water spiked with <i>P. aeruginosa</i>	10.08	9.92	0.20	0.10	0.10
(iv) River water spiked with <i>E. coli</i> & ozonised	12.09	12.51	12.50	11.69	11.96
(v) River water spiked with <i>P. aeruginosa</i> & ozonised	12.26	12.68	12.02	11.68	12.00

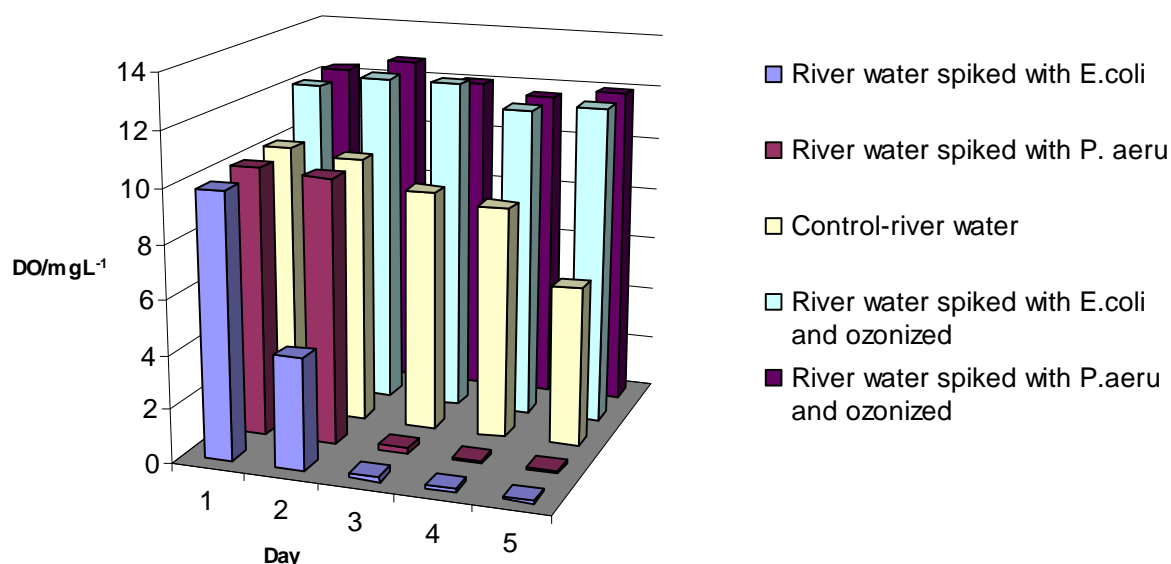
As presented in Table 3.42, the dissolved oxygen for ozonised samples is higher than the samples that are not ozonised. This was probably due to microorganisms present in the sample were inactivated by ozonation and therefore no oxygen was further consumed. Also due the decomposition of ozone to oxygen the amount of oxygen can increase.

**Table 3.43 Calculated BOD<sub>5</sub> values of Msunduzi River water under different treatments**

River water samples = ca.300 ml; Ozone =  $1.89 \times 10^{-5}$  M; Flow rate = 2 L/min. Ozonation duration = 15 minutes; Temperature =  $25 \pm 2$  °C.

Sample	BOD <sub>5</sub> /(mg/L)
(i) River water- Control	4.26
(ii) River water spiked with <i>E. coli</i>	9.76
(iii) River water spiked with <i>P. aeruginosa</i>	9.98
(iv) River water spiked with <i>E. coli</i> & ozonised	0.38
(v) River water spiked with <i>P. aeruginosa</i> & ozonised	0.76

The results presented in Tables 3.42 and 3.43 show that firstly the natural river water used as control has high BOD demand suggesting it has biological activity which consumes oxygen. The water sample not spiked with *P. aeruginosa* or *E. coli* the amount of oxygen used up by microorganism originally present in water was 4.26 mg/L. However, when the water was spiked by the facultative anaerobe *Escherichia coli* and *Pseudomonas aeruginosa* the amount of oxygen used up is almost doubled 9.76 mg/L and 9.98 mg/L, respectively showing that as the number of microorganisms increase, the demand for oxygen increases proportionally. It might not be entirely due to the fact that the water is heavily contaminated, but all microbes require oxygen for the growth.



