

# **THE ELECTROGENERATION OF HYDROXYL RADICALS FOR WATER DISINFECTION**

By

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in  
the Department of Chemistry, University of the Western Cape



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***DECEMBER 2006***

## ***DECLARATION BY CANDIDATE***

I, ***ZELO MANGOMBO***, declare that my thesis on “*THE ELECTROGENERATION OF HYDROXYL RADICALS FOR WATER DISINFECTION*” is my own work and that all the sources I have used or quoted, have been indicated and acknowledged by means of complete references.

**Signed:**



**Date:** December 2006

## ***ACKNOWLEDGEMENTS***

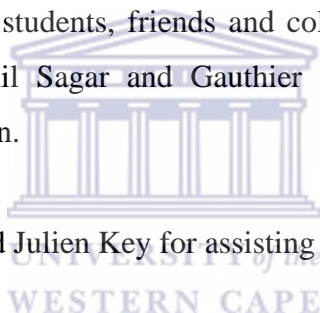
I express my thankfulness to Almighty God who made this project to be successful. To him be the glory and praise.

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## ***DEDICATION***

This thesis is dedicated:

- To my father Julien Zelo Mulemba Nganda, my mother Marie Masela Tekayiwula (deceased), my wife Meisie Mangombo, my children Moreen, Tshepo and Susan Masela Mangombo.
  
- To my brothers, sisters, nieces, nephews, uncle Kabeya Mwana Lenda (deceased) and Mbaya's family.
  
- To my aunty Odette Lukengu Mbaya and Najim Nkanga's family.



## ***ABSTRACT***

This study has shown that OH<sup>•</sup> radicals can be generated in an Fe/O<sub>2</sub> cell from the electrode products via Fenton's reaction and used for water disinfection. The cell system in which the experiments were carried out was open and undivided and contained two electrodes with iron (Fe) as the anode and oxygen (O<sub>2</sub>) gas diffusion electrode. Typically, 100 ml of Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O (0.5M) solution was used as a background electrolyte. OH<sup>•</sup> radicals were produced *in-situ* in an acidic solution aqueous by oxidation of iron (II), formed by dissolving of the anode, with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The H<sub>2</sub>O<sub>2</sub> was electrogenerated by reduction of oxygen using porous reticulated vitreous carbon (RVC) as a catalyst.

The analysis of the OH<sup>•</sup> radicals produced in the cell electrolyte involved dimethyl sulfoxide (DMSO) which was used as a scavenger of OH<sup>•</sup> radicals generating methane sulphinic acid (MSA). The MSA was converted to diazosulfone which was analysed by spectrophotometry.

The results show that: (i) More OH<sup>•</sup> radicals were produced at initial pH=3.0 as compared with the initial pH=2.5. (ii) Air can be used as an alternative to pure O<sub>2</sub> for OH<sup>•</sup> radical production in the Fe/O<sub>2</sub> battery. (iii) Higher currents and cell voltages occur with increasing running time of the battery which are consistent with the increasing pH and ferrous iron concentration of the electrolyte. (iv) The electrolyte solution continues to generate OH<sup>•</sup> radicals even after standing for several days. The disinfection efficiency of the Fe/O<sub>2</sub> battery electrolyte was investigated and compared with that of Fenton's reagent, chlorination and the solution produced from a DiaCell<sup>®</sup> electrolysis cell. The higher the OH<sup>•</sup> radical concentration produced in the systems tested, the more rapid is the *E.coli* inactivation for given contact times.

The relative killing efficiencies of *E.coli* have been shown to be Fenton reagent (94%), the Fe/O<sub>2</sub> electrolyte (87%) and chlorination (72%) after 60 min of contact time. The DiaCell<sup>®</sup> was found to be a more efficient means of disinfection (killing efficiency (96%)) of *E.coli* when compared to the Fenton reagent, the Fe/O<sub>2</sub> electrolyte and chlorine.

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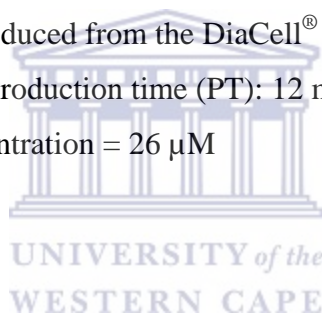
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## ***LIST OF ABBREVIATIONS***

WHO	World Health Organization
OH <sup>·</sup>	Hydroxyl radical
E.coli	Escherichia coli
VOCs	Volatile organic compounds
RO	Reverse Osmosis
O <sub>3</sub>	Ozone
DOC	Dissolved organic carbon
A	Surface area [m <sup>2</sup> ]
V	filtration rate [m <sup>3</sup> /(m <sup>2</sup> * h)]
Q	Throughput of water per hour [m <sup>3</sup> /h]
a	Operating hours per day
AOP	Advanced Oxidation Processes
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
Fe/O <sub>2</sub>	Iron/oxygen
U.V/vis	Ultraviolet visible
pH	The negative logarithm of the hydrogen ion activity measured at a defined temperature, usually at 25 °C
Na <sub>2</sub> SO <sub>4</sub>	Sodium sulfate
ORP	Oxidation-Reduction Potential (millivolts)
HF-CVD	Hot-filament chemical vapour deposition
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid (99.9 %)
SMCL	Secondary maximum contaminant level
mg/L	Milligrams per liter
Fe <sup>2+</sup> /Fe <sup>3+</sup>	Ferrous / Ferric ions
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
E <sub>cell</sub>	Cell potential at non-standard conditions
E <sup>0</sup> <sub>cell</sub>	Cell potential at standard conditions
R	Constant (8.31J/mole K)

T	Temperature (Kelvin scale)
F	Faraday's constant (96,485 C/mole e <sup>-</sup> )
n	Number of moles of electrons transferred in the balanced equation.
Q	Relation quotient for the reaction.
RVC	Reticulated Vitreous Carbon
A	Absorbance
c	Concentration (μM)
l	Path-length (cm)
ε	Molar absorption coefficient (ml/mol)
DMSO	Dimethyl Sulfoxide (99.9 %)
MSA	Methane sulphinic acid
λ <sub>max</sub>	Wavelength of maximum absorption
SHE	Standard hydrogen electrode
CH <sub>3</sub> ·	Methyl radical
R <sup>2</sup>	Correlation coefficients
μM	Micro-molar (10 <sup>-6</sup> M)
NB	Neutralizing Broth
TSA	Tryptone Soya Agar
TSB	Tryptone Soya Broth
CT	Contact time







Cholera epidemics and those caused by other water borne pathogens are continuing worldwide among rural communities in developing countries, including rural areas of South Africa [1]. Cholera is generally contracted from polluted water supplies; it causes vomiting and diarrhea that may result in rapid dehydration and sometimes death if not treated as soon as possible [2]. It is estimated that almost 1.3 billion people living in developing countries do not have access to safe drinking water [3]. This poses a serious health hazard, exposing many to the risk of waterborne diseases, caused by a lack of adequate water supply and sanitation facilities.

A survey of the rural communities in South Africa showed that few households have piped water supply while many others rely on communal standpipes and others upon river-water or ponds. In such environments, deaths, due to waterborne diseases, are a common occurrence, according to World Health Organization (WHO) reports [4,5].

Waterborne infectious diseases are transmitted primarily through contamination of the water sources with human and animal excreta. Use of such water for drinking or cooking, contact during washing or bathing may result in infection.

Fecally contaminated drinking water is a major path of transmission for bacterial, viral, and parasitic human pathogens. Where available source water is not microbiologically safe, many different home-based approaches to securing clean water have been attempted, including boiling, filtration, chemical treatment, and solar disinfection, even though the ongoing effectiveness of these approaches in the community, except via a few intervention studies, is unknown [5].

Purification of potable water containing pathogenic microorganisms requires specific treatment called “disinfection”; as disinfection reduces pathogenic microorganisms in water to levels safe by human health standards. However, the methods to segregate and identify the organisms are complex, expensive, time consuming, and explicit to each organism.

This makes determining and enumerating contamination from specific organisms in water supplies an overwhelming task beyond the ability of most municipal water supply authorities.

Therefore, the scale of the problem requires urgent action to establish effective disinfection regimes in rural areas of South Africa and the rest of the world. Although the disinfection of water by chlorination has been used for more than one hundred years, however, chlorination of non-piped rural water supplies is difficult in application [6,7,8,9].

In fact, chlorination is the most commonly used as a method of elimination disease-causing microorganisms in water but is often ineffective when used alone against many pathogenic bacteria, as at normal dosage rates do not kill all viruses, cysts or worms [10,11]. Also, chlorine solutions lose strength while standing or when exposed to air or sunlight. Unfavourable taste and odour associated with the use of chlorine when the concentration is great enough in drinking water also causes problems.

Use of chlorine is not associated with causing other diseases as WHO has recognized [12]. There are, however, problems of cost, monitoring, transport, purchasing and accurate dosing that cause failure of chlorine disinfection [13].

The water treatment disinfection process can be considered to have two purposes, firstly primary disinfection which is the elimination or inactivation of microbiological contaminants in the raw water supply [14], and secondly the provision of a residual in the distribution network [15].

Disinfection using hydroxyl radicals ( $\text{OH}^{\bullet}$ ) has emerged as a possible alternative to chlorine.  $\text{OH}^{\bullet}$  radicals, which are strong oxidizing agents and destroy all microbial growth in potable water treatment. [16,17].

The concept of using an iron/oxygen (Fe/O<sub>2</sub>) battery has been developed to generate highly reactive OH<sup>•</sup> radicals via Fenton's reaction [16,18]. The effectiveness of OH<sup>•</sup> radicals production via Fenton's reaction to disinfect *E.coli* in water has been demonstrated [16,17,19]. In addition to its disinfecting properties, OH<sup>•</sup> radicals have other benefits for water treatment, these include:

- ✓ Reduces total solids (clay, sand, color agents and organic matter)
- ✓ Increases efficiency of water clarification systems
- ✓ Reduces or eliminates pH adjustment problems
- ✓ Minimizes settled sludge volume (water quality standards)
- ✓ Eliminates undesirable matter from the water by oxidation.

The efficacy of using electrochemical systems to generate OH<sup>•</sup> radicals is largely dependent on cell configuration, electrode material, electrolyte composition, and other cell parameters such as pH, flow rate, temperature, voltage and current density [20, 21,22,23,24].

Study of the effect of the operating conditions in the Fe/O<sub>2</sub> battery as a disinfection technology design in which the OH<sup>•</sup> radicals produced are used for raw water disinfection has not been reported in the literature.

## 1.1 CONTRIBUTION OF THE WORK DESCRIBED IN THIS THESIS

The main contribution of this thesis is the investigation of a system for generating OH<sup>•</sup> radicals for raw water purification from the Fe/O<sub>2</sub> battery that does not require an external source power. This technique could be potentially useful in rural water supply disinfection in South Africa. The challenge is to produce an appropriate and sufficient amount of strong oxidant (OH<sup>•</sup> radicals), which via its applications to water-related problems can support South Africa's sustainable development and economic growth in order to promote a better quality of life for all.

## 1.2 OVERVIEW OF THE CHAPTERS

Chapter 1 introduces the problem of unsafe water. Thereafter the contribution of the work explained in this thesis is described.

Chapter 2 discusses the nature of unsafe water and effects of pollution. A review of disinfection methods is discussed, followed by the general aspects of bacterial water contamination and the various types of pathogens are highlighted.

The fourth and fifth parts of Chapter 2 deal respectively with properties and chemistry of the hydroxyl radical and the use of hydroxyl radicals in water. Methods of generating hydroxyl radicals, Fenton reaction and water treatment, photo-Fenton, electro-Fenton and water treatment are explained. Finally, a description of the iron/oxygen ( $\text{Fe}/\text{O}_2$ ) battery chemistry and energy, the electrochemistry related to the  $\text{Fe}/\text{O}_2$  battery, the use of boron-doped diamond electrodes in water electrolysis, aim and objectives of the study are given.

Chapter 3 covers construction and development of the  $\text{Fe}/\text{O}_2$  battery and the oxidation-reduction potential (ORP). The ultraviolet visible (uv/vis) calibration curves are explained followed by the experimental procedures.

Chapter 4 presents the results of the research. Introduction is given. This is followed by a detailed discussion of the effect of changing the electrolyte in the battery, the effect of initial electrolyte pH, the change in iron concentration in the  $\text{Fe}/\text{O}_2$  battery, the study of oxidation-reduction potential in the  $\text{Fe}/\text{O}_2$  battery, the use of air instead of oxygen for  $\text{OH}^\bullet$  radical production. Finally, conclusion and  $\text{OH}^\bullet$  radical production from the DiaCell are mentioned.

Chapter 5 shows the inactivation efficacy of  $\text{OH}^\bullet$  radicals on *E.coli* growth in water as compared to chlorination methods.

Chapter 6 describes conclusions and recommendation for further study.



**CHAPTER 2**  
UNIVERSITY *of the*  
WESTERN CAPE  
**LITERATURE REVIEW**

## 2.1 UNSAFE WATER AND HEALTH EFFECTS OF POLLUTANTS

Unsafe water is a substantial cause of human disease and death. Improvements in municipal supplies and distribution systems are important long-term solutions to safer drinking water in cities in developing countries including South Africa. Technology has greatly improved water quality. However, the difficulties of technology transfer to developing countries including South Africa have nowhere been better illustrated than in rural community water supply programs. Hence, the World Health Organization has continually insisted on high technical and sanitary standards for the rural community water supply systems to which they have contributed [25].

Raw water comes from different sources such as rivers, groundwater, precipitation (rain and snow), evaporation of seawater and lakes, etc. [26]. Water sources are usually contaminated by interactions with mineral weathering, biological processes and anthropogenic activities thus rendering the water unsuitable for drinking purposes.

Water affects basic human well-being and productivity through impacts on health and the expenditure of human energy to gather water. Insufficiency of water and lack of distribution systems can lead to extraordinary consumption of time and energy in gathering daily water needs.

It is well known that an enhancement in potable water supply will lead to a significant improvement in health. Major obstacles to human health in developing countries as well as South Africa are well known to include a large component relating to unsafe water, poor sanitation and inappropriate hygiene [27].

Rapid population growth and the lack of effective environmental protection in many developing countries including South Africa often lead to serious deterioration in the quality of water resources. Sewage discharge without sufficient treatment is the main source of organic impurities in surface water [28,29,30].

If water is used without proper disinfection, it may lead to diseases such as cholera. This is the problem that is associated with drinking water in rural communities of South Africa. It is estimated that over than 30 % of population in South Africa is need of secure access to safe drinking water. However, rural areas will require massive funding to establish full drinking water supply system coverage; such funding is not feasible within the near future [31]. Therefore, an alternative system that could address the problem is by implementing on-site generation of a strong disinfecting agent.

Moreover, new water legislation and policy in South Africa has been adopted to promote equity, sustainability, representatively and economic performance through the water management. The water legislation makes provision for water rights trading as an option for water allocation [32]. Water is now considered as a common asset. The right to use water in South Africa is granted to users, most of them have to be registered and licensed, and should pay for this right. The core concept of water management under the new dispensation is decentralization. Protective measures are meant to secure water allocation for basic human needs, ecological and development purposes [32].

In the meantime further refinements and evaluations of home-based efforts to purify and store water are needed. Thus, the available supply of high quality water and sufficient wastewater treatment capacity are essential to future economic development.

However, the presence of coliforms such as *Escherichia coli* in treated drinking water suggests either incomplete decontamination of source water, or recontamination of treated water, either of which puts the household at risk to waterborne diseases [33].

The World Health Organization has maintained that coliform bacteria should not be present in drinking water and that its presence suggests inadequate treatment, post-treatment contamination, or excessive nutrients [34].



## 2.2 GENERAL ASPECT OF BACTERIAL WATER CONTAMINATION

Various pathogenic microorganisms have been reported to be associated with the risk of waterborne diseases. They can reach the consumer either directly by drinking water or indirectly by contaminated food. In developing countries including South Africa particularly in the rural and farming areas where standards of public sanitation and water supplies are low and hygienic practices are substandard, diarrhea cases are normally high.

### 2.2.1 *Vibrio cholerae* bacteria (*Cholera disease*)

Cholera is a diarrhea disease caused by infection of the intestine with the bacterium *Vibrio cholerae*, as both children and adults can be infected. Cholera is usually transmitted through faecally contaminated water or food and remains an ever-present risk in many countries. The greatest risk occurs in over-populated communities, unsafe drinking-water, and increased person-to-person transmission. Thus, cholera can be an acute public health problem with the potential to cause many deaths [1,2].



### 2.2.2 *Escherichia coli* (*E.coli*) O157:H7

*E.coli* is a pathogen belonging to group of organisms called *Enterobacteriaceae*, which is informally referred to as enteric bacteria, family of rod-shaped bacteria that can grow fast by respiratory metabolism in the presence of oxygen (aerobically) and by fermentation in its absence (anaerobically). The ability to grow both aerobically and anaerobically classifies the *E.coli* bacteria as a facultative anaerobe [35].

*E.coli* can cause diseases in other body sites, and they increase between individual hosts, often after a relatively short extracorporeal residence, as they never find themselves in a static environment. It has been shown that certain strains of bacteria were responsible for infant diarrhea and gastroenteritis as an important public health discovery [35].

*E.coli* feed only sporadically in the large intestine and undertakes partial dehydration when canceled from their host. If deposited in a body of water they face the problems of nutrition at low concentrations of foodstuff [35]. Therefore, they can cause diarrhea, which can be quite severe and even fatal when drinking water is not chemically treated. Many disinfectants destroy these bacteria [36].

### 2.2.3 *Legionella bacteria*

The strain of this bacterium that causes Legionnaire's Disease is called *Legionella pneumophila*. These bacteria have always been found in non-marine aquatic environments such as lakes and ponds. The optimum growth temperature range for this bacterium is 20-45 °C. The organism has been found to possess the ability to survive in tap water at room temperature for over a year. *Legionella*, in their natural environments, grow within other organisms, i.e. with certain protozoan such as *Hartmanella vermiformis*, *Tetrahymena thermophila*, and *Acanthamoeba castellani*. The bacteria especially favor the average relative humidity level of sixty-five percent. This is because the humidity enables the bacteria to live for longer periods of time in airborne environment [37].

### 2.2.4 *Shistosomiasis and guinea worm or dracunculiasis*

*Shistosomiasis and dracunculiasis* are the two most important pathogens in which they spend a part of their life cycle in an intermediate aquatic host or hosts. They are transmitted by routes of infection through parasitic worms which depend on aquatic intermediate hosts to complete their life cycles. Therefore the degree of sickness depends on the number of adult worms which are infecting the patient [38].

Some common microorganisms found in domestic wastewater and the diseases associated with them are presented in Table 2.1.

Table 2.1. Infectious agents pathogenic present in raw domestic wastewater [39]

Organism	Disease
<b>Bacteria</b> Escherichia coli Legionella pneumophila Leptospira Salmonella typhi Samonella Shigella Vibro cholerae Yersinia enterolitica	Gastroenteritis Legionellosis Leptospirosis Typhoid fever Salmonellosis Shigellosis Cholera Yerinosis
<b>Virus</b> Adenovirus Enteroviruses Hepatitis A Norwalk agent Reovirus Rotavirus	Respiratory disease Gastroenteritis, heart anomalies, meningitis Infectious hepatitis Gastroenteritis Gastroenteritis Gastroenteritis
<b>Protozoa</b> Balantidium Cryptosporidium Entamoeba histolytica Giardia lamblia	Balantidium Cryptosporidium Amebiasis Giardia lamblia
<b>Helminths</b> Ascaris lumbricoides Enterbius vericularis Fasciola hepatica Hymenolepis nana Taenia saginata T.solium Trichuris trichuira	Ascariasis Enterbiasis Fascioliasis Hymenolepiasis Taeniasis Taeniasis Trichuriasis

## 2.3 REVIEW OF DISINFECTION METHODS

### 2.3.1 *Boiling*

Microorganisms that might be lurking in water will be killed if the water is boiled long enough [40]. Boiling will also drive out some of the volatile organic compounds (VOCs) that might also be in the water.

This method works well to make water that is contaminated with living organisms safe to drink, but because of the inconvenience and full requirements, it is not routinely used to purify drinking water except in emergencies.

Boiling also does not remove many other water contaminants. In fact boiling is liable to concentrate contaminants (like lead, asbestos, mercury, and many toxic organic chemicals) that are not vaporized as the relatively pure water vapour boils off [40].

### 2.3.2 *Distillation*

To remove impurities from water by distillation, the water is boiled causing the water to be vaporized as steam leaving the non-volatile contaminants behind. The steam is then cooled until it condenses, and the resulting distillate drips into a container. Salts, sediment, and metals anything that was not evaporated remains in the distiller and must be removed. Volatile Organic Compounds (VOCs) are a good example of a contaminant that distillation will not remove. A carbon filter or other device must be used with a distiller to ensure the complete removal of all contaminants.

A good distillation unit produces very pure water. This is one of the few ways to remove nitrates, chloride, and other salts that carbon filtration cannot remove. Distillation also kills microorganisms in the water. Distillation takes time to purify the water; uses power all the time unit is operating, and requires cleaning [41].

### 2.3.3 *Reverse Osmosis*

Water pressure is used to force water molecules through a membrane leaving the contaminants behind. Purified water is collected from the clean side of the membrane. Water containing the concentrated contaminants is flushed down the drain from the contaminated side. Reverse osmosis (RO) removes salt and most other inorganic material present in water. For that reason, RO lends it self to in places where the drinking water is brackish (salty) or contains nitrates that are difficult to remove by other methods.

With good quality carbon filters to remove any organic materials that get through to the RO membrane, the purity of the treated water approaches that produced by distillation.

Microscopic bacteria and parasites (including viruses) can be removed by RO units, but any defect in the membrane would allow these organisms into the filtered water [41].