**Figure 3.38** 3-D plot of dissolved oxygen levels in river water under different treatments

In water samples spiked by *Escherichia coli* or *Pseudomonas aeruginosa* and then ozonised the amount of oxygen used up is as low as 0.3 mg/L and 0.32 mg/L respectively which shows that microorganisms that were originally present in water as well as the microorganisms spiked are all effectively destroyed or deactivated resulting in termination of growth and reproduction, therefore no depletion of available oxygen.

**Table 3.44 COD Determination – Titration values**

Sample	Titration values
River water-control	5.73 ml
Deionised water	6.90 ml
COD (mg/L)	9.36 mg/L

The contribution of microbes towards COD is small and hence the COD determinations were not done with spiked samples. When COD and BOD values were compared to allow for differentiation between biologically oxidizable matter and biologically inert matter, BOD values were found to be lower 4.26 mg/L as presented in Table 3.43 than COD values 9.36 mg/L as presented in Table 3.44, which shows that some materials such as cellulose reacted with dichromate present in COD tests, but not the oxygen present under biological conditions.

## 4.0 Conclusions

The choice of any technology depends on its advantages and limitations in addition to cost considerations. The cost of ozone disinfection systems depend on the manufacturer, the site location, the capacity of the plant, and the characteristics of the wastewater to be disinfected. Ozonation costs are generally high in comparison with other disinfection techniques, such as chlorination. In general, costs are largely influenced by site-specific factors, and thus, the estimates that follow are typical values and can vary from site to site. Obviously, ozonation costs will be relatively lower when comparatively clean water source to be treated for drinking purposes.

Typical cost estimate of ozone disinfection to disinfect one mgd of wastewater (BOD content < 30 mg/L and SS content < 30 mg/L) are the annual costs including labour (US\$ 12 000), power 90 kW and other (filter replacements, compressor oil, spare dielectric, etc) (US\$ 6 500), destruct unit small -around 30 cfm (US\$ 800), large - around 120 (US\$ 1 000-1 200), non-component costs, (US\$ 35000), engineering (US\$ 12 000-15 000) and contingencies 30%. Capital costs include oxygen feed gas and compressor (US\$ 245 500) and contact vessels -500 gpm (US\$ 4 000 – 5 000) (U.S EPA, 1999).

The cost of chlorine disinfection systems also depend on the manufacturer, the site, the capacity of the plant, and the characteristics of the wastewater to be disinfected. A study conducted by the Water Environment Research Foundation in 1995 for the average dry weather flow of 1 million gallons per day showed an estimated cost of \$49,300 per year. (A chlorine dose of 5 to 20 mg/L was used from a 1-ton gas cylinder.) The annual costs include power consumption, cleaning supplies, miscellaneous equipment repairs, and personnel costs. Generally, the total cost of chlorination will increase by about 30 to 50% when adding the dechlorination step. In addition, hypochlorite compounds are more expensive than chlorine gas.

Based on the current studies, the populations of *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria were found to be quite susceptible to ozone. By ozonation

effective inactivation of the microbial count was achieved. From the results for the three strains investigated, with excess ozone concentrations maintained, the kinetics of inactivation of microbes followed *pseudo* first-order kinetics. Further the order with respect to ozone was also first-order in all three studies, thus resulting overall second order kinetics. The overall second order rate constants for inactivation of *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were  $4.90 \times 10^4$ ,  $4.58 \times 10^4$  and  $4.20 \times 10^3$  respectively.

Using  $1.89 \times 10^{-5}$  M ozone and 2 L/min flow rate, for *Escherichia coli* and *Pseudomonas aeruginosa* strains the half lives for inactivation were 0.49 and 1.10 minutes, while for the gram-positive *Bacillus subtilis* it was 2.55 minutes. Under the identical conditions for *Escherichia coli* and *Pseudomonas aeruginosa*, the log inactivation achieved in six minutes were two and one respectively, while for the gram-positive *Bacillus subtilis* it was approximately 1 log in 30 minutes. This was because *Bacillus subtilis* was more resistant form of bacterium, as it has ability to form a tough, protective endospore allowing it to tolerate extreme environmental conditions. Nevertheless ozone has a high germicidal effectiveness against the strains.