#### 2.3.4 *Ultra Violet light*

Ultraviolet light (UV) has been used fairly extensively for disinfection of small community water supplies [42]. The efficiency of the UV disinfection is dependant on the intensity of the light and the exposure of the microorganisms to the light. The majority of microorganisms require fairly low UV doses for inactivation. UV light decreases in efficiency as contamination (especially turbidity and some substances in solution such as iron and organic compounds) increases.

Water passes through a chamber transparent to a source of Ultra Violet (UV) Light. UV light kills bacteria and deactivates viruses but the tough cryptosporidia cysts are fairly resistant to UV light [42]. Any turbidity in the water can shadow pathogens, protecting them from the light. UV light is not effective against any non-living contaminant, lead, asbestos, organic chemicals, chlorine etc. UV is typically used as a final purification stage in some filtration systems. If contaminants in addition to bacteria and viruses are to be removed, a good quality carbon filter or reverse osmosis system in addition to the UV system must be used.

UV disinfection of water is normally achieved by passing water through a tube lined with UV lamps. This gives efficient disinfection after a contact time of few seconds. The lamps may continue to produce blue light when they are worn out and no longer produce disinfection light.

Disinfection with UV light does not give rise to tastes and odors. There is no requirement for consumable chemicals, maintenance is straightforward and there is no danger of overdosing. UV light does not leave a residual effect in the water [40].

### 2.3.5 Ozonation

Ozone ( $O_3$ ) is a very reactive and unstable gas with a short half-life with respect to conversion to oxygen. Ozone is a strong oxidizing agent, and will oxidize all bacteria, molds, yeast pores, most organic material and viruses. Ozone is a disinfectant that effectively kills biological contaminants and oxidizes and precipitates iron, and manganese so they can be filtered out of the solution. Ozone oxidizes and breaks down many organic chemicals as well, but ozone treatment creates its own set of undesirable byproducts that can be harmful to health if they are not controlled e.g. formaldehyde and bromate [42].

Oxidation reactions of ozone in water occur via two pathways, namely direct oxidation by molecular ozone and indirect oxidation by free radical species. These radical species can be formed by decomposition of ozone and from reaction of ozone with some inorganic and organic compounds. Ozone decomposition in water forms hydroxyl radicals and is accelerated by a number of free radicals and anions.

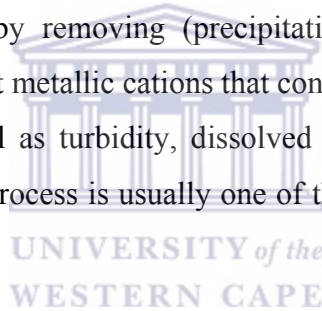
Ozone is slightly soluble in water. It is an efficient disinfectant, and because it is unstable does not leave residual in water unlike chlorine. For this reason it is impossible to overdose with ozone. It contributes to the bleaching of color and removal of tastes and odors.

Ozone is a very efficient disinfectant and is more effective than chlorine inactivating cryptosporidium oocysts and viral agents. There are significant disadvantages in the use of ozone; it does not provide residual protection against recontamination during distribution and as ozone affects biological stability, it may encourage regrowth of bacteria. The lack of a residual in ozonation may be dealt with by employing regular booster ozonation during distribution. Ozonation is much more expensive than chlorination [43].

### 2.3.6 *Coagulation and Flocculation*

The process of coagulation can be described as the combination of small particles into large aggregates. The process of chemical coagulation-flocculation enhances the removal of colloidal particles by destabilizing them, chemical precipitating them and accumulating the precipitated material into larger floc particles that can be removed by gravity settling or filtering [44]. The settled floccules are referred to as sedimentation. Sedimentation is problematic in terms of sludge disposal [45].

Coagulation with aluminum or iron (III) salt results in the formation of insoluble, positively charged aluminium or iron hydroxide (or polymeric aluminum –or iron –hydro complexes) that effectively attract negatively charged colloidal particles. Coagulation-Flocculation or precipitation using lime, lime soda ash and caustic soda is used to soften water, usually ground water by removing (precipitating) calcium, magnesium, iron, manganese and other polyvalent metallic cations that contribute to hardness. Reduction of microbial contaminants as well as turbidity, dissolved colloidal organic matter is also achieved in this process. This process is usually one of the first steps in a series of water purification steps [46].



### 2.3.7 *Filtration*

There are many types of filtration strategies and combination of strategies used. The basic concept behind all filters is fairly simple. The contaminants are physically prevented from moving through the filter either by screening them out with very small pores or in same case by trapping them within the filter matrix attracting them to the surface of filter [40]. The different types of filter are described below:

### 2.3.7.1 *Slow sand filtration*

Slow sand filtration has been used throughout the world for the last 150 years. Although slow sand filtration technology has been widely used in Europe since the early 1900s, its current use in North America has been primarily limited to smaller communities in New England and is still a popular method of treating municipal water supplies [47]. The filters are easily constructed and the process is simple to operate. The process consumes no chemicals or electrical energy during normal operation. However due to high filtration rate, large areas of filter bed may be required.

Slow sand filtration is a biological process characterized by smaller sand particles (0.25 to 0.4 mm in diameter) and a lower filtration rate (0.1 to 0.3 m/h) in which turbidity, colour and microbiological contaminants can be removed to levels acceptable for potable water.

Filtration rates of approximately 0.04 to 0.16 gallons per minute per square foot of filter surface area are typical. Therefore, a relatively large surface area is necessary to accommodate a realistic flow rate (for example, a 10-gpm flow rate requires between 60 and 250 square feet of filter surface area). It will not remove all microorganisms, but removes a significant amount due to the formation of a rich biological matrix called a "Schmutzedecke" [47].

This layer consists of a wide assortment of life forms including algae, rotifers, and many other organisms. These organisms assimilate microorganisms (protozoans, bacteria and virus) thus reducing their numbers as water passes through the biologically active matrix. Slow sand filters are cleaned by draining the filter and scraping the top inch of sand (which includes the "Schmutzedecke"). However, this destroys the "Schmutzedecke" and requires a re-ripening period that can take weeks. While one filter is being cleaned, the other is on-line to continue the filtration process.



In recent years, a new method of cleaning slow sand filters called "wet harrowing" has been developed that simplifies the cleaning process. Also, the creation and use of polyethylene filter vessel structures have made the task of building slow sand filters much easier. It must be recognized that slow sand filters have their limitations in that they cannot remove high turbidity, high levels of microorganisms, nor chemical contamination from water [4].

However Global Water uses slow sand filters wherever this relatively simple water treatment technology can accommodate local water conditions. When properly operated, disinfection of the filtrate should not be necessary but it is typically practiced to combat subsequent regrowth and contamination of the treated water. Additional treatment processes are required for hard and unusually turbid water, as well as water high in dissolved solids, such as sodium, nitrite, nitrate, sulphate and fluoride.

With water supplies that are high in organic content, the filtration process is not effective in complex iron removal, in which organic compounds may be bound to iron ions and cannot be removed by an aeration/ filtration process [4,47].

Typically, water that may produce organic complexing of iron should have a level of organic carbon over 2 mg/L and may also contain ammonia. Water containing a level of dissolved organic carbon (DOC) over 5 mg/L may cause taste, odour and colour problems. Slow sand filtration cannot effectively remove dissolved organic carbon. A carbon filter should be used in addition to the slow sand filter [47].

With the recent issuance of the Surface Water Treatment Rule by the U.S. Environmental Protection Agency and new filter requirements for all surface water systems to ensure removal of Giardia cysts, there is renewed interest in slow sand technology.

### 2.3.7.2 *Rapid sand filtration*

Rapid sand filtration is a technique that is now commonly used in rural developing areas. It is a sophisticated process usually requiring power operated pumps for backwashing or cleaning the filter bed, and flow control of the filter outlet.

The period between backwashes depends on the quality of water being filtered, usually daily. The purpose of backwashing is to remove the suspended material that has been deposited in the filtration bed during filtration cycle.

The filter process operates based on two principles: mechanical straining of suspended matter and physical adsorption. Water fills the pores of the filter medium and the microorganisms are adsorbed on the surface of the grains or trapped in the openings. The key to this process is the relative grain size of the filter medium [48]. The performance of a rapid filter depends on the following parameters: filtration rates, influent characteristics (i.e. particle size, distribution etc) and filter medium characteristics, which control the removal of the particles and their release upon backwashing.

Generally, it is true that the treatment effect can be improved by reduced filtration rates (not less than 2,5 m/h), smaller granulation size of the filter medium, increasing depth of the filter bed, increasing size of the flocs and decreasing the concentration of particles to be retained. The range of filter beds is between 1 and 2 m.

The operating head is between 1.5 and 2.5m [48]. The required filter surface area can be determined according to the following relationship:

$$A=Q/(a*v)$$

Where A= surface area [m<sup>2</sup>], v = filtration rate [m<sup>3</sup>/(m<sup>2</sup>\*h)]; Q = throughput of water per hour [m<sup>3</sup>/h]; a = operating hours per day [48].

Combined with coagulation, flocculation and sedimentation, rapid sand filtration is a very efficient treatment process for the removal of microorganisms.

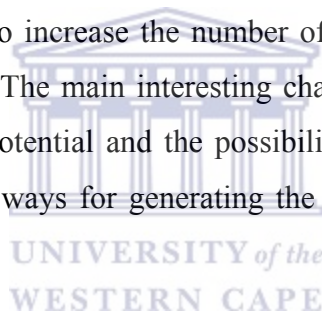
Besides of the advantages that these filters are effective in removing suspended solids and require a minimal land for construction, they present the following disadvantages:

- Rapid sand filters have a high capital and operational costs
- Uses energy for pumping

Requires a high degree of training for plant operators, for monitoring, operation and maintenance [48].

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

Advanced oxidation processes involving hydroxyl radicals, represent a new treatment concept and have gained increasing interest in treatment of industrial and drinking wastewater to remove organic and inorganic contaminants. The common target of many groups working worldwide is to increase the number of applications of these processes and to improve the efficiency. The main interesting characteristics of hydroxyl radicals are their very high oxidation potential and the possibility of its generation by different ways [49]. The most common ways for generating the hydroxyl radicals are explained below:



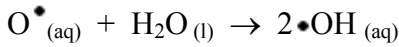
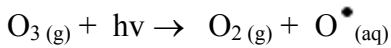
#### 2.3.8.1 *Anodic oxidation*

In an electrochemical treatment organic compounds can be oxidized indirectly or directly by the anodes [50]. An indirect anodic process via the production of oxidants such as hydroxyl radicals, ozone, etc destroys the organics and toxic microbial pollutants present in wastewater. A direct process in which the pollutants are first adsorbed on the anode surface and then oxidized by an electron transfer reaction [51].

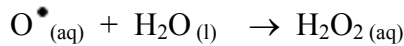
### 2.3.8.2 *UV / O<sub>3</sub> Process*

The UV/O<sub>3</sub> system is an effective method for the oxidation and destruction of organic compounds in water. The advanced oxidation process with UV radiation and ozone is initiated by the photolysis of ozone. The photodecomposition of ozone leads to two hydroxyl radicals, which if they do not react, recombine producing hydrogen peroxide.

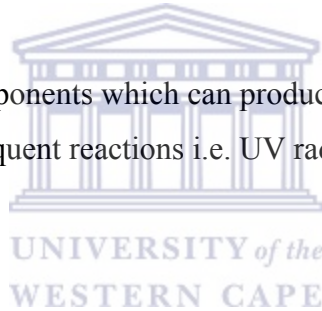
The reaction mechanism starts with photodecomposition of ozone molecules by UV to form oxygen radicals, which then combine with water to form hydroxyl radicals [52].



Oxygen radicals can also react with water to form hydrogen peroxide [53].



This system contains three components which can produce hydroxyl radicals and /or to oxidize the pollutants for subsequent reactions i.e. UV radiation, ozone and hydrogen peroxide.

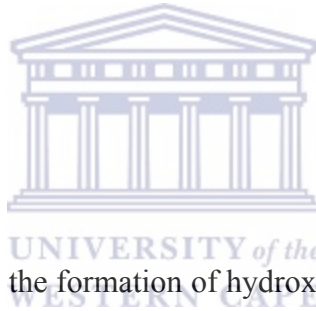
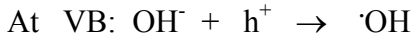
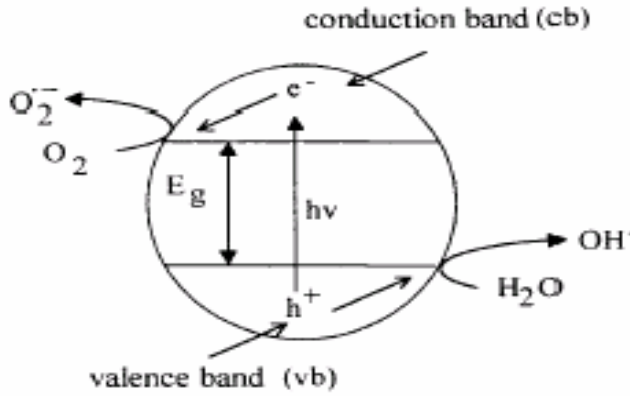


### 2.3.8.3 *UV / TiO<sub>2</sub> (Heterogeneous photo catalysis) Process*

The heterogeneous photocatalytic process consists of utilizing the near UV radiation to photo excite a semiconductor catalyst TiO<sub>2</sub> in presence of oxygen and water. Under these circumstances oxidizing species (hydroxyl radicals) are generated.

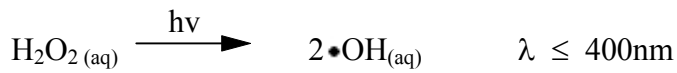
The photo catalysis process involves light energy from ultraviolet radiation (light) in the form of photons, excites the electrons on the surface of the TiO<sub>2</sub> suspended in the contaminated water, moving them from the valence band to the conduction band [54]. The result of the energy change is the formation of positive holes on the surface of the TiO<sub>2</sub>, and free electrons. Hydroxyl radicals form which can oxidize organic chemicals and kill microorganisms.

**Figure 2.1** The photo catalytic semiconductor particle illustrated by TiO<sub>2</sub> is shown below [55]:



#### 2.2.8.4 *H<sub>2</sub>O<sub>2</sub> / UV Process*

The H<sub>2</sub>O<sub>2</sub> / UV system involves the formation of hydroxyl radicals by hydrogen peroxide photolysis.

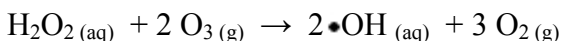


It presents the advantage compared when working with ozone in that it provides a cheap and sure source of radicals also eliminating the problem of handling of ozone.

The major drawback of this process is that if the solution contains strongly absorbing substance which can compete with hydrogen peroxide for the radiation, thus cloudy water or water containing compounds absorbing UV radiation can present problems being treated by this method [56].

### 2.3.8.5 $O_3/H_2O_2$ Process

Addition of hydrogen peroxide to ozone offers another way to accelerate the decomposition of ozone, leading to the formation of hydroxyl radicals. The reaction of this process is illustrated below:



This process does not depend on the UV radiation absorption to activate the ozone or hydrogen peroxide molecules. Its greatest advantage is to be able to work with turbid water without problems [57].

### 2.3.9 Chlorine Dioxide

Chlorine dioxide is a gas that is soluble in water. Chlorine dioxide is a more powerful oxidizing agent than chlorine and its disinfection action is less pH-dependant than that of chlorine.

The oxidation-reduction reaction of chlorine dioxide is as follows:



Other important half reactions are:



Therefore the total chlorine dioxide redox potential is  $0.954 \text{ V} + 0.76 \text{ V} = +1.714 \text{ V}$ , whereas for chlorine



Chlorine dioxide removes taste and odors effectively [58]. Chlorine dioxide is not a chlorinating reagent so no chloroform or other trihalomethanes are formed [59].

Chlorine dioxide has proved to be an excellent biocide and an effective oxidant in drinking water, cooling water, wastewater, and odor-control applications. Chlorine dioxide achieved faster kill of microorganisms at lower concentrations than did other chlorine-based sanitizers [60].

Chlorine dioxide can remove pesticides particularly Aldrin and methoxychlorine. Herbicides such as paraquat and diquat are eliminated by chlorine dioxide within a few minutes at a pH higher than 8 [61]. Chlorine dioxide is unstable and cannot be conveniently transported or stored over long periods. It always generated on site because of its risk of rapid decomposition [62].

### 2.3.10 Chlorine

Chlorine addition is the most common method for potable water production due to its effectiveness at inactivating several types of pathogens and its reactively low cost [63]. For almost a century the predominant method of disinfection of water has been by the use of chlorine compounds. Chlorination is effected by adding chlorine gas, or alternatively sodium or calcium hypochlorite to the water. It also produces a residual that further protects the water from contamination after treatment.

Chlorination can however, cause problems by producing destructive byproducts when being administered to water containing traces of organic material [64]. A disadvantage with all chlorine chemical treatment methods is that the chemicals decompose over time and therefore have limited shelf lives [65].

## 2.4 PROPERTIES AND CHEMISTRY OF THE HYDROXYL RADICAL

### 2.4.1 Properties of hydroxyl radicals

Hydroxyl radical ( $\text{OH}^\bullet$ ) is a highly reactive transient that can rapidly oxidize with most organic compounds in water. A number of studies have been focused on its role as a strong oxidizing agent for wastewater treatment [66, 67]. For example, the destruction of aniline and chloroaniline with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) electrogenerated in situ at gas diffusion electrodes [68]. The destruction of Chlorophenoxy herbicides by  $\text{H}_2\text{O}_2$  with iron salts for complete mineralization [69]. Earlier studies have reported the electrogeneration of hydroxyl radicals using boron-doped diamond as electrodes [70].  $\text{OH}^\bullet$  radicals have high oxidation potential, second only to elemental fluorine (see Table 2.2) [71].

**Table 2.2** Comparative oxidation potentials at pH=0 and temperature = 25 °C

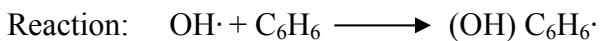
<i>Species</i>	<i>Volts</i>
Fluorine	3.0
Hydroxyl radical	2.8
Ozone	2.1
Hydrogen peroxide	1.8
Potassium permanganate	1.7
Chlorine dioxide	1.5
Chlorine	1.36
Oxygen	1.2
Bromine	0.80
Iodine	0.54

Thus it can be seen that the hydroxyl ( $\text{OH}^\bullet$ ) radicals are powerful disinfectant agents, which can destroy all known microorganisms in water [27,71,72]. The half-life of  $\text{OH}^\bullet$  radicals in aqueous solution is approximately  $10^{-9}$  seconds [84]. The  $\text{OH}^\bullet$  radicals react rapidly with most organic substances in water. Its chemical reactions are of four types as follows:



### 1<sup>0</sup> Addition

When the OH<sup>•</sup> radicals are added to an unsaturated compound such as aliphatic or aromatic to yield a free radical product (cyclohexadienyl radical).



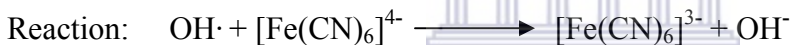
### 2<sup>0</sup> Hydrogen Abstraction

When the OH<sup>•</sup> radicals are combined with an alcohol compound, hence an organic free radical and water are produced.



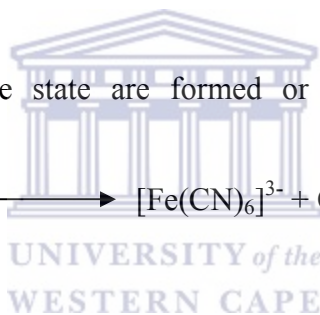
### 3<sup>0</sup> Electron transfer

Where ions of higher valence state are formed or an atom or free radical if a mononegative ion is oxidized.



### 4<sup>0</sup> Radical interaction

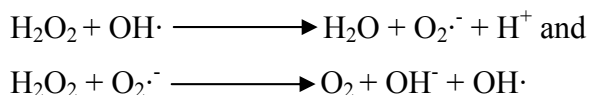
When the OH<sup>•</sup> radicals react with another OH<sup>•</sup> radicals or with an unlike radical to generate a stable product. This reaction requires no activation energy.



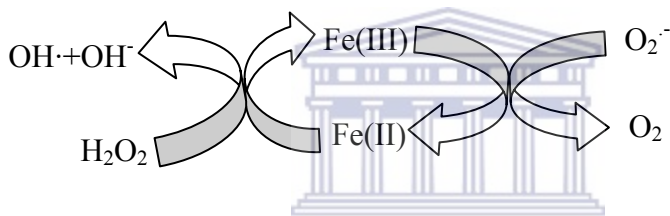
## 2.4.2 Chemistry of the hydroxyl radicals

### *Haber-Weiss reaction/Fenton's reaction*

The Haber-Weiss reaction cycle involves hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), superoxide anion ( $\text{O}_2^{\cdot-}$ ) and hydroxyl radical ( $\text{OH}\cdot$ ). Its cycle consists of the following two reactions:

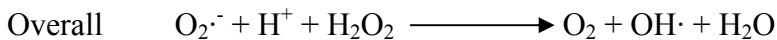


$\text{Fe}^{2+}$  can catalyze this cycle:  $\text{Fe}^{3+}$  is reduced to  $\text{Fe}^{2+}$  by superoxide, followed by oxidation by  $\text{H}_2\text{O}_2$  to form  $\text{OH}\cdot$  and  $\text{OH}^-$  (see Figure 1) [73].