The rate of inactivation of microbes primarily depended on the ozone concentration and the effect of temperature is marginal. The ozone treatment is more effective in disinfection under slightly acidic condition. This confirms that disinfection is mainly through direct attack of molecular ozone on the microbe.

Ozone has proved be a powerful oxidant able to achieve inactivation. Ozone significantly reduced the BOD and COD values of river water samples. After ozone treatment, the only significant residual is the dissolved oxygen. This study demonstrates that ozone is a preferable disinfectant for the treatment of the effluents for the destruction of pathogens.

## 5.0 References

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### Appendix A: Calculation for [O<sub>3</sub>] in the gas

In a typical example:

Voltage: 40 V and flow rate = 2 L min<sup>-1</sup>

[Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>]: 0.05208 M

Volume of. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used: 0.29 ml

Moles Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used: 0.05208 mol/L x 0.00029 L = 1.510 x 10<sup>-5</sup> mol

Moles O<sub>3</sub>: [1.510 x 10<sup>-5</sup> / 2 (stoichiometric factor)] x 10 (dilution factor)  
= 7.550 x 10<sup>-5</sup> mol

Mass of O<sub>3</sub>: 7.550 x 10<sup>-5</sup> x 3 x 15.999 g mol<sup>-1</sup> = 0.00362 g

Concentration of O<sub>3</sub>: 0.00362 g x 0.25 (Flow rate = 2 L min<sup>-1</sup>, time = 2 min)  
= 0.906 mg L<sup>-1</sup>

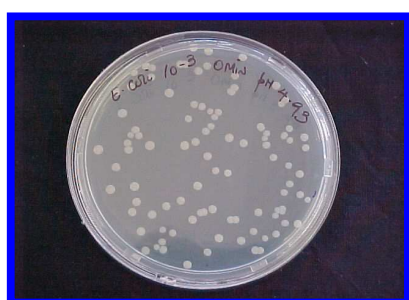
Concentration of O<sub>3</sub> in M and molecules L<sup>-1</sup> = 0.906 x 10<sup>-3</sup> g L<sup>-1</sup>/48 gmol<sup>-1</sup>  
= 1.8875 x 10<sup>-5</sup> M  
= 1.8875 x 10<sup>-5</sup> M x 6.02 x 10<sup>23</sup> molecules mole<sup>-1</sup>  
= 1.1363 x 10<sup>19</sup> molecules L<sup>-1</sup>

NB: The concentration of ozone calculated above is the concentration of ozone in the gas which had been bubbled into the KI solution.

## Appendix B: *Escherichia coli* (DH5 $\alpha$ )

### Appendix B1: Representative nutrient agar plates of *E. coli* as function of time using ozone disinfectant: pH 4.93

Conditions:  $1.89 \times 10^{-5}$  M; Flow rate: 2 L/min; [*Escherichia coli*] =  $10^8$  CFU/ml. Growth medium: Nutrient agar plate. Time (min) = (a - c), control experiments: a: 0, b: 3, c: 9, d: 1, e: 2, f: 3, g: 4 & h: 5. Dilution factor = (a - c): 1000, d: 100, e: 100, f: stock, g: stock, h: stock.



a



b



c



d



e



f

**h****g**

**Appendix B2: Representative nutrient agars plates of *E. coli* as function of time using ozone disinfectant: pH 5.93**

Time (min) = (a - d), control experiments: a: 0, b: 3, c: 6, d: 9, e: 1, f: 2, g: 3 & h: 4.