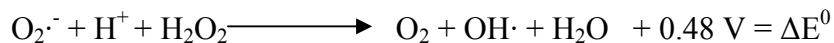
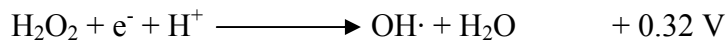
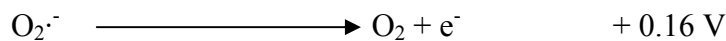
**Figure 2.2.** The Haber-Weiss cycle/Fenton's reaction. *the*  
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The thermodynamic condition for the reducibility of superoxide in acidic solution is as follows [74]:



$$E^0 (\text{O}_2/\text{O}_2^{\cdot-}) = -0.16 \text{ V}$$

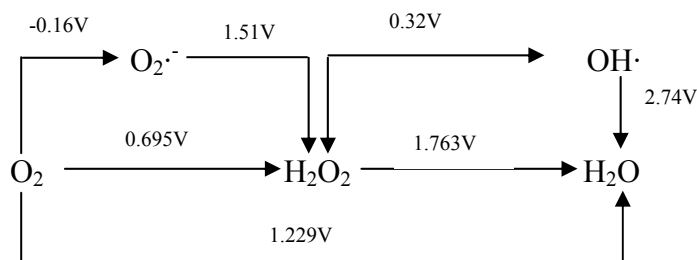
$$E^0 (\text{OH}\cdot/\text{H}_2\text{O}) = 0.32 \text{ V}$$



$$\Delta G^0 = -nF\Delta E^0$$

$$\Delta G^0 = -11.1 \text{ kcal/mol or } -46.3 \text{ kJ/mol}$$

The corresponding redox potential diagram for oxygen in acid solution with the concomitant proton transfers involved oxygen activation chain are depicted in Figure 2.3.



**Figure 2.3.** Redox diagram for oxygen activation chain.

Figure 2.3 has shows the redox reactions which can occur among oxygen species. Iron can react with molecular oxygen or with its reduced species, leading to the formation of hydroxyl radicals if two conditions are respectively filled: (i) ion is reducible into its ferrous state; (ii) the ferrous ion has a redox potential allowing the Fenton reaction, i.e. the transfer of one electron to  $\text{H}_2\text{O}_2$ .

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## 2.5 USE OF HYDROXYL RADICALS IN WATER TREATMENT

The hydroxyl radicals ( $\text{OH}^\cdot$ ) are powerful, non-selective chemical oxidants. When generated in solution they are responsible for the oxidation of organic compounds mostly by hydrogen abstraction generating free organic radicals (see equation (i)).

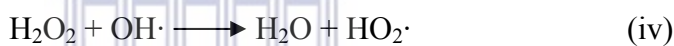
The organic radicals react rapidly with environmental molecular oxygen to form peroxyradicals (equation (ii)), starting a series of oxidative degradation reactions that can lead to the complete mineralization of the organic contaminants in polluted water [75].



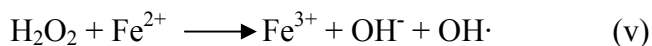
## 2.6 METHODS OF GENERATION OF HYDROXYL RADICALS

The most recent advances in water purification concern the oxidation of persistent organic compounds dissolved in water, generally refractory to common biological detoxification processes. Methods based on chemical and photolytic catalysis have been included in a group of new technologies denominated Advanced Oxidation Processes (AOPs), which generate highly degrading OH<sup>•</sup> radicals. These radicals are produced by different combinations of ozone, hydrogen peroxide, UV radiation and titanium dioxide, and also by the combination of hydrogen peroxide with ferrous ions (Fenton's reaction).

Furthermore, the recombination of radical-radical (see equation (iii)) and hydroperoxyl radicals production in presence of excess of H<sub>2</sub>O<sub>2</sub>, (equation (iv)), which are much less reactive and do not appear to contribute to the oxidative degradation of organic substrates.



The above mentioned combined methods for OH<sup>•</sup> radicals generation, have been reported by several authors for the decomposition of a wide variety of organic contaminants [5,17,18] and are of special interest since some of them use solar energy. OH<sup>•</sup> radicals can be produced from the Fenton reagents, according to the equation (v) below [20]:



## 2.7 CHEMISTRY OF THE FENTON REACTION

The Fenton reaction, source of OH<sup>•</sup> radicals, was first discussed in 1894 by H.J.H.Fenton [20,102]. Although the Fenton reaction has been studied for more than 100 years it is still a subject of controversy [76]. The kinetics and mechanisms involved are very complex [76] and dependent upon a large number of factors such as pH, reactant concentrations,

complexing agents and temperature. Nevertheless it remains a very important reaction both in biological systems, organic chemistry and water treatment [77]. It is used extensively to treat wastewaters, sludges and contaminated soils for the purposes of

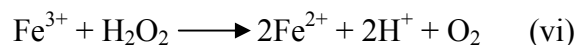
- Organic pollutant destruction
- Toxicity reduction
- Biodegradability improvement
- BOD/COD removal
- Odor and colour removal

The Fenton reaction has also been proposed for use in water disinfection. The Fenton reagent, the reaction of iron oxide with hydrogen peroxide can effectively catalyze the oxidation of organic compounds in contaminated water, see section 2.8 [78].

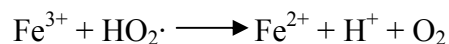
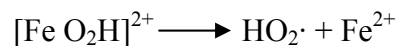
Although it is a simplification of the system, the Fenton reaction can be appreciated and understood most easily when the reactants used are the stoichiometry of the following equation with non-complexing anions (such as perchlorate  $\text{ClO}_4^-$ ) between pH 3-6 in aqueous solution.



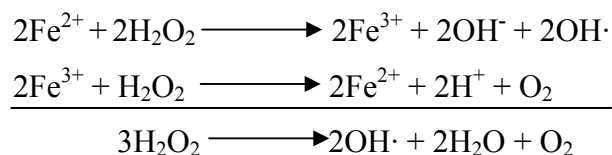
Under these conditions, it is considered that the following reaction also occurs:



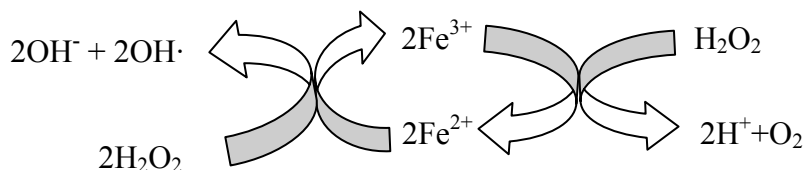
The above reaction proceeds via the following intermediate steps:



Thus combining 2x (v) with (vi)

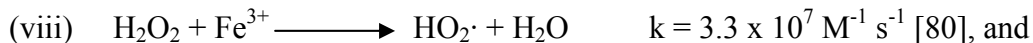
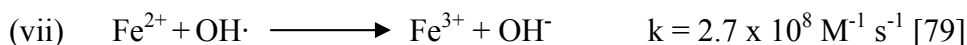


Hence hydrogen peroxide is decomposed using  $\text{Fe}^{2+}/\text{Fe}^{3+}$  as a catalyst.

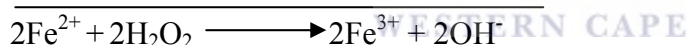
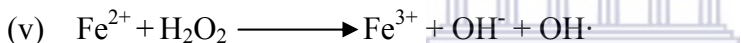


The above idealized situation is complicated by the very fast reactions of hydroxyl radical which approach the diffusion rate.

At 25 °C, for example:



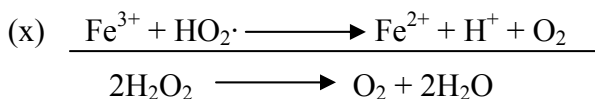
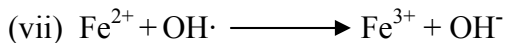
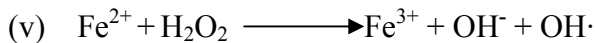
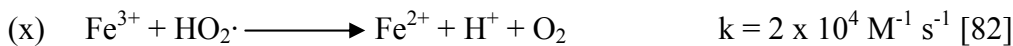
Thus under conditions where excess  $\text{Fe}^{2+}$  is present reactions (a) and (c) are dominant and the system is no longer catalytic in iron.



The overall rate of oxidation follows pseudo second-order kinetics:

$$-d [\text{Fe}^{2+}]/dt = d [\text{Fe}^{3+}]/dt = -2 d [\text{H}_2\text{O}_2]/dt = 2 k [\text{H}_2\text{O}_2] [\text{Fe}^{2+}]$$

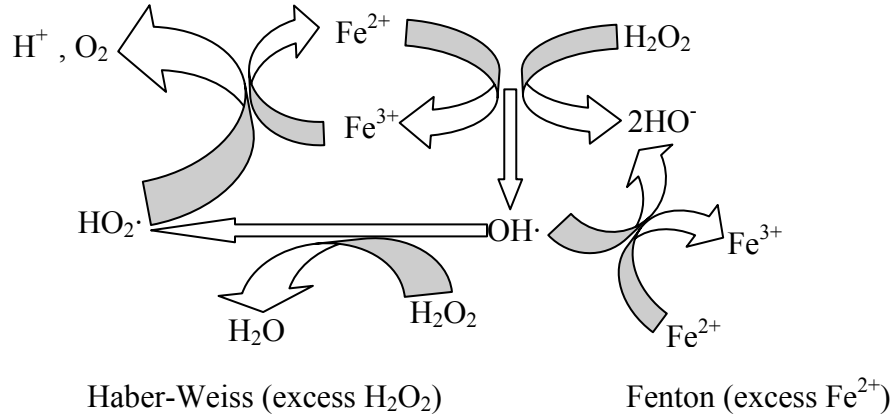
Under conditions where excess  $\text{H}_2\text{O}_2$  is present reactions (a) and (d) are dominant combined with, at 25 °C



The oxidation follows pseudo first-order kinetics:

$$-d[\text{Fe}^{2+}]/dt = k_{\text{obs}} [\text{H}_2\text{O}_2]$$

This later system is known as the Haber-Weiss cycle [83]. The total system can be summarized in a diagram described as follows:



**Figure 2.4.** Diagram of total system for Haber-Weiss cycle.

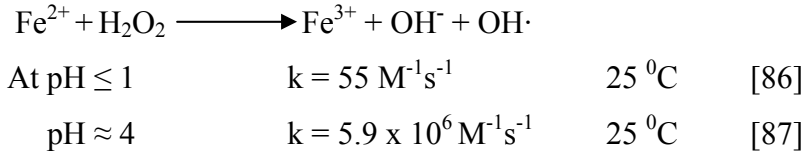
Additionally the self reaction of hydroxyl radicals (reaction (ix)) is also a factor. Thus even in the absence of other scavengers the life time of the hydroxyl radical is extremely short ( $\leq 10^{-9}$  seconds [84]). Although it is possible to calculate steady state  $\text{OH}^\cdot$  radical concentrations, these are not significant [85] in terms of practical application. What is significant is the total amount of hydroxyl radicals produced in the solution. The added scavenger and/or probe concentrations will dominate the change in hydroxyl radical concentration.

$$\text{Then } d[\text{OH}^\cdot]/dt = F - k_p[\text{OH}^\cdot][\text{P}] - \sum k_s[\text{OH}^\cdot][\text{S}] \quad [85]$$

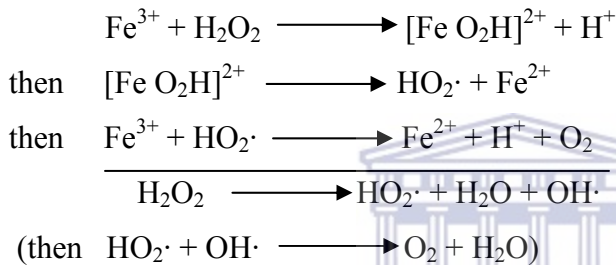
Where  $F$  = formation rate of hydroxyl radical;  $P$  = probe and  $S$  = scavengers (these include  $\text{Fe}^{2+}$ ,  $\text{H}_2\text{O}_2$ , and  $\text{OH}^\cdot$  radical). Thus as  $[P]$  increases the term  $-k_p[\text{OH}^\cdot][P]$  will dominate the loss terms. Under these conditions, the total moles of  $P$  reacted will be stoichiometrically related to the time dependent total of moles of  $\text{OH}^\cdot$  radical formed [85]. Hence all analytical methods involving analysis of “[ $\text{OH}^\cdot$ ]” with probes, actually measures the total amount of  $\text{OH}^\cdot$  radicals produced. Of course, this applies to Fenton water treatment processes, since the scavengers are the pollutants in the water and again

the total amount of pollutant oxidized (removed) depends upon the time dependent total of OH<sup>•</sup> radicals produced in the solution.

The rate of production of OH<sup>•</sup> radical in the Fenton reaction is very dependent upon pH for the reaction:



pH 4-6 Fe(OH)<sup>+</sup> becomes the dominant species. It should be noted that the Fe<sup>3+</sup>/ H<sub>2</sub>O<sub>2</sub> system (with no Fe<sup>2+</sup> initially present) also produces a Fenton-like oxidation process. The overall of the reaction becomes:



Hence overall reaction: 2H<sub>2</sub>O<sub>2</sub> → O<sub>2</sub> + 2H<sub>2</sub>O

And -d [H<sub>2</sub>O<sub>2</sub>]/dt = k<sub>obs</sub> [H<sub>2</sub>O<sub>2</sub>] [80,81]

Thus the Fe<sup>3+</sup>/H<sub>2</sub>O<sub>2</sub> can be used in water treatment [77] as an alternative to that of Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>.

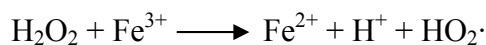
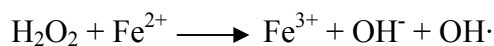
## 2.8 FENTON REACTION AND WATER TREATMENT

The combination of hydrogen peroxide and iron (II) salt, which is known as Fenton's reagent, is an effective oxidant of various organic compounds because the OH<sup>•</sup> radicals generated can rapidly oxidize most organic compounds in water [88,89,90].

The degradation mechanism of organic substrates is more complex, involving nonchain hydroxyl radical oxidation, direct hydrogen peroxide oxidation, and oxidation by other radical species. Therefore, the formation yield of hydroxyl radicals for each iron (II) ion



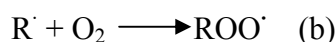
oxidized by hydrogen peroxide is a function of pH, and the oxidation of organic substances involves such radicals under acidic conditions (Steps 1-6: a-b).



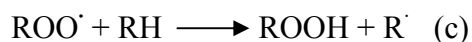
Step 1. Oxidation with Fenton's reagents.



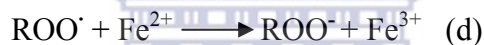
Step 2. Rapid reaction of organic radicals with environmental molecular oxygen to produce organic peroxy radicals.



Step 3. Abstraction of hydrogen atoms from organic peroxy radicals to produce organic hydroperoxides and other organic radicals.



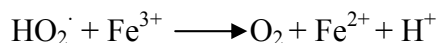
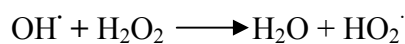
Step 4. Reduction of peroxy radicals in the presence of iron (II) ions



Step 5. Decomposition of hydroperoxides to generate chain-continuing radicals, in the presence of iron salts.



Step 6. Decomposition of hydrogen peroxide catalyzed by iron salts in dilute acid solution.



Fenton's reagent shows a powerful degrading action on refractory organic substances such as aromatic compounds and surfactants (steps 1-6: a-e).

Thus, Fenton's reagent is currently applied in industrial waste-water treatment. Strong COD reduction is achieved that showing extended bond degradation. Therefore, Fenton's reagent can improve biological degradation of organic compounds in water [88,89,90].

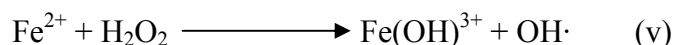
Depending of the chemical structure of the organic molecules, the hydroxyl radical reaction pathway can be one of addition reactions, subtraction reactions of a combination of both, leading to the mineralized end products [91].

## 2.9 PHOTO-FENTON REACTION AND WATER TREATMENT

Although the toxicity of organic compounds is not as high as heavy metal in contaminated water, however, the high incident concentrations (mg/L) may terminate abundant bacteriological populations in municipal biological wastewater treatment plants. The common biological wastewater treatment is not possible. Advanced Oxidation Processes (AOPs) are a viable alternative. The most commonly used AOPs utilized H<sub>2</sub>O<sub>2</sub>, O<sub>3</sub> or O<sub>2</sub> as oxidants [88,89].

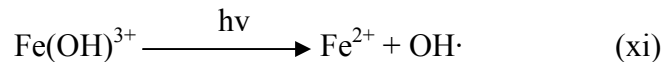
Fenton's reagent is classified as AOP, since hydroxyl radicals are produced as a result of the reaction between H<sub>2</sub>O<sub>2</sub> and Fe<sup>2+</sup> or Fe<sup>3+</sup> that constitute this process. The OH· radicals are one of the most powerful oxidants known to remove organic compounds in drinking water. The rate and extent of the production of these species can be significantly increased by using the combined systems UV/TiO<sub>2</sub>/ H<sub>2</sub>O<sub>2</sub> and UV/ Fe<sup>3+</sup>/ H<sub>2</sub>O<sub>2</sub> (photo-Fenton reaction) that are considered as the most promising for the remediation of contaminated waters [90].

The active species generated, mainly hydroxyl radicals (OH·), are the initiator of a chain of reactions that leads to the total mineralization of the organic compounds to carbon dioxide (CO<sub>2</sub>), H<sub>2</sub>O and other inorganic compounds. The Fenton reagents are mixture of hydrogen peroxide and ferrous ion which produces OH· radicals according to the equation (a) below [90]:



In fact, the Fenton reaction efficiency can be enhanced in the presence of UV irradiation [91]. The iron product in equation (v) can be photoreduced by regenerating Fe<sup>2+</sup> and

producing more OH<sup>·</sup> radicals (see equation xi). This study demonstrated that light strongly accelerates the Fenton reaction, suggesting a significant improvement in the removal of organic compounds in contaminated water.

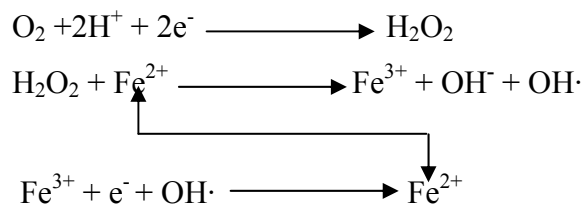


The combination of Fenton reaction and UV irradiation (equation xi) is known as photo-Fenton reaction. Thus, its applications for water treatment have been investigated by several researchers. Therefore, the photo-Fenton reaction was considered as an alternative and very promising technique because of its great affinity for wastewater treatment process [89,90,91].

## 2.10 ELECTRO-FENTON REACTION AND WATER TREATMENT

Electro-Fenton reaction is considered as AOP, since hydroxyl radicals are electrosynthesized in aqueous solutions by simultaneous process reduction of oxygen (O<sub>2</sub>) and ferric (Fe<sup>3+</sup>) ions and followed by complete mineralization of the initial pollutants. However, the viability of the electro-Fenton process to generate both of the Fenton reagent species (Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>) was evaluated as a potentially more efficient alternative to the classical Fenton's reaction to produce hydroxyl radical, which can react rapidly with organic compounds in contaminated water. The production of OH<sup>·</sup> radicals is a two step process (Figure 2.5) [92,93]:

- (a) electrochemical reduction of dioxygen into O<sub>2</sub><sup>·-</sup> and formation of H<sub>2</sub>O<sub>2</sub> in the acidic medium;
- (b) reaction of H<sub>2</sub>O<sub>2</sub> with Fe<sup>2+</sup> ions yielding OH<sup>·</sup> radicals. In these conditions, Fe<sup>3+</sup> ions are electrochemically



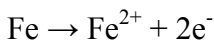
**Figure 2.5.** Diagram for OH<sup>·</sup> radical production, based on the electro-Fenton process.

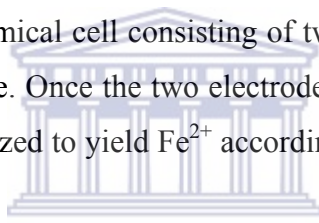
The electro-Fenton methods can be considered very efficient and much cleaner techniques than chemical ones for improving the quality of water resources and eliminating organic compounds in water.

## 2.11 THE IRON/OXYGEN (Fe/O<sub>2</sub>) BATTERY CHEMISTRY AND ENERGY

The Fe/O<sub>2</sub> battery acts as a voltaic cell to generate electricity spontaneously [94]. The system has a cathode of porous carbon (RVC), which acts as a catalyst for reduction of oxygen to hydrogen peroxide. Na<sub>2</sub>SO<sub>4</sub> has been used as a background electrolyte [95]. The oxygen (at flow rate of 20 ml/min) dissolves in water forming a gas diffusion electrode producing H<sub>2</sub>O<sub>2</sub> in the aqueous solution [96].

The Fe/O<sub>2</sub> battery is electrochemical cell consisting of two electrodes at which oxidation and reduction (redox) take place. Once the two electrodes are connected the Fe acting as an anode is spontaneously oxidized to yield Fe<sup>2+</sup> according to the reaction showed below, at pH=0 [72]:



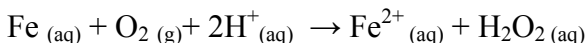


E<sup>0</sup> (V) = +0.44

Whilst at the cathode, the oxygen is reduced to H<sub>2</sub>O<sub>2</sub> by releasing its two electrons (meaning oxygen was reduced and dissolved in water) according to the reaction as follows (pH=0) [97]:

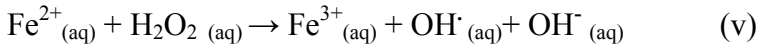


Reticulated Vitreous Carbon (RVC) acts as a gas diffusion catalytic surface for the above reaction. Then the overall cell reaction is [98]:



The cell potential is 1.128 V. The Fenton reaction then occurs in the electrolyte between the two electrode products, ferrous ion (Fe<sup>2+</sup>) is combined with the electrogenerated H<sub>2</sub>O<sub>2</sub>

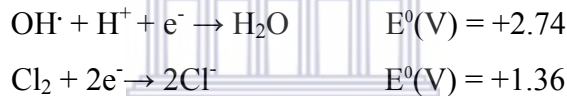
to produce OH<sup>•</sup> radicals and other species according to the equation (v) (see section 2.6 as discussed previously) [99,100]:



Both Fe<sup>2+</sup>/Fe<sup>3+</sup> catalyze the reaction. In the presence of H<sub>2</sub>O<sub>2</sub>, thus the Fenton reaction occurs quickly. Some research suggests that Fe<sup>2+</sup> may be preferred as OH<sup>•</sup> radicals have been produced at high rates of application according to the equation discussed above.

Fe<sup>3+</sup> species can be reduced when they are combined with H<sub>2</sub>O<sub>2</sub> to yield Fe<sup>2+</sup> and other species (see section 2.6) such as hydroperoxyl radical (HO<sub>2</sub><sup>•</sup>), which is weaker oxidant than OH<sup>•</sup> radical in solution [99,100]. Hence OH<sup>•</sup> radicals are produced continuously and Fe<sup>2+</sup> acts as a catalyst and H<sub>2</sub>O<sub>2</sub> is consumed.

The OH<sup>•</sup> radicals produced in the equation (v) are powerful oxidizing agents. The oxidation potential of OH<sup>•</sup> radicals compared to the oxidation potential of chlorine (Cl<sub>2</sub>) is described below:



The high oxidation potential of OH<sup>•</sup> radicals causes them to act as powerful disinfectant agents for water treatment [22,101]. This cell system has an advantage of providing direct conversion of chemical energy to electrical energy without an external power source. In order to establish the efficacy of the cell and to produce a high amount of OH<sup>•</sup> radicals some parameters are considered, which include [99,100]:

- Choice of electrode material
- Electrolyte
- pH of the solution
- Electrode gap
- Flow rate of the gas (oxygen/air)
- Temperature
- Time of battery operation.

## 2.12 ELECTROCHEMISTRY RELATED TO THE Fe/O<sub>2</sub> BATTERY

### 2.12.1 Cell potential

The cell potential (voltage) is the difference in potential between the two electrodes of a cell. When the two electrodes are connected the electric current is transferred from the anode to the cathode. An electrical circuit provides a pathway for electrons to flow continuously in order to achieve equilibrium in the electrochemical cell [102,103].

#### 2.12.1.1 Standard cell potential

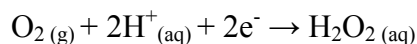
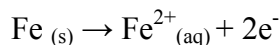
The standard cell potential is the potential that has been obtained from the sum of two standard electrode potentials in the cell, “ $E^0_{\text{cell}}$ ”, under standard conditions i.e. concentrations of 1 mole (1M) per liter and pressures of 1 atmosphere (1atm) at 25 °C [102,103].

The overall standard cell potential ( $E^0_{\text{cell}}$ ) is the sum of the reduction potential and oxidation potential in the electrochemical cell system. Therefore, the standard cell potential is given as follows:

$$E^0_{\text{cell}} = E^0_{\text{reduction}} + E^0_{\text{oxidation}}$$

The standard cell potential ( $E^0_{\text{cell}}$ ) of the Fe/O<sub>2</sub> battery is a cell reaction that:

- The half-reactions for each process are as follows:



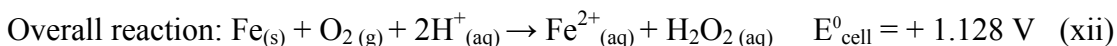
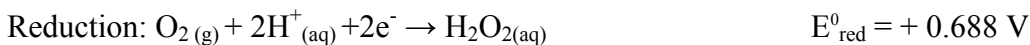
- The standard potential for the oxidation half-reaction:

$$E^0_{\text{oxidation of iron}} = + 0.44 \text{ V}$$

- The standard potential for the reduction half-reaction:

$$E^0_{\text{reduction of oxygen}} = + 0.688 \text{ V}$$

Then the overall standard cell potential ( $E^0_{\text{cell}}$ ) is as follows:

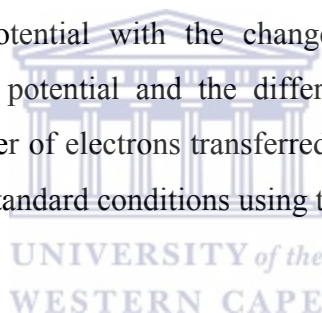


The positive standard cell potential shows that the cell undergoes a spontaneous chemical reaction.

### 2.12.1.2 *Non-standard cell potential*

The non-standard cell potential is the potential between the two electrodes, “ $E_{\text{cell}}$ ”, in the cell when the conditions are not the same as in the standard state i.e. concentration not 1molar (1M), pressures not 1atmosphere (1atm.) at different temperature.

Nevertheless, the new cell potential with the changed conditions is calculated by determining first the standard potential and the different parameters such as Q (the reaction quotient), n (the number of electrons transferred in the reaction) and finally, the cell potential ( $E_{\text{cell}}$ ) at the non-standard conditions using the Nernst equation [102,103].



### 2.12.2 **Nernst equation**

The Nernst equation is written as [102,103]:

$$E_{\text{cell}} = E^0_{\text{cell}} - (RT/nF) \ln Q \quad (\text{xiii})$$

Where,

$E_{\text{cell}}$  = Cell potential at non-standard conditions

$E^0_{\text{cell}}$  = Cell potential at standard conditions

R = Constant (8.31J/mole K)

T = Temperature (Kelvin scale)

F = Faraday's constant (96,485 C/mole  $e^-$ )

n = Number of moles of electrons transferred in the balanced equation.

Q = Reaction quotient for the reaction.

e.g.  $aA + bB \rightarrow cC + Dd$ .

$$Q = [C]^c \times [D]^d / [A]^a \times [B]^b$$

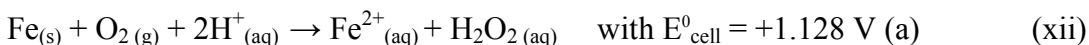
At 25°C, the equation (xiii) is modified to:

$$E_{\text{cell}} = E^{\circ}_{\text{cell}} - (0.0257 / n) \ln Q \quad (\text{xiv})$$

And when  $\text{Log}_{10}$  is introduced, the equation (xiv) is written as:

$$E_{\text{cell}} = E^{\circ}_{\text{cell}} - (0.0592 / n) \text{Log}_{10} Q. \quad (\text{xv})$$

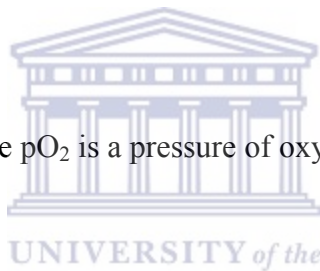
For Fe/O<sub>2</sub> battery, the cell potential at the non-standard conditions is predicted as the overall cell reaction of the Fe/O<sub>2</sub> battery and the cell potential at standard conditions according to the previous equation (xii) below:



The new cell potential is calculated by determining the value of the reaction quotient Q using the equation (xii) above.

$$Q = \frac{[\text{Fe}^{2+}] \times [\text{H}_2\text{O}_2]}{[\text{Fe}] \times p\text{O}_2 \times [\text{H}^+]^2}$$

Where  $p\text{O}_2$  is a pressure of oxygen.



On the other hand, H<sub>2</sub>O<sub>2</sub> and Fe are not included in the calculation. They are both respectively pure liquid and pure solid; therefore, their activities are equal to one. Then Q becomes:

$$Q = \frac{[\text{Fe}^{2+}]}{p\text{O}_2 \times [\text{H}^+]^2} \quad (\text{b})$$

At 25 °C, the cell potential ( $E_{\text{cell}}$ ) at the non-standard is:

$$E_{\text{cell}} = E^{\circ}_{\text{cell}} - (0.0592/n) \text{Log } Q \quad (\text{xvi})$$

Since equations (a) and (b) are substituted in the equation (xvi), with  $n = 2$  moles of electrons (as the number of moles of electrons transferred in the balanced equation), then  $E_{\text{cell}}$  will become as noted below [35,65]:



$$E_{\text{cell}} = + 1.128\text{V} - (0.0592/2) \text{Log} \frac{[\text{Fe}^{2+}]}{p\text{O}_2 \times [\text{H}^+]^2} \quad \text{Or}$$

$$E_{\text{cell}} = + 1.128\text{V} - (0.0296) \text{Log} \frac{[\text{Fe}^{2+}]}{p\text{O}_2 \times [\text{H}^+]^2} \quad (\text{xvii})$$

The dependence of the cell potential on  $[\text{Fe}^{2+}_{(\text{aq})}]$  and  $[\text{H}^+_{(\text{aq})}]^2$  concentration and flow rate of oxygen will be investigated as well as the use of air instead of oxygen.

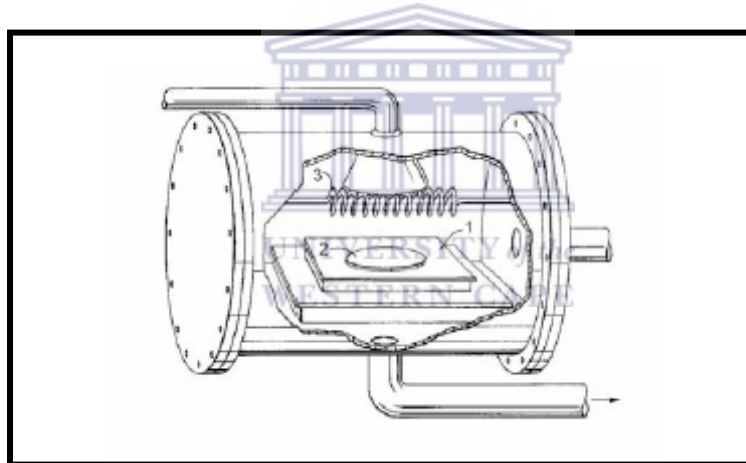
### 2.13 THE USE OF BORON-DOPED DIAMOND ELECTRODES IN WATER PURIFICATION VIA THE GENERATION OF HYDROXYL RADICALS

Diamond is an excellent candidate for electrode material when properly doped to acquire an adequate conductivity, as it is the case of boron-doped diamond (BDD) thin films. Diamond coating of 0.1 to 1 $\mu\text{m}$  thickness on silicon discs are used as electrodes in electrolysis cell such as the Diacell<sup>®</sup>. The Diacell<sup>®</sup> is an electrolyser concept based on BDD/Si electrodes, which has potential for different water treatment applications beside disinfection, mainly the COD reduction in industrial wastewater treatment [104]. The Diacell<sup>®</sup> (type 106:01-010) is built with two compartments containing bipolar BDD/Si electrodes (diamond coatings on both faces), negative on one side and positive on the other side. BDD electrodes in water electrolysis oxidize organic and inorganic compounds. This reactivity is associated with the production of hydroxyl radicals, and may also involve the production of chlorine, ozone, peroxo-disulfates, peroxo-dicarbonates and hydrogen peroxide, dependent upon the solutes in water, which are all disinfectants [105].

### 2.13.1 *Synthesis of boron doped diamond (BDD)*

Synthetic diamond was first produced by C.V. Burton in the laboratory in 1950 [106]. Boron is incorporated in diamond to obtain a semiconducting material by the high-pressure, high-temperature technique.

The procedure is described as a hot-filament chemical vapour deposition (HF-CVD). Since these first innovations, a number of CVD methods have been developed for diamond film growth. CVD is one of the best-known methods for diamond production [107]. BDD thin film has been synthesized with the HF-CVD technique. The HF-CVD reactor (figure 2.6) is a vacuum chamber supplied with carefully controlled flow process gases [107]. The principle of the HF-CVD technique is based on the thermal activation of the gas phase.



**Figure 2.6** Diagram of Hot-filament CVD reactor: 1) heater, 2) substrate and 3) filament.

### 2.13.2 *Synthesis of thin-film diamond by HF-CVD*

Diamond is the hardest known material. It is a metastable form of carbon at ambient temperature and pressure. With the four polymorphisms of carbon, diamond is the most compact and strongly bonded structure. The diamond has a great interest from its wide range of extreme properties such as high mechanical hardness, high value of thermal conductivity ( $2.10^{-3} \text{ W m}^{-1} \text{ K}^{-1}$  at room temperature), broad optical transparency, biological compatibility and low electron affinity. In addition, diamond is an electrical insulator but becomes a semiconductor when doped with boron (band gap of 5eV). Finally, it is very resistant to chemical corrosion [107].

The principle of the CVD method is to produce a quantity of monoatomic hydrogen in a gas containing a low concentration of hydrocarbon. The diamond synthesis can be classified in accordance with the mode of gas activation [107].

### 2.13.3 *Doping of diamond*

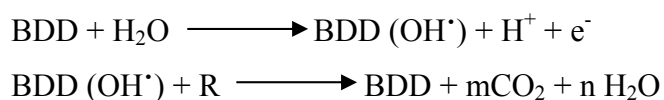
Intrinsically, diamond is an insulator. To make it a conductor, it can be doped with boron, fluorine or nitrogen. With increasing concentration of the doping, the insulator behavior of diamond changes to that of a semiconductor and then into metallic behavior. Two types of doping are possible: positive and negative. Positive doping consists of substituting some carbons by an atom having a similar size and one less electron than carbon, (*e.g.* boron) [108]. The doping atoms reside at an electronic level just above the valence band of diamond.

In the case of negative, doping atom has an extra electron (*e.g.* nitrogen). The boron doping atoms, which are electron acceptors, have free electrons in the dopant band and holes or vacancies in the valence band, to sustain the flow of current [108]. The electrical resistivity of BDD films also depends on the doping level in the diamond coating.

#### 2.13.4 *Electrochemical properties and applications of synthetic diamond films*

The overpotential for oxygen evolution on BDD electrodes aqueous acidic solution creates a potential window sufficiently positive to allow the formation of hydroxyl radicals (i.e.  $\geq + 2.7$  V) [109]. The properties of BDD such as high anodic stability in strongly acidic medium, high oxygen evolution overpotential and formation of hydroxyl radicals produces powerful oxidants with high redox potential for waste water treatment. Although biological treatment is the most economic process for waste water treatment, it is not applicable to refractory organic pollutants, and electrochemical oxidation becomes a very attractive alternative. The oxidation of some model organic pollutants has been investigated at BDD electrodes [109]. All the oxidations can be achieved with high current efficiency. Recently, there have been many reports on the use of BDD electrodes in the general area of waste water treatment [110,111]. Compared to graphite or glassy carbon electrodes, the inertness of diamond causes very good resistance toward corrosive conditions, which is very useful in electrochemistry (molten salts, batteries, fuel cells). The production of hydroxyl radicals on BDD electrodes also has potential for use in water disinfection [70].

Comninellis and co-workers have proposed a mechanism (see equations below) for the oxidation of organics with oxygen evolution, which assumes that both organic oxidation and oxygen evolution are taking place on a BDD via intermediation of hydroxyl radicals, generated from the discharge of water [111].



## 2.14 AIMS AND OBJECTIVES OF THE PROJECT

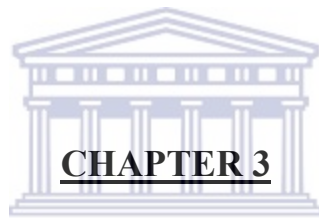
### 2.14.1 *Aims*

The project is aimed at the electrogeneration of disinfectants capable of treating a large variety of raw water sources by the method of Fenton's reaction in a Fe/O<sub>2</sub> battery that does not require an external power source of mains electricity. A comparison of the disinfection efficiency of the Fe/O<sub>2</sub> battery, a DiaCell (also producing hydroxyl radicals), and chlorine will also be carried out.

### 2.14.2 *Objectives of the project*

The objectives of this project are described as follows:

1. To develop/construct and test an appropriate Fe/O<sub>2</sub> electrochemical cell battery system for the disinfection of water.
2. To investigate a method of determining the hydroxyl radicals produced in the Fe/O<sub>2</sub> battery.
3. To establish the characteristics of the battery i.e. study the variables that affect the production of hydroxyl radicals, and optimization of the conditions for maximum production.
4. To establish the relative disinfection efficiencies of the Fe/O<sub>2</sub> battery, DiaCell<sup>®</sup> technology and chlorine by their effect on *Escherichia coli* growth in water.



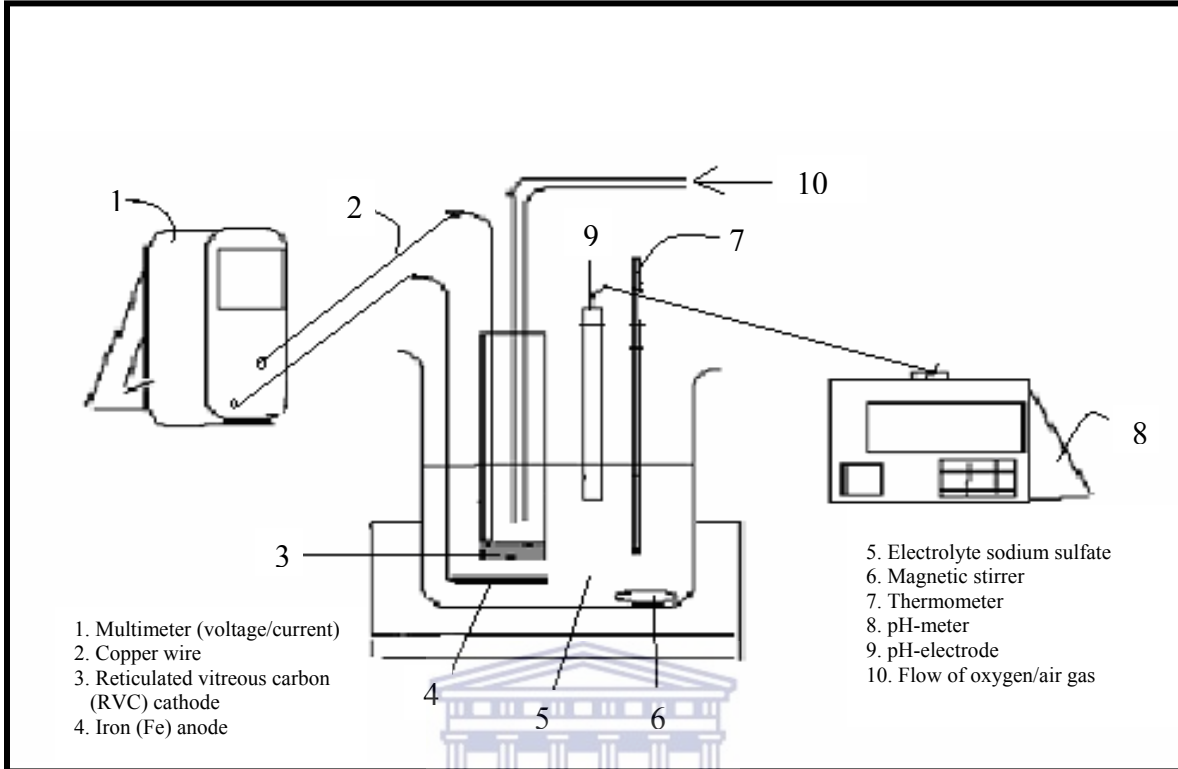
**CHAPTER 3**

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**EXPERIMENTAL METHODS**

### 3.1 CONSTRUCTION AND DEVELOPMENT OF THE IRON/OXYGEN (Fe/O<sub>2</sub>) BATTERY

The Fe/O<sub>2</sub> battery is a cell, which is easy to construct and has the potential of producing OH<sup>•</sup> radicals via Fenton's reaction without using an external power source and could be used in rural communities where there is lack of electricity. The main advantage of using electrochemical OH<sup>•</sup> radical generation is that the reactants are produced *in situ*, the cell system generates OH<sup>•</sup> radicals spontaneously in the solution.

The Fe/O<sub>2</sub> battery has been generally described in section 2.11. The electrode products Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> generate OH<sup>•</sup> radicals in aqueous media via the Fenton reaction [112,113]. The cell system in which the experiments were carried out was open and undivided and contained in a 150ml glass beaker. Typically, 100 ml of Na<sub>2</sub>SO<sub>4</sub>.10H<sub>2</sub>O (0.5M) solution was used as a background electrolyte. The anode was an iron disc of area 5 cm<sup>2</sup> and 5 mm thick and the cathode a porous carbon (RVC) disc of 10 mm thickness of area 3 cm<sup>2</sup>. A magnetic stirrer ensured mixing of the Fenton reagent electrode products. The pH was determined using a pH-meter (model HANNA instruments, HI 8520). The current and voltage were monitored using a multimeter (model PROVA<sup>®</sup>, Dual Channels RS-232C). See the Fe/O<sub>2</sub> battery cell diagram (Figure 3.1) and experimental set up (photographs 3.1).



**Figure 3.1.** Diagram of Fe/O<sub>2</sub> battery system.





**Photographs 3.1.** Fe/O<sub>2</sub> battery experimental set up (a), (b) and (c)

### 3.2 OXIDATION-REDUCTION POTENTIAL (ORP)

ORP of a solution is a measure of the equilibrium potential, relative to the standard hydrogen electrode, developed at the interface between a noble metal electrode and the aqueous solution containing electro-active redox species.

The ORP has the advantage for water system monitoring in that it can be measured easily to give an mV-value estimate of water disinfection potential. Recent research has shown that with ORP value between **650-700mV** and the water pH is **6.5-7.0** that bacteria such as *Escherichia coli*, *Salmonella* and certain fungi are all killed after due contact time in water [35]. Increasing ORP becomes an approach to water disinfection since it reflects the antimicrobial potential of water quality.