Dilution factor = (a - d): 1000, e: 100, f: 10, g: stock, h: stock



a



b



c



d



e



f

**g****h**

**Appendix B3: Representative nutrient agars plates of *E. coli* as function of time using ozone disinfectant: pH 6.96**

Time (min) = (a - d), control experiments: a: 0, b: 3, c: 6, d: 9, e: 1, f: 2, g: 3, h: 4, i: 5, j: 6, k: 7, l: 8. Dilution factor = (a - d): 1000, e: 1000, f: 1000, g: 100, h: 100, i: stock, j: stock, k: stock, l: stock



a



b



c



d



e



f

**g****h****i****j****l****k**

**Appendix B4: Representative nutrient agars plates of *E. coli* as function of time using ozone disinfectant: pH 7.93**

Time (min) = (a - c), control experiments: a: 0, b: 3, c: 9, d: 1, e: 2, f: 4, g: 6, h: 7, i: 8, j: 10. Dilution factor = (a - c): 1000, d: 1000, e: 100, g: stock, h: 10, i: stock, j: stock.



a



b



c



d



e



f

**g****h****i****J**

**Appendix B5: Representative nutrient agars plates of *E. coli* as function of time using ozone disinfectant: pH 9.16**

Time (min) = (a - d), control experiments: a: 0, b: 3, c: 6, d: 9, e: 1, f: 2, g: 3, h: 4, i: 5, j: 6, k: 7, l: 8, m: 9, n: 10. Dilution factor = (a - d): 1000, d: 1000, e: 1000, g: 1000, h: 100, i: 10, j: 10, k: stock, l: stock, m: stock, n: stock.



a



b



c



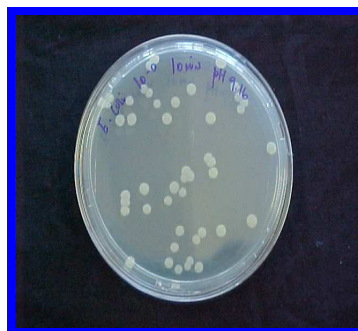
d



e



f

**g****h****j****i****k****l****m****n**

## Appendix C: *Pseudomonas aeruginosa*

### Appendix C1: Representative nutrient agar plates of *P. aeruginosa* as function of time using ozone disinfectant: pH 4.91

Conditions:  $1.89 \times 10^{-5}$  M; Flow rate: 2 L/min; [*P. aeruginosa*] =  $10^8$  CFU/ml. Growth medium: Nutrient agar plate. Time (min) = (a - d), control experiments: a: 0, b: 3, c: 6, d: 9, e: 1, f: 2, g: 3, h: 4, i: 5. Dilution factor = (a - d): 100, e: 100, f: 100, g: 100, h: 100, i: stock, j: stock.



a



b



c



d



e



f

**g****h****i****j**

**Appendix C2: Representative nutrient agar plates of *P. aeruginosa* as function of time using ozone disinfectant: pH 6.00**

Time (min) = (a - b), control experiments: a: 0, b: 3, c: 1, d: 2, e: 3, f: 4, g: 5, h: 6.

Dilution factor = (a - b): 100, c: 100, d: 10, e: stock, f: stock, g: stock, h: stock



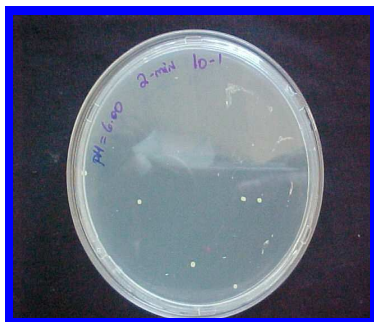
a



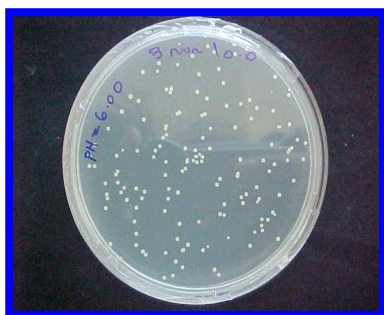
b



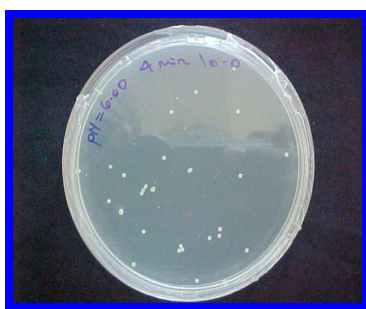
c



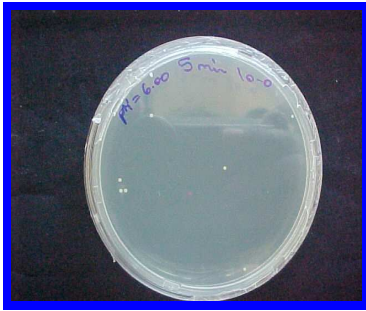
d



e



f

**g****h**

**Appendix C3: Representative nutrient agar plates of *P. aeruginosa* as function of time using ozone disinfectant: pH 6.96**