The ORP was calibrated by saturating pH buffers with Quinhydrone solution to make stable millivolts (mV) standard solutions. The procedure was as follows:

- Connect the ORP electrode to a suitable pH Meter, set the millivolt scale.
- Immerse the electrode in the pH buffers (7.00 and 4.01) mixed with Quinhydrone for calibration. The meter should read between +240 and +280 mV.
- Rinse the electrode thoroughly with distilled water, and immerse it in the sample from the Fe/O<sub>2</sub> battery. Record the mV values.

### 3.3 ULTRAVIOLET VISIBLE (UV/Vis) CALIBRATION CURVES

A series of calibration solutions were prepared. A quartz glass sample cuvette with a pathlength of 1cm was filled with the calibration solution. The absorbance of the solution was measured at a fixed wavelength using a GBC 920 UV/Visible spectrophotometer. The procedure was as follows: The cuvette was filled with blank (prepared) and the instrument was turned to read zero. The sample was then put in the cuvette for measurement. The blank was prepared as explained under procedure 3.4.2.4.

Solution absorbance varies experimentally according to Beer- Lambert law, as it is proportional to the molar concentration. The Beer's law formula can be written in the simple form as [114]:

$$A = \epsilon c l$$

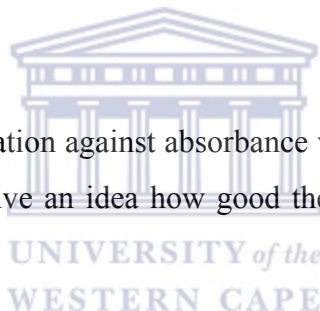
Where A = Absorbance; c = concentration (mg/ml); l = path-length (cm) and  $\epsilon$  = molar absorption coefficient (ml/mol). However,  $\epsilon$  can be calculated from Beer-Lambert law when the molar concentration of the standard compound is known [114].

Therefore,

$$\epsilon = A / c l$$

The system should obey Beer's law over a wide concentration range, and the linear calibration graph should be reproducible and insensitive to small changes in instrumental characteristics.

A calibration curve of concentration against absorbance was drawn. The gradient and the co-relational constant  $R^2$  can give an idea how good the calibration is. If  $R^2 = 0.999$  is acceptable.



### 3.4 EXPERIMENTAL PROCEDURES

#### 3.4.1 Materials and chemical reagents

All chemical reagents and solvents were purchased from commercial sources and used without further purification. Sodium sulfate decahydrate ( $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ ), ferrous sulfate, and hydroxylammonium were purchased from Saarchem, sulfuric acid, hydrogen peroxide, sodium acetate, and 2,2 bipyridal (2,2-dipyridyl) chloride were purchased from Merck, Dimethyl Sulfoxide (DMSO), and Diazonium salt (Fast Blue BB salt) were obtained from Sigma-Aldrich, toluene and butanol were obtained from Kimix, pyridine was purchased from B&M Scientific, Methane Sulphinic Acid (MSA) was obtained from

Chemistry Department (University of the Western Cape/South Africa). All solutions and reagents were prepared with distilled water and all glassware used was washed with distilled water and then autoclaved at 121°C for 20 min.

Reticulated Vitreous Carbon (RVC) is a microporous, glassy carbon electrode material with high surface area that combines with rigid structure, high resistance to temperature in non-oxidizing environments, high porosity and low resistance to fluid flow [72,115]. RVC was used as an electro catalyst; vitreous carbon has a low density, low thermal expansion, high thermal and electric conductivities, and high corrosion resistance [72,115]. Pletcher and *al.* have confirmed the ability of RVC to catalyse generation of hydrogen peroxide via the reduction of oxygen in aqueous solution [72,97,115].

### 3.4.2 Preparation of chemical reagents and analysis methods

#### 3.4.2.1 Preparation of Sodium sulfate (electrolyte)

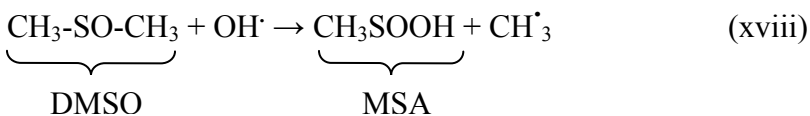
The  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  was used to prepare a 0.5 M solution that was employed as an electrolyte. The solution was maintained at room temperature (RT). 100 ml of this standard solution was adjusted to pH 2.5-3 with sulfuric acid (0.1 N  $\text{H}_2\text{SO}_4$ ). The solution was then ready for the appropriate Fe/O<sub>2</sub> battery experiment.

#### 3.4.2.2 Colorimetric assay for methane sulphinic acid (MSA)

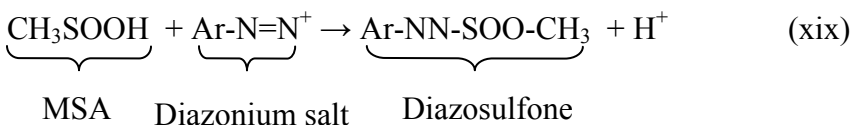
Babbs, *et al.* have reported the method of quantifying OH<sup>•</sup> radicals in solution [116,117]. The method is based on the use of DMSO to scavenge OH<sup>•</sup> radicals in the solution to yield methane sulphinic acid (MSA) and the methyl radical (CH<sub>3</sub><sup>•</sup>), see Reaction 1, equation (xviii) [116]. OH<sup>•</sup> radicals are quite difficult to detect in solution because of their short lifetime and low steady-state concentration. Hence, MSA is the primary product and is a stable in solution. The Diazonium salt “Fast Blue BB salt” is added to

the solution containing MSA to yield a diazosulfone (see Reaction 2, equation (xix)) [116,118], which can be extracted into an organic solvent and its absorbance measured by vis/uv spectrophotometry.

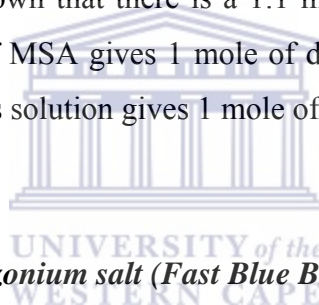
**Reaction 1:**



**Reaction 2:**



The reactions 1 and 2 have shown that there is a 1:1 molar ratio between OH<sup>·</sup> radicals and diazosulfone i.e. 1 mole of MSA gives 1 mole of diazosulfone. 1 mole of hydroxyl radical generated in the aqueous solution gives 1 mole of MSA.



3.4.2.3 **Preparation of Diazonium salt (Fast Blue BB salt)**

0.3119 g of Fast Blue BB was diluted in 50 ml of volumetric and flask filled up to the mark with distilled water to make a concentration of 15 mM. Only 0.2 ml of this solution was used in each analysis from freshly prepared Fast Blue BB. The Diazonium salt solution is stable only for 8 hours and has to be kept in the dark place, at room temperature in order to avoid its decomposition. After adding Fast Blue BB salt solution to the MSA solution, the bright yellow colour observed in the solution is the *diazosulfone* (see Reaction 2, section 3.4.2.2). The diazosulfone is stable in the range of pH 5-9 and, the diazosulfone is easily extracted into organic solvents.

#### 3.4.2.4 *Preparation of blank (reference)*

The blank contained all reagents at the same concentrations as in the test sample but without the cell solution containing the OH<sup>•</sup> radicals. It was used as the reference solution in the vis/uv spectrometer.



### 3.4.3 ANALYTICAL PROCEDURE FOR OH<sup>·</sup> RADICAL DETERMINATION

Diazosulfone was extracted into organic solvents by the following procedure:

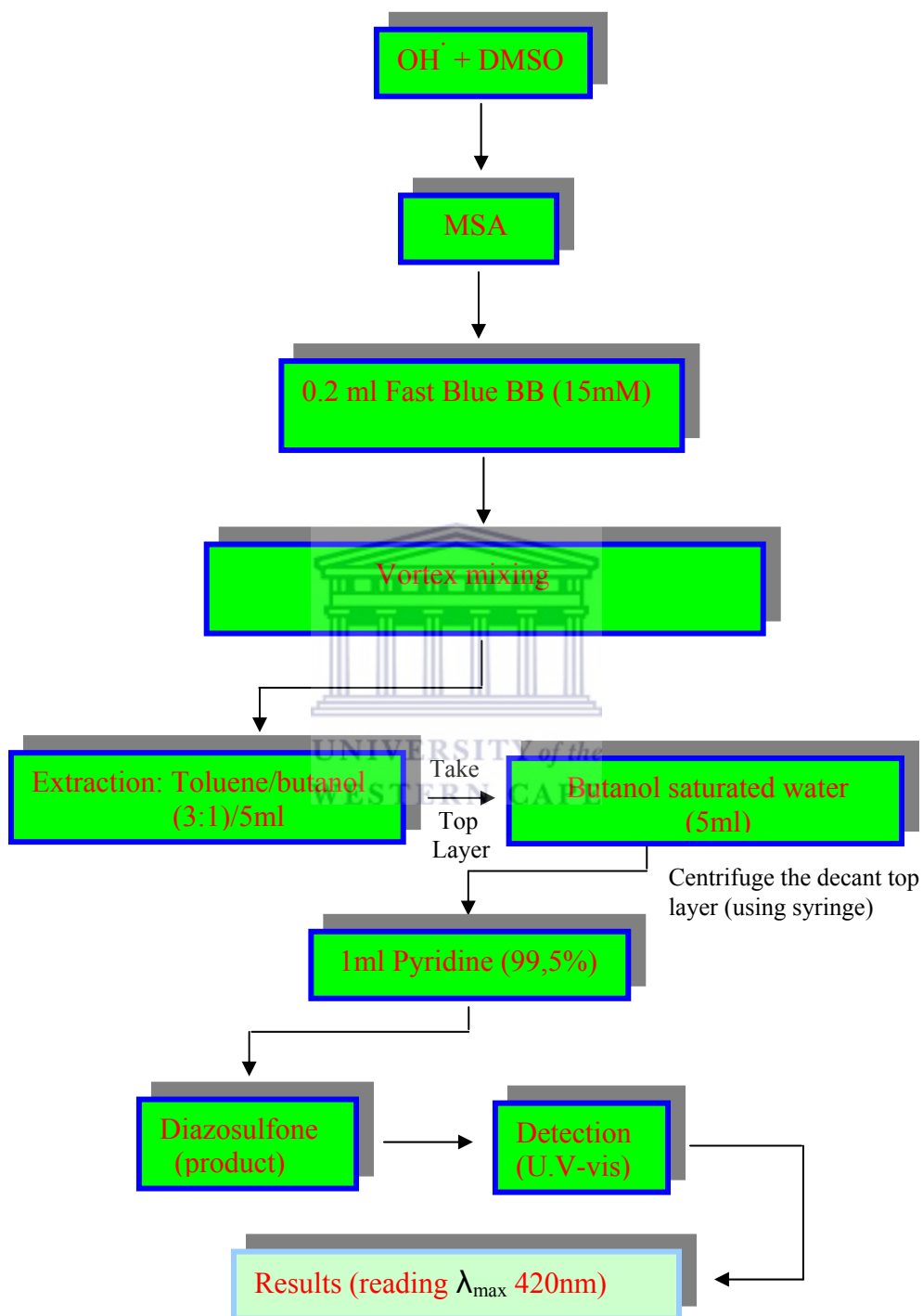


Figure 3.2. Analytical procedure for OH<sup>·</sup> radical determination.

### 3.4.4 TEST OF THE LITERATURE METHOD OF OH<sup>•</sup> RADICALS ANALYSIS

The aim of this test was to check the analysis method used by generating known amounts of OH<sup>•</sup> radicals in aqueous solution from Fenton reagents of known concentration [18,112,113]. Initially, calibration with standard MSA solutions was carried out.

#### 3.4.4.1 Preparation of standard methane sulphinic acid (MSA) solutions

Standard MSA solutions in distilled water were made up to volume in a 50ml graduated flask as a standard solution. Five standard solutions (labeled A-E) with different concentrations (10, 25, 50, 75 and 100 µM) were prepared. The initial pH of each standard MSA solution was adjusted to 2.5 with sulfuric acid (0.1 N H<sub>2</sub>SO<sub>4</sub>). 10 ml of the final solution was used for analysis purposes while the experiment was carried out at room temperature (25 °C).

These five standard solutions (labeled A-E) were ready for extraction analysis using the method that is described in section 3.4.3. The blank of each solution was prepared in the same method as standard solutions [18,112,113]. A series of calibration solutions for spectrophotometric analysis were prepared (see table 3.1).

**Table 3.1** Standard MSA solution (labeled A-E) with final concentrations (µM)

Solution	A	B	C	D	E
Final concentration of MSA (µM)	10	25	50	75	100

Knowing the MSA concentrations (table 3.1), therefore the effectiveness of the colorimetric assay by extraction of *diazosulfone*, was assessed when 0.2 ml of the Diazonium salt *Fast Blue BB salt* (15 mM) was added to each MSA solution (see Reaction 1 and 2, section 3.4.2.2).



The diazosulfone was extracted by using different organic solvents toluene-butanol 3:1 (3 ml), butanol saturated-water (5 ml) and pyridine (1 ml). The extraction procedure of diazosulfone (product) with organic solvents was explained in the previous section 3.4.3.

Vortex mixing and centrifugation were used respectively for 1 min and 5 min to mix up the solution and separate the two layers formed in the solution. The first layer was transferred into clean vials for further analysis whilst the second layer was discarded. Diazosulfone was extracted with organic solvents and bright yellow solution was obtained. Photograph 3.2 shows the extracted diazosulfone solutions (A, B and C) before spectrophotometric analysis.

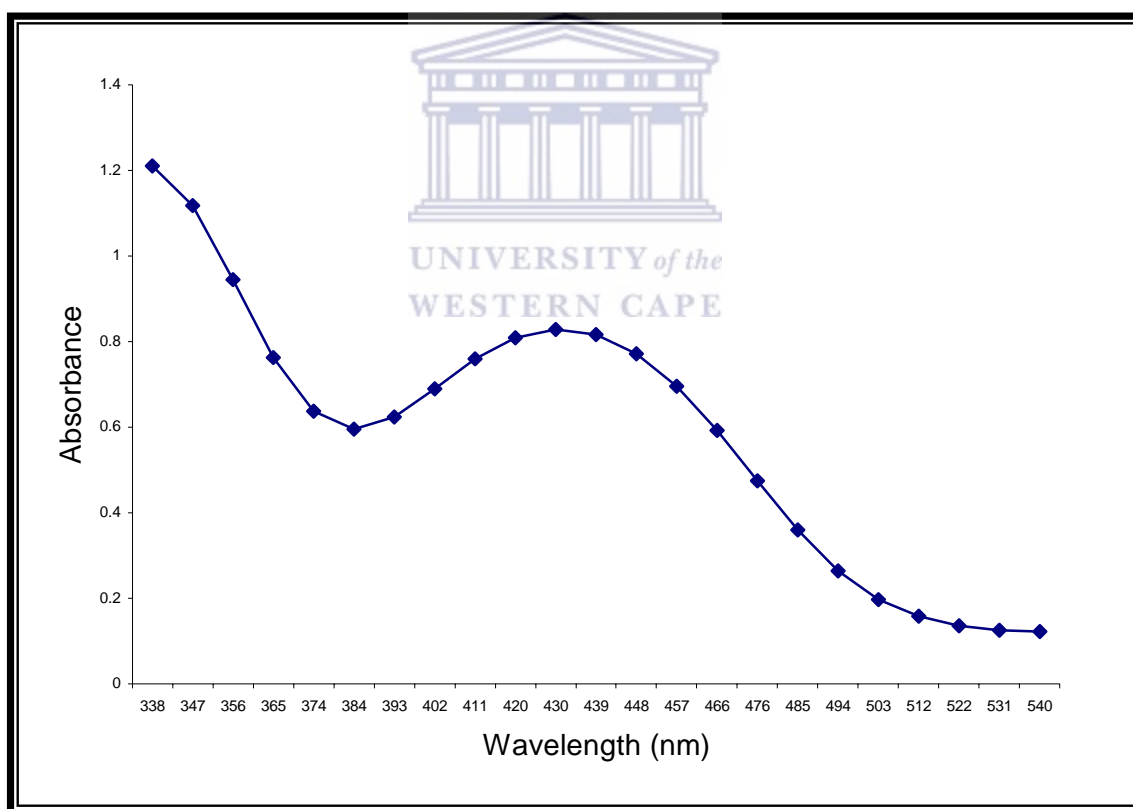


**Photograph 3.2** Extracted diazosulfone solutions (A, B and C) at different concentrations (10, 25 and 50  $\mu\text{M}$ ) respectively.

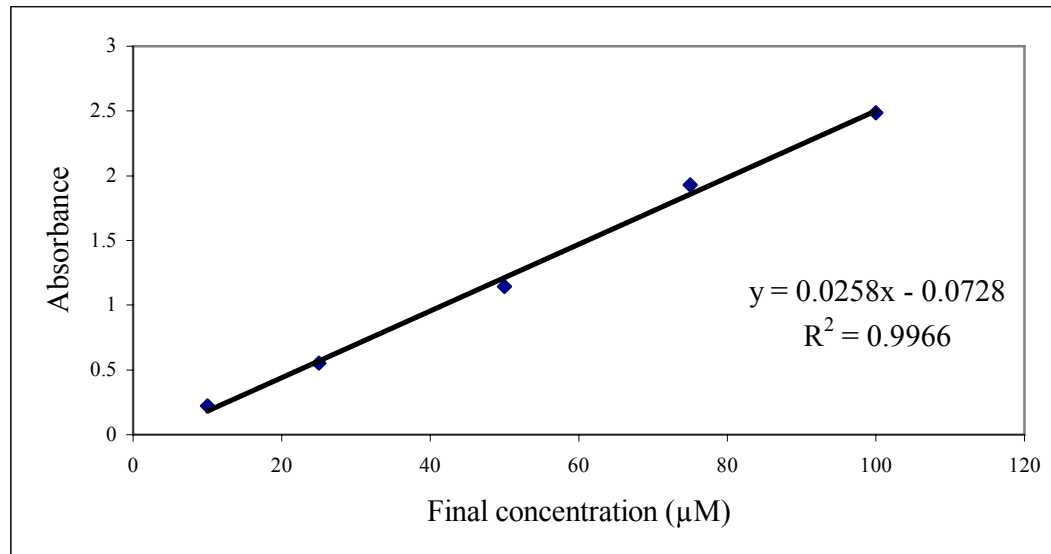
The results obtained are an average of three readings and are shown in table 3.2 below. Figure 3.3 shows the vis/uv absorption spectrum of diazosulfone. Figure 3.4 illustrates the graph of standard MSA solutions: absorbance ( $\lambda_{\max}$  420 nm) versus final concentration ( $\mu\text{M}$ ).

**Table 3.2** Visible absorbance (420nm) and final concentration ( $\mu\text{M}$ ) of standard MSA

Solution	A	B	C	D	E
Final concentration of MSA ( $\mu\text{M}$ )	10	25	50	75	100
Absorbance (420nm)	0.2197	0.5527	1.2449	1.9308	2.4864



**Figure 3.3** A typical vis/uv absorption spectrum of diazosulfone (solution C) between 336-540 nm.



**Figure 3.4** Graph of standard MSA solutions: absorbance ( $\lambda_{\max}$  420 nm) versus final concentration ( $\mu\text{M}$ ), see Table 3.2.

#### 3.4.4.2 Preparation of Fenton generated standard MSA solutions

A typical standard solution was prepared as follows:

- (i) 0.18 ml of DMSO (99,9%) was added to each sample of 0.1 M  $\text{H}_2\text{O}_2$ , different volumes of  $\text{H}_2\text{O}_2$  were taken using a micropipette and made up to volume in 50 ml volumetric flask as shown table 3.3.
- (ii)  $\text{FeSO}_4$  (1 mM) was also made up in dilute DMSO (50 mM) in 100 ml volumetric flask.
- (iii) 2 ml of each  $\text{H}_2\text{O}_2$ /DMSO solution (mixture (i)) was slowly added to 8 ml of each  $\text{FeSO}_4$ /DMSO solution (mixture (ii)) saturated with Argon gas to prevent oxidation of the ferrous ion at rate of 0.5 ml/min in a 100 ml beaker while stirring [116,118].
- (iv) 2 ml of the solution (iii) was added to 8 ml of pure DMSO. The pure DMSO is added in order to facilitate the extraction in organic solvents during the analysis.

However, ratios below 2:8 gave lower absorbances and above 2:8 there was no detection of diazosulfone. Hence, the ratio 2:8 was used in all experiments.

It is assumed that stoichiometric amounts of OH<sup>•</sup> radicals are produced according to the equation below (v) and all product OH<sup>•</sup> radical is scavenged by the DMSO to produce MSA. Similar conditions were used as in section 3.4.4.1.



The series of calibration solutions for spectrophotometric analysis the Fenton reagents were prepared by using the dilutions with final concentrations as shown in the table 3.3.

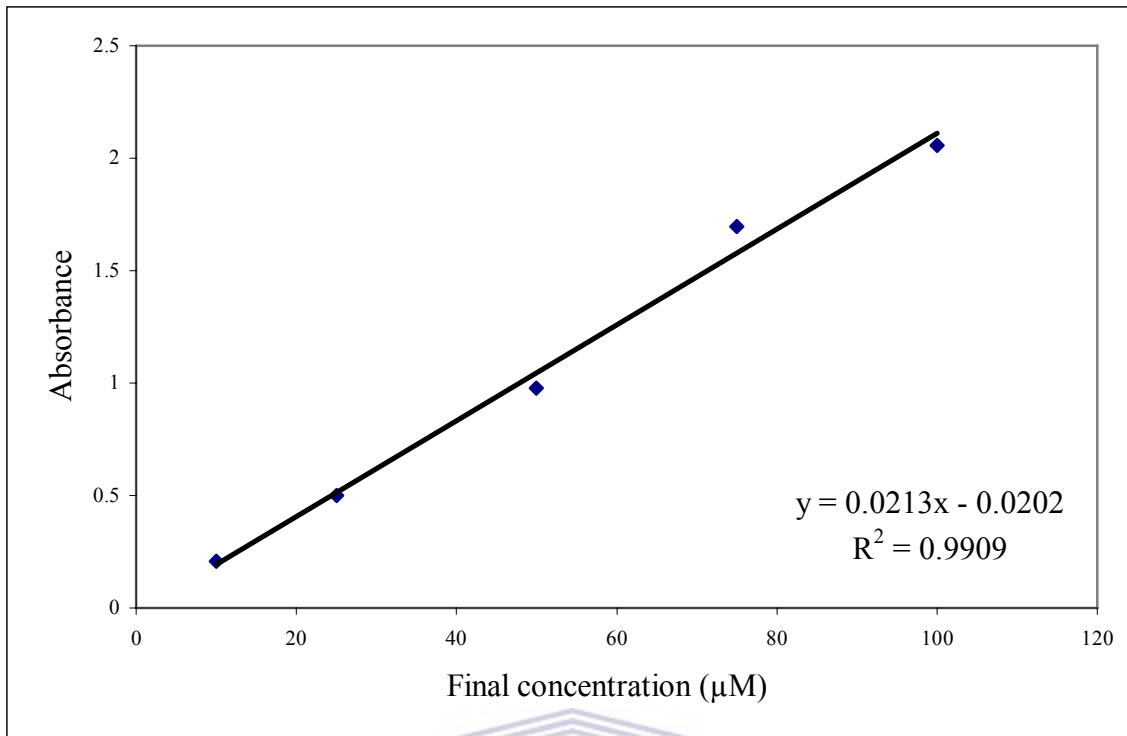
**Table 3.3** Dilution series of Fenton generated standard MSA with final concentrations

Solution	A	B	C	D	E
Volume of 0.1 M H <sub>2</sub> O <sub>2</sub> (ml)	0.0125	0.0250	0.050	0.0625	0.125
Final concentration of MSA (μM)	10	25	50	75	100

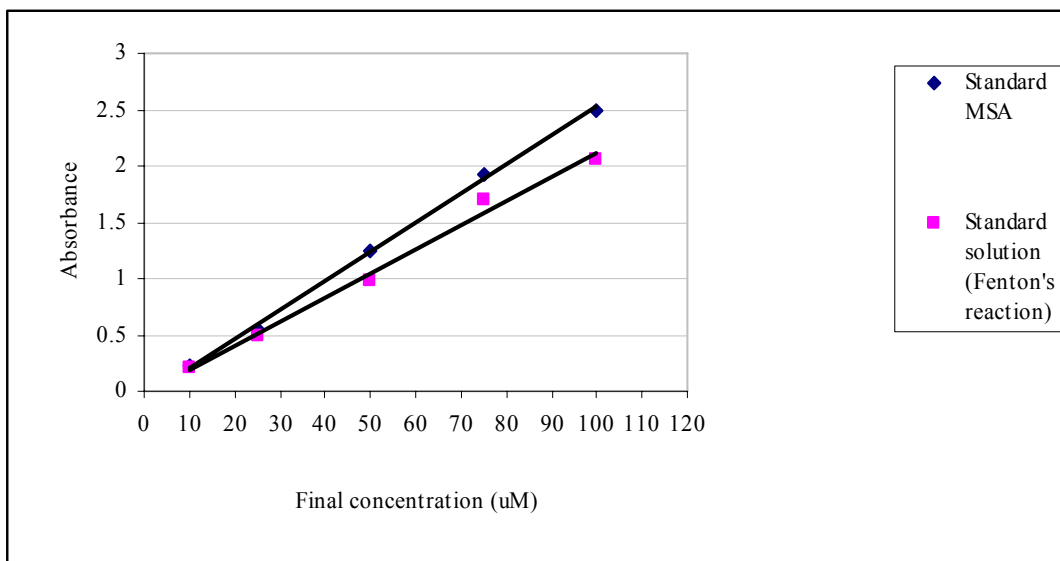
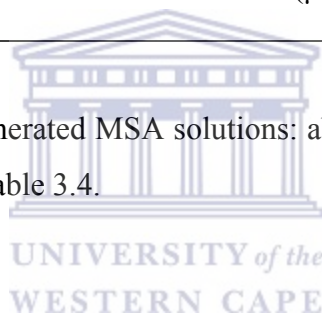
Diazosulfone was produced and extracted by different organic solvents (section 3.4.3), then finally its absorbance measured at 420 nm (visible range). OH<sup>•</sup> radicals were generated in the Fenton's reaction; there is interrelationship of 1:1 molar ratio between diazosulfone and OH<sup>•</sup> radicals (see section 3.4.4.2). The results shown (see Table 3.4) are the average of three readings absorbance (λ<sub>max</sub> 420 nm) using three different samples of the same solution and final concentration (μM) of Fenton generated standard MSA.

**Table 3.4** Visible absorbance (λ<sub>max</sub> 420nm) and final concentration of Fenton generated standard MSA

Solution	A	B	C	D	E
Final concentration of MSA (μM)	10	25	50	75	100
Absorbance (λ <sub>max</sub> 420nm)	0.2088	0.4989	0.9769	1.6970	2.0567



**Figure 3.5** Graph of Fenton generated MSA solutions: absorbance ( $\lambda_{\max}$  420 nm) versus final concentration (μM), see Table 3.4.



**Figure 3.6** Calibration curves of standard MSA and Fenton generated MSA solution: absorbance ( $\lambda_{\max}$  420 nm) versus final concentration ( $\mu\text{M}$ ).

The two calibration curves of both show good linearity (see figures 3.3 and 3.5) with correlation coefficients ( $R^2$ ) of 0.9966 (standard MSA) while slightly lower ( $R^2$ ) constant of 0.9909 for Fenton generated MSA solution. The graphs are linear in the range of concentrations studied and therefore obey Beer's law. However, the two graphs show deviations with the Fenton generated MSA solutions showing lower readings than those standard MSA (figure 3.6). Nevertheless since the operation of the Fe/O<sub>2</sub> battery for disinfection purposes depends upon orders of magnitude of OH<sup>•</sup> radical produced rather than exact amounts, the analytical method is applicable for this project.

#### 3.4.4.3 Measurement of pH and flow rate

##### 3.4.4.3.1 *Measurement of pH*

The initial pH of all standard solutions was adjusted with sulfuric acid before further extraction in organic solvents during the analysis processes.

The final pH of the electrolyte in the Fe/O<sub>2</sub> battery was measured when the pH-electrode was placed into the electrolyte solution in the anode chamber while the battery was running and recorded for an interval of a desired time.

##### 3.4.4.3.2 *Measurement of flow rate*

Flow rate of 20 ml/min of O<sub>2</sub> or air in the cathode chamber was maintained constant in all experiments to electrogenerate H<sub>2</sub>O<sub>2</sub> and measured using a burette soap bubble meter.

#### 3.4.4.4 Iron determination by colorimetric spectrophotometry

The method for determining the iron content in solution based on the formation of the pink colored complex of iron with 2,2-dipyridyl as per the method of *White, 2000* [119,120]. Iron sulfate ( $\text{FeSO}_4$ ) was used to make up the standard solution.

Two reagent solutions were made up in the following procedure: (i) first solution ( $\text{S}_1$ ): 7g of  $\text{CH}_3\text{COONa}$  (sodium acetate) and 0.02g of 2,2-dipyridyl reagent were mixed with distilled water into 50 ml of volumetric flask. (ii) Second solution ( $\text{S}_2$ ) was prepared as follows: 4g of hydroxylammonium chloride ( $\text{HONH}_2\text{Cl}$ ) was dissolved in distilled water and making the volume up to 50 ml in a standard graduated flask.  $\text{S}_2$  solution reduces  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ .

Then the analysis for  $\text{Fe}^{\text{total}}$  was carried out as follows: 1.6 ml of  $\text{S}_2$  is added to 2 ml of the iron solution followed by 2 ml of  $\text{S}_1$ . The absorbance of each solution was measured using vis/uv spectroscopy at  $\lambda_{\text{max}}$  540 nm.

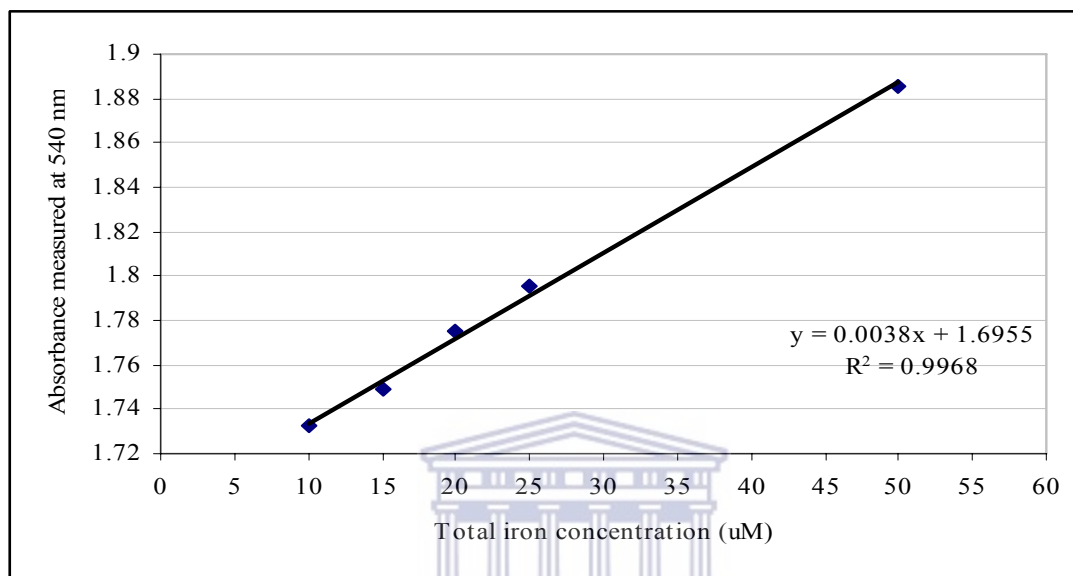
The results obtained (see Table 3.5) are the average of three readings absorbance ( $\lambda_{\text{max}}$  540 nm) using three different samples of the same solution.

**Table 3.5** Total iron final concentrations ( $\mu\text{M}$ ) and its absorbance measured at 540 nm

Solution	Final concentration ( $\mu\text{M}$ ) of $\text{Fe}^{\text{total}}$	Absorbance of $\text{Fe}^{\text{total}}$
<b>A</b>	10	1.7325
<b>B</b>	15	1.7490
<b>C</b>	20	1.7753
<b>D</b>	25	1.7952
<b>E</b>	50	1.8853

<b>Blank</b>	0.0000	0.0000
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Figure 3.7 represents the graph Fe<sup>total</sup> concentration (μM) in the solution with respect to absorbance (λ<sub>max</sub> 540 nm), see table 3.5. The blank was prepared under similar conditions as standard solution.



**Figure 3.7** Graph of Fe<sup>total</sup> concentration (μM) with respect to absorbance (λ<sub>max</sub> 540 nm), see Table 3.5.

Knowing the absorbance, the iron concentration can be calculated by using the Beer's Law equation (see Figure 3.7),  $y = 0.0038x + 1.6955$ , where  $y$  is the absorbance and  $x$  is the concentration (μM). Ferric iron (Fe<sup>3+</sup>) concentration can also be calculated as the difference between the total and ferrous iron concentration when two separate analyses are carried out, firstly using S<sub>1</sub> alone to get ferrous ion concentrations and then S<sub>2</sub> and S<sub>1</sub> for total iron.





## **CHAPTER 4**

## **RESULTS AND DISCUSSION**

## 4.1 INTRODUCTION

A study of the effects of electrolysis time on hydroxyl radical production with some variable parameters in the Fe/O<sub>2</sub> battery cell was undertaken. These parameters included sodium sulfate and sodium chloride concentration. The flow rate, inter-electrode gap, surface area of anode and cathode were kept unchanged.

During the operation (see section 2.11) of the Fe/O<sub>2</sub> battery where Fe acts as an anode and O<sub>2</sub> acts as a cathode, OH<sup>•</sup> radicals were electrogenerated in the solution. The two electrodes were separated with an interelectrode gap of 2 mm [112, 113]. The shorter gap will favor higher currents. O<sub>2</sub> gas was bubbled at flow rate of 20 ml/min forming electrogenerated H<sub>2</sub>O<sub>2</sub> in the solution while Fe was oxidized to form Fe<sup>2+</sup>. The interelectrode gap of 2mm was kept unchanged.

The cell was allowed to run at room temperature for a desired period (e.g. 7.5, 15, 30 and 60 min) before analysis of the electrolyte produced. In order to prevent the precipitation of Fe (III) in the solution, the initial pH of the solution was adjusted to  $\leq 3$  with sulfuric acid (0.1 N) or perchloric acid (0.1 N) depending on the electrolyte used in the system. The ratio: 2:8 (2 ml of electrolyte: 8 ml of DMSO) was used throughout during each analysis for OH<sup>•</sup> radical content of the electrolyte since this ratio gave the highest absorbance of diazosulfone at  $\lambda_{\max}$  420 nm. This ratio was established during the Fenton reagent reactions to produce MSA (see section 3.4.4.2). The current, voltage and pH change in the battery were investigated.

## 4.2 EFFECT OF CHANGING THE ELECTROLYTE IN THE BATTERY

Three electrolyte solutions were compared: Na<sub>2</sub>SO<sub>4</sub> (0.5 M), Na<sub>2</sub>SO<sub>4</sub> (0.05 M) and NaCl (0.5 M) solution. The sulfuric acid (0.1 N) was used to adjust the initial pH of both Na<sub>2</sub>SO<sub>4</sub> solutions to 2.5. The perchloric acid (0.1 N) was used to adjust initial pH of NaCl

to 2.5. The interelectrode gap of 2 mm was kept constant. Each experiment was run for a total of 60 minutes at room temperature (25 °C).

During the experiments electrolyte samples were collected at various times and quenched immediately in a solution of DMSO. The analysis method employed was similar to that used in section 3.4.4.2. The average of triplicate results for each set of experiment is indicated below in table 4.1a-4.1c.

**Table 4.1a** Final concentration of OH<sup>•</sup> radicals (μM) using 0.5 M Na<sub>2</sub>SO<sub>4</sub> solution with battery running time (min) at 25 °C and pH 2.5

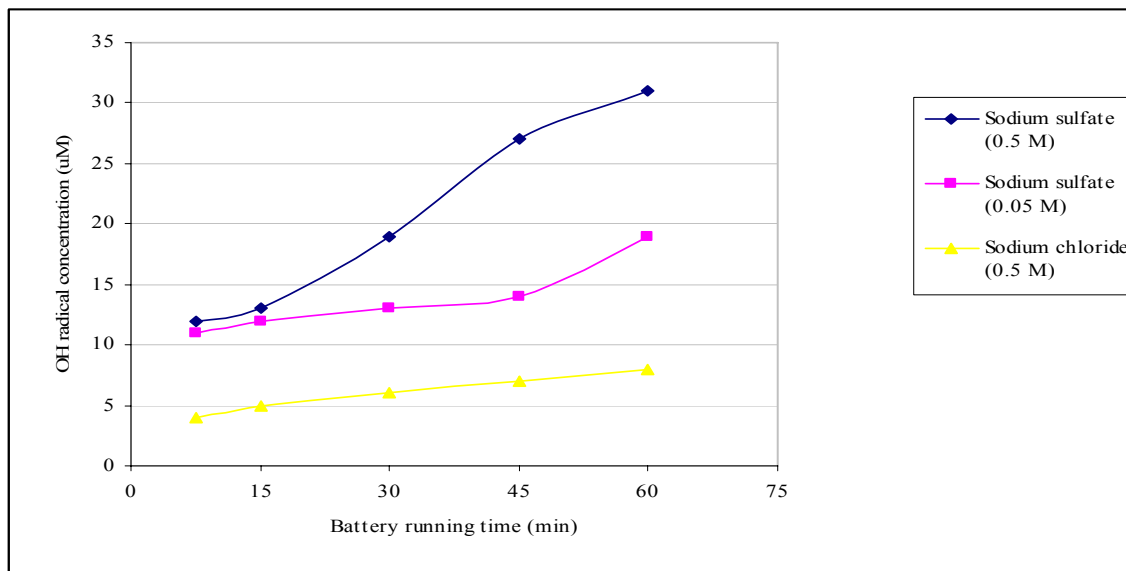
Electrolyte	Na <sub>2</sub> SO <sub>4</sub> (0.5 M)				
Time (min)	7.5	15	30	45	60
Absorbance (420 nm)	0.2255	0.2565	0.3823	0.5553	0.6483
OH <sup>•</sup> concentration (μM)	12	13	19	27	31

**Table 4.1b** Final concentration of OH<sup>•</sup> radicals (μM) using 0.05 M Na<sub>2</sub>SO<sub>4</sub> solution with battery running time (min) at 25 °C and pH 2.5

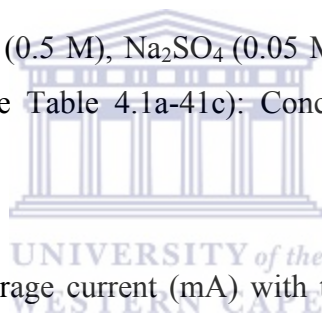
Electrolyte	Na <sub>2</sub> SO <sub>4</sub> (0.05 M)				
Time (min)	7.5	15	30	45	60
Absorbance (420 nm)	0.2046	0.2365	0.2538	0.2762	0.3762
OH <sup>•</sup> concentration (μM)	11	12	13	14	19

**Table 4.1c** Final concentration of OH<sup>•</sup> radical (μM) using 0.5 M NaCl solution with battery running time (min) at 25 °C and pH 2.5

Electrolyte	NaCl (0.5 M)				
Time (min)	7.5	15	30	45	60
Absorbance (420 nm)	0.0688	0.0875	0.1065	0.1273	0.1514
OH <sup>•</sup> concentration (μM)	4	5	6	7	8

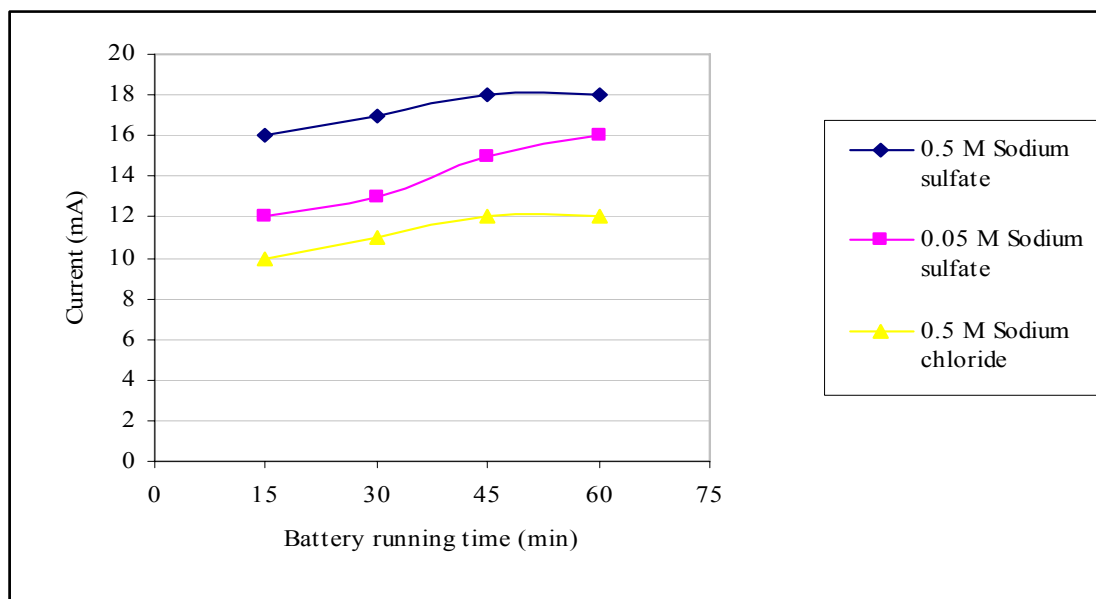


**Figure 4.1** Graphs for Na<sub>2</sub>SO<sub>4</sub> (0.5 M), Na<sub>2</sub>SO<sub>4</sub> (0.05 M) and NaCl (0.5 M) electrolyte solutions at initial pH 2.5 (see Table 4.1a-41c): Concentration of OH<sup>•</sup> radical (µM) versus time.