Time (min) = (a - c), control experiments: a: 0, b: 3, c: 9, d: 1, e: 2, f: 3, g: 4, h: 5, i: 6.

Dilution factor = (a - c): 100, d: 100, e: 100, f: stock, g: stock, h: stock, i: stock



a



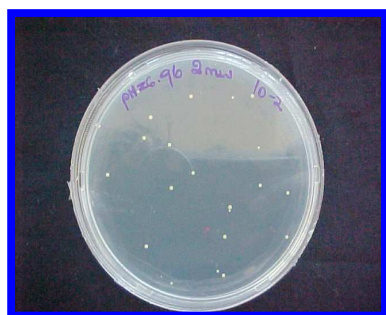
b



c



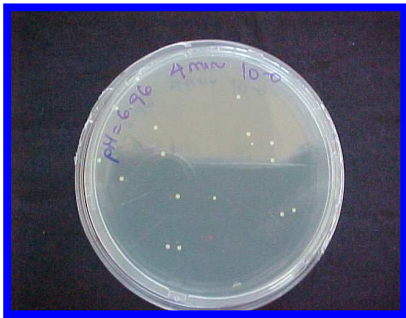
d



e



f

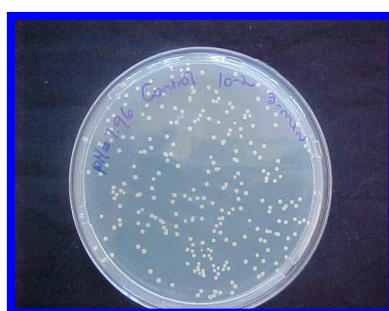
**g****h****i**

**Appendix C4: Representative nutrient agar plates of *P. aeruginosa* as function of time using ozone disinfectant: pH 7.96**

Time (min) = (a - c), control experiments: a: 0, b: 3, c: 6, d: 1, e: 2, f: 3, g: 4, h: 5, i: 6, j: 7, k: 8. Dilution factor = (a - c): 100, d: 100, e: 100, f: 100, g: stock, h: stock, i: stock, j: stock, k: stock



a



b



c



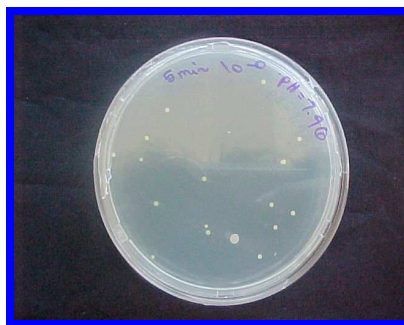
d



e



f

**g****h****i****j****k**

**Appendix C5: Representative nutrient agar plates of *P. aeruginosa* as function of time using ozone disinfectant: pH 9.28**

Time (min) = (a - c), control experiments: a: 0, b: 3, c: 9, d: 1, e: 2, f: 3, g: 4, h: 5, i: 6, j: 7, k: 8. Dilution factor = (a - c): 100, d: 100, e: 100, f: 100, g: 100, h: 10, i: stock, j: stock, k: stock.



a



b



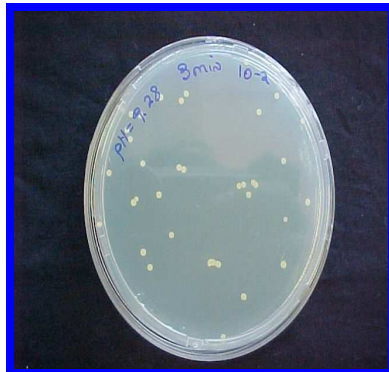
c



d



e



f

**g****h****i****j****k**