**Table 4.2** Variation of the average current (mA) with time (min) for Na<sub>2</sub>SO<sub>4</sub> (0.5 M), Na<sub>2</sub>SO<sub>4</sub> (0.05 M) and NaCl (0.5 M) solution at 25 °C and pH 2.5

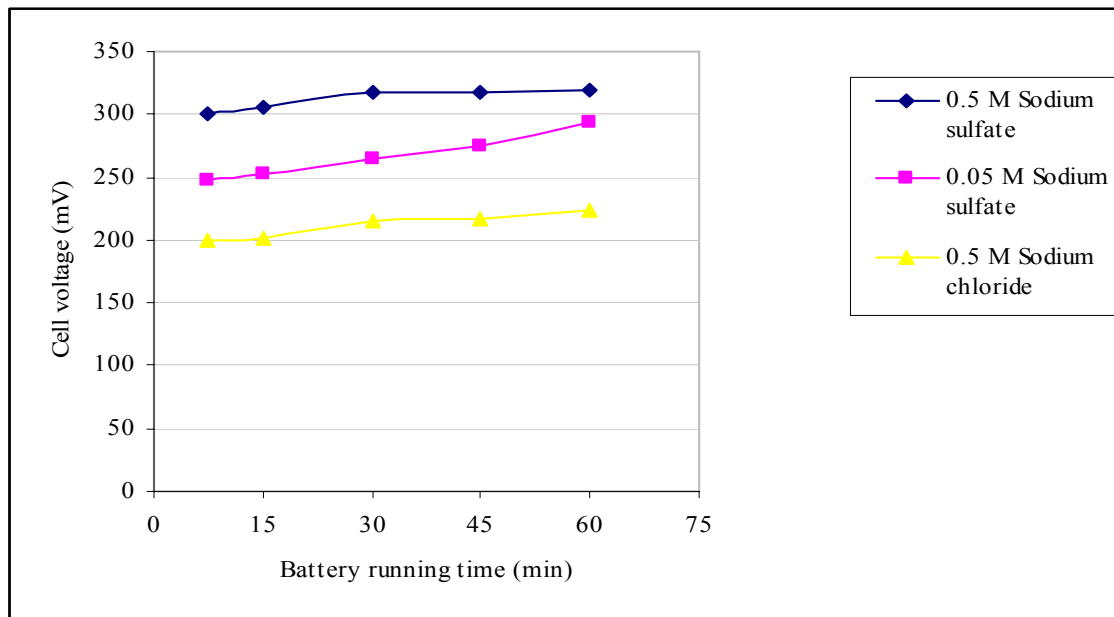
Time (min)	Na <sub>2</sub> SO <sub>4</sub> (0.5M) current (mA)	Na <sub>2</sub> SO <sub>4</sub> (0.05M) current (mA)	NaCl (0.5M) current (mA)
7.5	15	11	9
15	16	12	10
30	17	13	11
45	18	15	12
60	18	16	12



**Figure 4.2** Graph for the average current (mA) with time (min) for  $\text{Na}_2\text{SO}_4$  (0.5 M),  $\text{Na}_2\text{SO}_4$  (0.05 M) and  $\text{NaCl}$  (0.5 M) solution at  $25^\circ\text{C}$  and pH 2.5 (see Table 4.2).

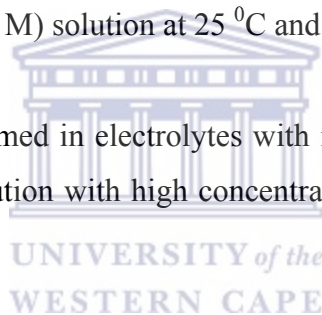
**Table 4.3** Variation of the average cell voltage (mV) with time (min) for  $\text{Na}_2\text{SO}_4$  (0.5 M),  $\text{Na}_2\text{SO}_4$  (0.05 M) and  $\text{NaCl}$  (0.5 M) solution at  $25^\circ\text{C}$  and pH 2.5

Time (min)	$\text{Na}_2\text{SO}_4$ (0.5M) voltage (mV)	$\text{Na}_2\text{SO}_4$ (0.05M) voltage (mV)	$\text{NaCl}$ (0.5M) voltage (mV)
7.5	300	248	200
15	305	253	202
30	317	265	215
45	317	275	217
60	319	293	223



**Figure 4.3** Graph for the average cell voltage (mV) with time (min) for Na<sub>2</sub>SO<sub>4</sub> (0.5 M), Na<sub>2</sub>SO<sub>4</sub> (0.05 M) and NaCl (0.5 M) solution at 25 °C and pH 2.5 (see Table 4.3).

These experiments were performed in electrolytes with initial pH=2.5. The results show that the electrolyte Na<sub>2</sub>SO<sub>4</sub> solution with high concentration (0.5M) produced most OH<sup>•</sup> radicals.



Increase of OH<sup>•</sup> radical with time is due to increase in concentration of reactants i.e. Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>. Thus Na<sub>2</sub>SO<sub>4</sub> (0.5 M) solution was used as a background electrolyte in the Fe/O<sub>2</sub> battery for all other experiments.

#### 4.3 EFFECT OF INITIAL ELECTROLYTE pH

These experiments were performed in order to determine the effect of different initial pH in the electrolyte under similar conditions as explained in section 4.2. The Na<sub>2</sub>SO<sub>4</sub> (0.5 M) was used as a background electrolyte. Sulfuric acid (0.1 N) solution was used to adjust the initial pH 2.5-3.0. Table 4.4 shows the results of experiments when 0.5 M Na<sub>2</sub>SO<sub>4</sub> was used as the electrolyte at initial pH 2.5.

Table 4.5 indicates the data of the experiment when 0.5 M Na<sub>2</sub>SO<sub>4</sub> was used as the electrolyte at initial pH 3.0. All the experiments were conducted at room temperature.

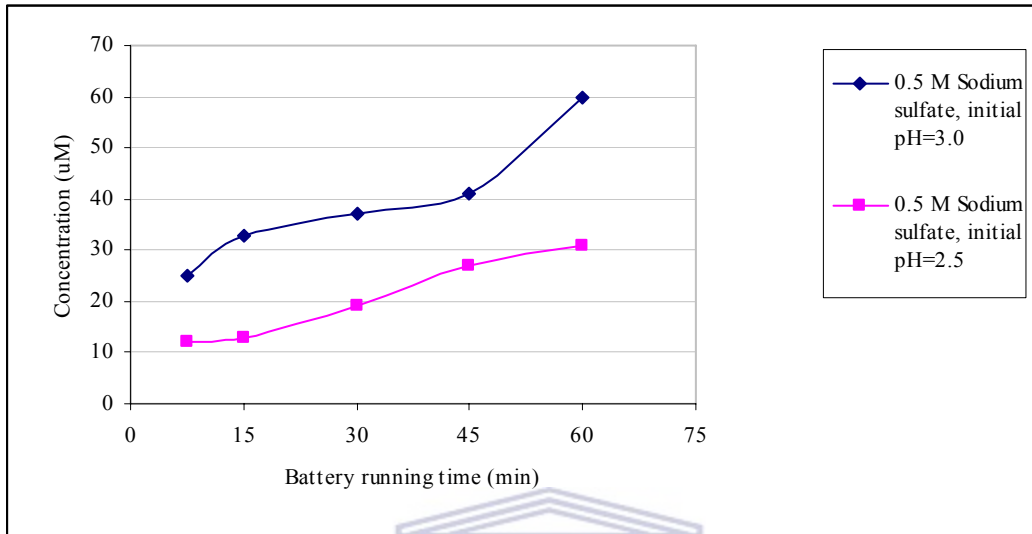
**Table 4.4** Production of OH<sup>•</sup> radicals, change of voltage, current, and pH with battery running time. Electrolyte: Na<sub>2</sub>SO<sub>4</sub> (0.5M) and initial pH= 2.5

Solution	Time (min)	Absorbance ( $\lambda_{\max}$ 420nm)	Voltage (mV)	Current (mA)	Final concentration ( $\mu$ M)	Final pH
<b>A</b>	7.5	0.2255	300	15	12	2.59
<b>B</b>	15	0.2565	305	16	13	2.65
<b>C</b>	30	0.3823	317	17	19	2.78
<b>D</b>	45	0.5553	317	18	27	2.85
<b>E</b>	60	0.6483	319	18	31	2.90

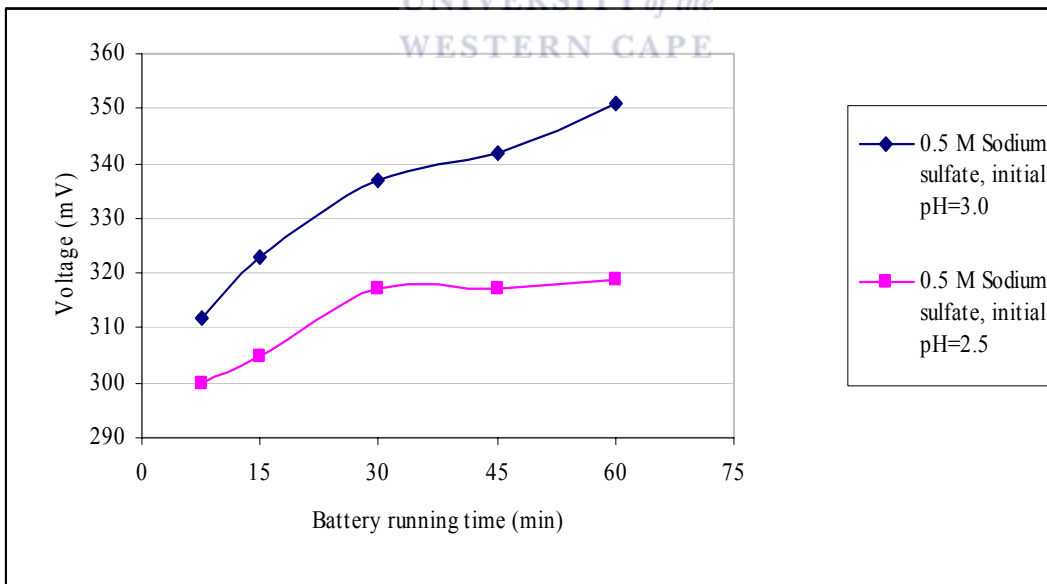
**Table 4.5** Production of OH<sup>•</sup> radicals, change of voltage, current, and pH with battery running time. Electrolyte: Na<sub>2</sub>SO<sub>4</sub> (0.5M) and initial pH= 3.0

Solution	Time (min)	Absorbance ( $\lambda_{\max}$ 420nm)	Voltage (mV)	Current (mA)	Final concentration ( $\mu$ M)	Final pH
<b>A</b>	7.5	0.5205	312	19	25	3.09
<b>B</b>	15	0.6742	323	20	33	3.16
<b>C</b>	30	0.7648	337	21	37	3.31
<b>D</b>	45	0.8501	342	22	41	3.45
<b>E</b>	60	1.2560	351	23	60	3.50

Figure 4.4 shows the change of OH<sup>-</sup> radical concentration with battery running time. The change of voltage, current and the effect on pH with battery running time are shown respectively in figures 4.5, 4.6 and 4.7.

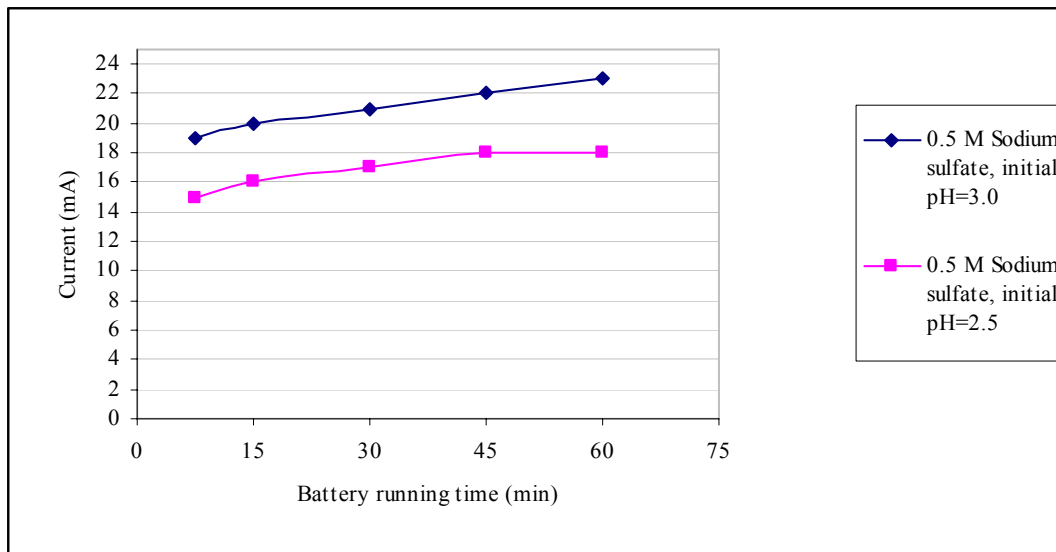


**Figure 4.4** Change of OH<sup>-</sup> radical production with battery running time (Table 4.4 and Table 4.5)

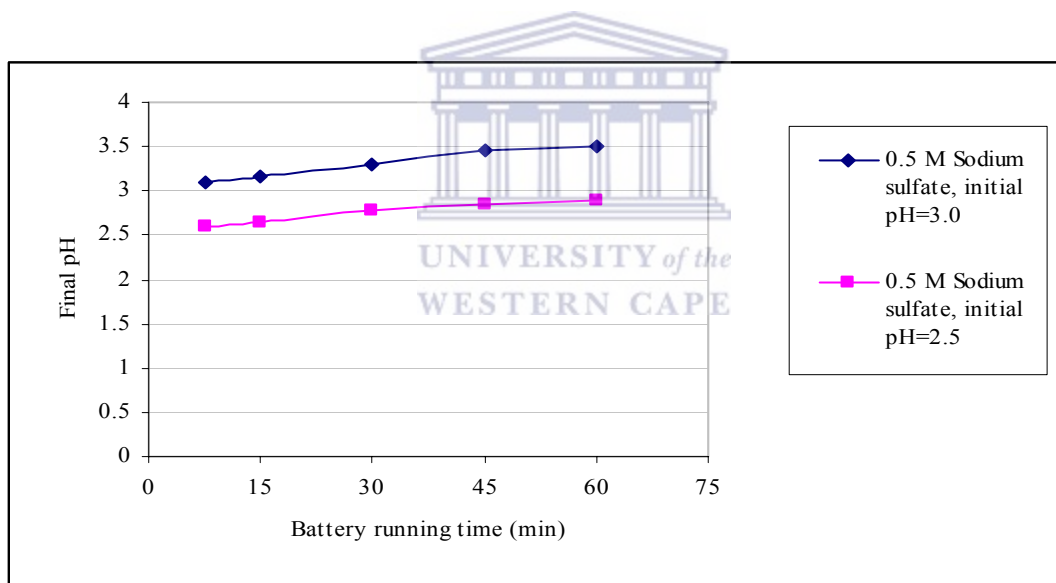


**Figure 4.5** Change of voltage (mV) with battery running time (Table 4.4 and Table 4.5)





**Figure 4.6** Change of current (mA) with battery running time (Table 4.4 and Table 4.5)



**Figure 4.7** pH changes with battery running time (Table 4.4 and Table 4.5)

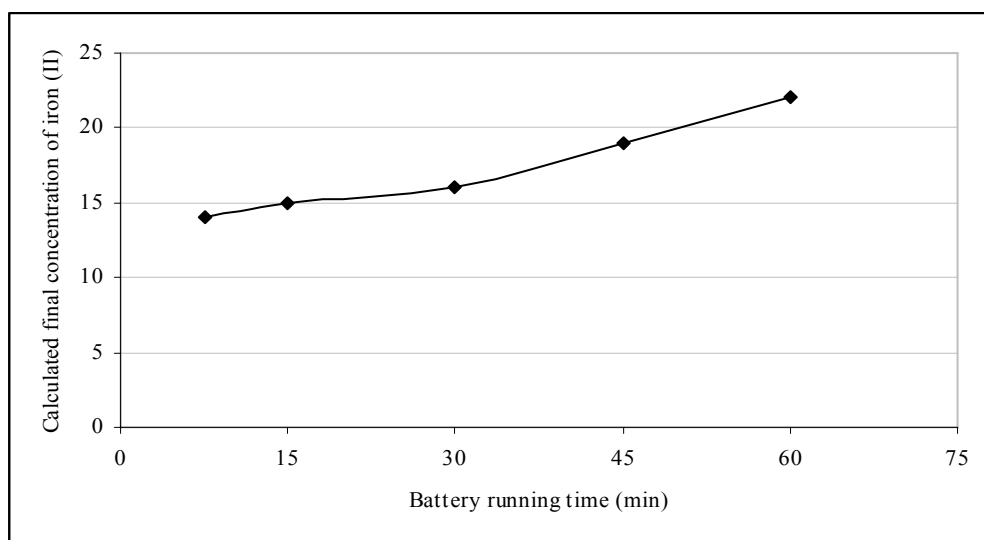
#### 4.4 CHANGE IN IRON CONCENTRATION IN THE Fe/O<sub>2</sub> BATTERY

The analysis of iron concentration has been described previously in section 3.4.4.3 using colorimetric spectrophotometry. Hence, the same procedure and calculations have been used for the Fe/O<sub>2</sub> battery cell. The analysis for Fe<sup>2+</sup> was carried out as follows: distilled water is added to the iron solution followed by S<sub>1</sub>. Iron concentration is given by the equation for best-fit line,  $y = 0.0038x + 1.6955$  (see figure 3.7, section 3.4.4.4) where  $y$  is the absorbance at  $\lambda_{\max}$  540 nm and  $x$  is the concentration of the iron solution.

Thus, table 4.6 shows the results obtained for the absorbance of Fe<sup>2+</sup> detected at wavelength ( $\lambda_{\max}$  540 nm) and the calculated final concentration of Fe<sup>2+</sup> ( $\mu\text{M}$ ) using equation indicated above. Figure 4.8 indicates the graph of final concentration of Fe<sup>2+</sup> ( $\mu\text{M}$ ) against time.

**Table 4.6** Absorbance ( $\lambda_{\max}$  540 nm) and calculated final concentrations ( $\mu\text{M}$ ) for ferrous ion (Fe<sup>2+</sup>) in the solution with time (min)

Solution	Time (min)	Absorbance ( $\lambda_{\max}$ 540nm)	Final concentration ( $\mu\text{M}$ ) of Fe <sup>2+</sup>
<b>A</b>	7.5	1.7458	14
<b>B</b>	15	1.7512	15
<b>C</b>	30	1.7550	16
<b>D</b>	45	1.7657	19
<b>E</b>	60	1.7766	22



**Figure 4.8** Graph of final concentrations ( $\mu\text{M}$ ) for ferrous ion ( $\text{Fe}^{2+}$ ) versus time (min), Table 4.6.

The current with time (figure 4.6, Table 4.4 and 4.5) increases due to the increase in the concentration of charge carriers in the electrolyte, in particular the  $\text{Fe}^{2+}$  concentration increases [94,122].

The  $\text{Fe}^{2+}$  concentration increases with time (figure 4.8, Table 4.6) due to the reaction ( $\text{Fe} \rightarrow \text{Fe}^{2+} + 2\text{e}^-$ ) at the anode, the Fenton reaction appears to be catalytic in iron under these conditions, otherwise  $\text{Fe}^{2+}$  concentration would not increase [123].

The pH increases with time (figure 4.7, Table 4.4 and 4.5) due to the following cathodic reaction:  $\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}_2$ , in which  $\text{H}^+$  ions are removed in the solution as  $\text{H}_2\text{O}_2$  is produced [93,97].

Increasing initial pH from 2.5 to 3.0 (see Table 4.4 and 4.5) increased  $\text{OH}^\bullet$  radical production (figure 4.4, Table 4.4 and 4.5) since the Fenton reaction rate is higher at  $\text{pH} \geq 3.0$ , see Chapter 2 (section 2.7) [87]. Similarly as pH increases with time (figure 4.7) so does Fenton reaction rate producing more  $\text{OH}^\bullet$  radicals.

The cell potential (figure 4.5) is initially low due to very low concentration of  $\text{Fe}^{2+}$  but then increases slightly with time. Referring to the Nernst equation (see section 3.12.2, equation (xvii)), with increasing pH in the electrolyte and with  $\text{Fe}^{2+}$  concentration also increasing results in an increase in cell potential with time.

Currents are higher in the 0.5 M  $\text{Na}_2\text{SO}_4$  than in 0.5 M  $\text{NaCl}$  since the charge carrier concentration is greater. Currents increase with time as the total concentration of charge carriers increases as the cell reaction proceeds.

The initial  $\text{pH} \leq 3.0$  (see Table 4.4 and 4.5) was essential to sustain the ferrous ion in the solution as the formation of  $\text{OH}^\cdot$  radicals by the reaction of  $\text{H}_2\text{O}_2$  with  $\text{Fe}^{2+}$  is the limiting step in the overall reaction rate of oxidation of  $\text{Fe}^{2+}$  for Fenton's reaction [18]. However, the effect of pH for initial pH values higher than 3.0 was not further investigated since a significant amount of  $\text{Fe}^{3+}$  precipitates at  $\text{pH} > 3.0$ .

#### 4.5 STUDY OF OXIDATION-REDUCTION POTENTIAL (ORP) IN THE $\text{Fe}/\text{O}_2$ BATTERY



The ORP of the electrolyte solution will depend upon a combination of all the redox couples present e.g.  $\text{Fe}^{2+}/\text{Fe}^{3+}$ ,  $\text{H}_2\text{O}_2/\text{O}_2$ ,  $\text{O}_2/\text{H}_2\text{O}$ ,  $\text{OH}^\cdot/\text{H}_2\text{O}$  etc. In general the more positive the ORP the more oxidizing the environment and thus more disinfecting is the solution [124].

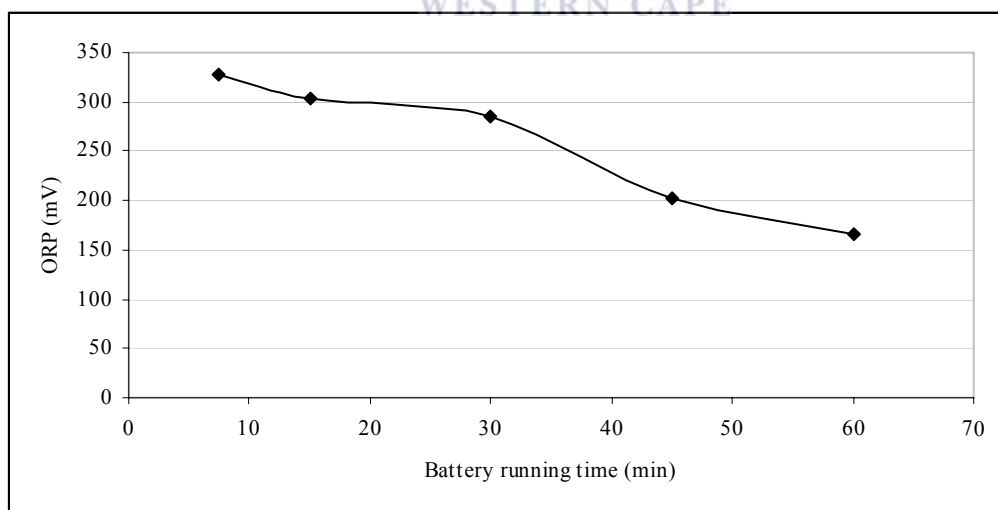
The aim of these experiments was to study the change in ORP of the electrolyte with battery running time. Also in order to test whether the electrolyte remained active (in terms of Fenton generated  $\text{OH}^\cdot$  radicals) the battery was run for 3 hours and then disconnected. The electrolyte was repeatedly analysed after standing for intervals of up to ten days (see figure 4.10a).

Similar conditions were employed in all experiments (see section 4.3). Electrolyte:  $\text{Na}_2\text{SO}_4$  (0.5M), initial pH=3.0, flow rate: 20 ml/min, interelectrode gap: 2 mm, and temperature = 25 °C.

Table 4.7a illustrates the ORP changes with battery running time (figure 4.9). The results in table 4.7b (figure 4.10a) indicate the ORP changes after 3 hours running time, followed by periods of standing with battery disconnected (figure 4.10b).

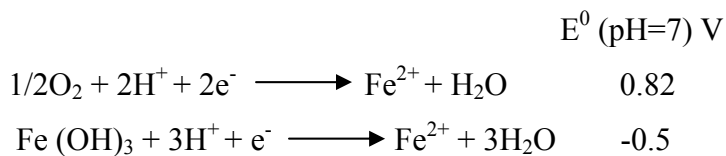
**Table 4.7a** Results of ORP changes with battery running time after 60 min

Solution	Time (min)	ORP (mV)
<b>A</b>	7.5	327
<b>B</b>	15	304
<b>C</b>	30	285
<b>D</b>	45	203
<b>E</b>	60	165



**Figure 4.9** ORP changes with battery running time after 60 min.

The ORP values decrease gradually after the cell was run for 60 min (figure 4.9). Several redox couples will contribute probably involving:



as well as others. As the pH increases the forward reactions become less favourable and the  $E^0$  value less positive or more negative. Thus the overall ORP drops with increasing pH (and hence decreases with time).

**Table 4.7b** Results of ORP with periods of battery disconnected (after running time: 3 hours)

Solution	Disconnection time (after day)	Absorbance ( $\lambda_{\text{max}}$ 420nm)	ORP (mV)	Final concentration ( $\mu\text{M}$ )	pH
<b>A</b>	1	0.6483	309	31	5.0
<b>B</b>	2	0.5641	312	27	4.7
<b>C</b>	5	0.4930	315	24	4.6
<b>D</b>	8	0.3923	319	19	4.5
<b>E</b>	10	0.3682	321	18	4.2

**Figure 4.10a** ORP with periods of battery disconnected (running time: 3 hours).

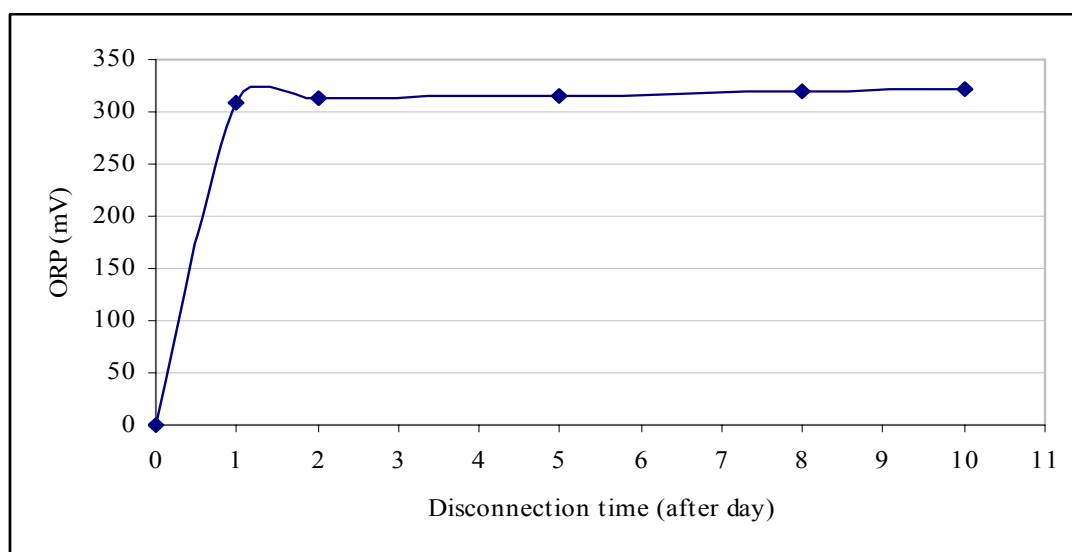
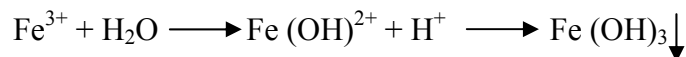
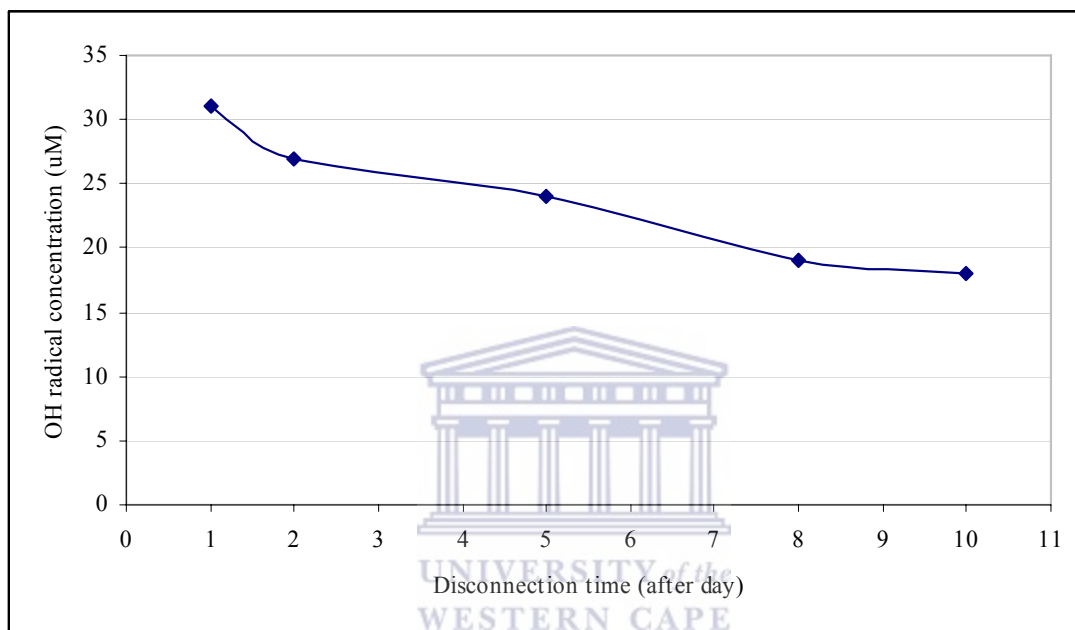


Figure 4.10a (Table 4.7b) shows that ORP increases with disconnection time after the battery was run for 3 hours. This increase is probably due to iron (III) hydrolysis responsible for the decrease in pH as per the following equation:



Eventually  $\text{Fe}(\text{OH})_3$  precipitates which can be seen to occur while the battery is switched off [18,112,113].



**Figure 4.10b** Changes in  $\text{OH}^\bullet$  radicals concentration with standing time

The results above (figure 4.10b, Table 4.7b) indicate that  $\text{OH}^\bullet$  radical concentration decreases gradually with disconnection time while ORP increases since the pH drops the production of  $\text{OH}^\bullet$  radicals will also drop as the rate of Fenton reaction decreases when the pH changes from 2.5 to  $\geq 4$  [87]. What is significant is that the  $\text{Fe}/\text{O}_2$  battery electrolyte continues to generate  $\text{OH}^\bullet$  radicals even after long periods of being disconnected [87].

#### 4.6 USE OF AIR INSTEAD OF OXYGEN (O<sub>2</sub>) FOR OH<sup>•</sup> RADICAL PRODUCTION IN THE Fe/O<sub>2</sub> BATTERY

The purpose of the experiment was to establish the amount of OH<sup>•</sup> radical production in the Fe/O<sub>2</sub> battery when air instead of oxygen is used in the gas diffusion electrode.

The experiments were performed at initial pH=3 (section 4.3): Electrolyte: Na<sub>2</sub>SO<sub>4</sub> (0.5M), Flow rate: 20 ml/min and interelectrode gap: 2 mm. The cell was run for 60 minutes in the room temperature.

Table 4.8a and 4.8b (Figure 4.11) show the results of the experiments with final concentration (μM) of OH<sup>•</sup> radical production using air and oxygen when the cell was run for 60 minutes.

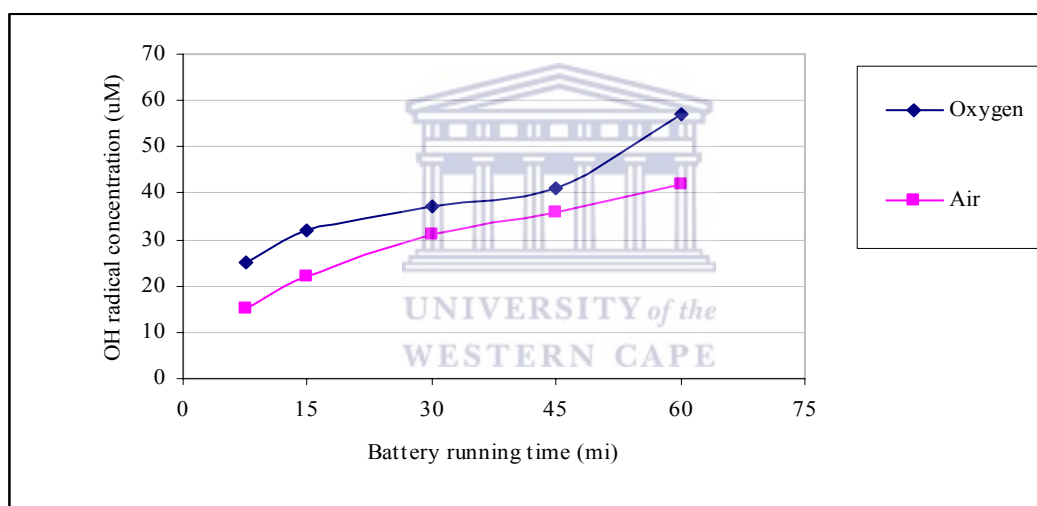
**Table 4.8a** Results of OH<sup>•</sup> radical production using air with battery working time

Solution	Time (min)	Absorbance (λ <sub>max</sub> 420nm)	Voltage (mV)	Current (mA)	Final OH <sup>•</sup> concentration (μM)	Final pH
<b>A</b>	7.5	0.2970	304	14	15	3.05
<b>B</b>	15	0.4440	317	15	22	3.11
<b>C</b>	30	0.6386	318	16	31	3.22
<b>D</b>	45	0.7469	319	17	36	3.35
<b>E</b>	60	0.8669	319	18	42	3.44



**Table 4.8b** Results of OH<sup>•</sup> radical production using oxygen with battery working time

Solution	Time (min)	Absorbance ( $\lambda_{\max}$ 420nm)	Voltage (mV)	Current (mA)	Final OH <sup>•</sup> concentration ( $\mu\text{M}$ )	Final pH
A	7.5	0.5115	312	19	25	3.09
B	15	0.6682	323	20	32	3.16
C	30	0.7603	337	21	37	3.31
D	45	0.8458	342	22	41	3.45
E	60	1.2010	351	23	57	3.50



**Figure 4.11** Concentration ( $\mu\text{M}$ ) of OH<sup>•</sup> radical production using air and oxygen with battery working time (min), Table 4.8a and 4.8b.

These results above (see Tables 4.8a and 4.8b, figure 4.11) indicate simply that air can be used as an alternative to pure O<sub>2</sub>.

#### 4.7 CONCLUSIONS

- A higher amount of OH<sup>•</sup> radicals was produced at initial pH=3.0 as compared with the initial pH=2.5.
- Both air and oxygen used in the gas diffusion electrode were investigated in the Fe/O<sub>2</sub> battery. The results show that air can be used as an alternative to pure O<sub>2</sub>.
- Higher currents and cell voltages occur with increasing running time of the battery which are consistent with the increasing pH and Fe<sup>2+</sup> concentration of the electrolyte with running time.
- Increasing electrolyte concentration (Na<sub>2</sub>SO<sub>4</sub>) increases OH<sup>•</sup> radical production. Na<sub>2</sub>SO<sub>4</sub> is a better electrolyte than NaCl for OH<sup>•</sup> radical production.
- The electrolyte solution continues to generate OH<sup>•</sup> radicals even after standing for several days.

#### 4.8 HYDROXYL RADICAL PRODUCTION FROM THE DIACELL<sup>®</sup>

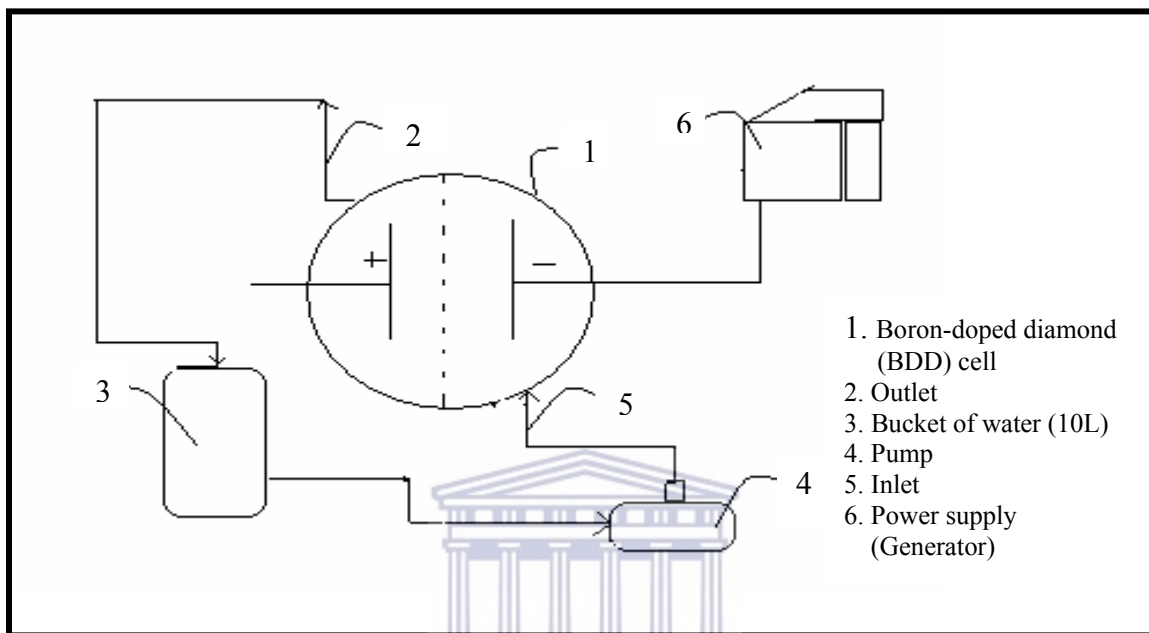
The purpose of this experiment was to test whether the DiaCell<sup>®</sup> produces OH<sup>•</sup> radicals and the method used previously in the Fe/O<sub>2</sub> battery for the analysis of OH<sup>•</sup> radicals production in aqueous solution generated in the DiaCell<sup>®</sup>. The DiaCell<sup>®</sup> electrolyzer uses boron-doped diamond (BDD) electrodes was employed in this study using tap water [70].

The water flows through the Diacell<sup>®</sup> from the bottom to the top in order to optimize the evacuation of the gases formed at the electrode surfaces [102], see figure 4.12 and photograph 4.1.

The procedure used was as follows: 10 L batches of tap water were circulated using on-line pump through DiaCell<sup>®</sup> for 4 min. The flow rate of 42 ml/second was maintained constant in all experiments. Samples were collected at 1 min intervals for a period of 4 min. The samples were analyzed by the method used under similar conditions as described earlier in chapter 3 (see section 3.4.4.2).

Table 4.9 (figure 4.13) shows the results of OH<sup>•</sup> radicals production from DiaCell<sup>®</sup> using tap water as electrolyzed at pH 7. The final concentration of OH<sup>•</sup> radicals (μM) was calculated by using the same equation described in chapter 3 (section 3.4.4.2, figure 3.5).

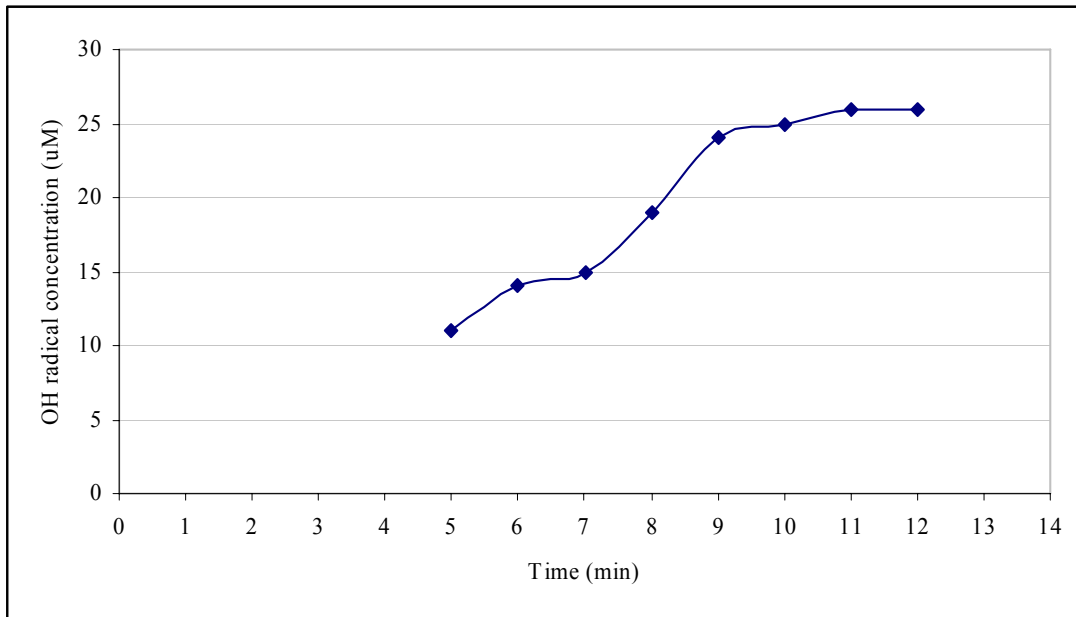
**Figure 4.12** Diagram of the Electrolyser Diacell<sup>®</sup>



**Photograph 4.1** Electrolyser Diacell<sup>®</sup> (type 106:01-010)

**Table 4.9** Production of OH<sup>•</sup> radicals from DiaCell<sup>®</sup> using the electrolyzed tap water at pH 7. Temperature = 25 °C; Flow rate = 42 ml/second

Solution	Time (min)	Absorbance ( $\lambda_{\text{max}}$ 420nm)	Voltage (V)	Current (A)	Final OH <sup>•</sup> concentration ( $\mu\text{M}$ )
<b>A</b>	5	0.2149	10.01	0.46	11
<b>B</b>	6	0.2693	10.01	0.46	14
<b>C</b>	7	0.2994	10.01	0.46	15
<b>D</b>	8	0.3846	10.01	0.46	19
<b>E</b>	9	0.4990	10.01	0.46	24
<b>F</b>	10	0.5061	10.01	0.46	25
<b>G</b>	11	0.5239	10.01	0.46	26
<b>H</b>	12	0.5258	10.01	0.46	26



**Figure 4.13** Final concentration of OH' radical production from DiaCell<sup>®</sup> against time (min), Table 4.9.

The results above (figure 4.13, Table 4.9) show that the DiaCell<sup>®</sup> is able to produce OH' radicals using the electrolyzed tap water at pH 7 with constant flow rate of 42 ml/second under similar conditions as section 4.3.

The results must be treated with caution since although the chlorine concentrations are very low ( $\leq 2 \mu\text{M}$ ) in the tap water after passing through the DiaCell for 10 min). Chlorine does interfere with the analysis method at higher concentration (e.g. at  $50 \mu\text{M}$  Chlorine had an absorbance of 0.1590 ( $\lambda_{\text{max}}$  420nm) indicating an "OH' radical" concentration but the values obtained were not constant over repeated experiments. Also the species present may not have been OH' radical but more likely a longer lived powerful oxidant species. The nature of that species is the subject of much controversy [105].

In this chapter, the  $\text{OH}^\bullet$  radical production was studied. The ability of the  $\text{OH}^\bullet$  radicals to destroy microorganisms has to be evaluated. Hence *Escherichia coli* (*E.coli*), a well-known indicator bacterium, was chosen as the experimental microorganism for the disinfection experiments [125].

The disinfection ability of  $\text{OH}^\bullet$  radicals produced in the Fe/O<sub>2</sub> battery and DiaCell<sup>®</sup> technology have been further evaluated quantitatively and then compared with those of the chlorination method in chapter 5.



The logo of the University of Western Cape, featuring a classical building with columns and a pediment, is centered in the background.

**CHAPTER 5**

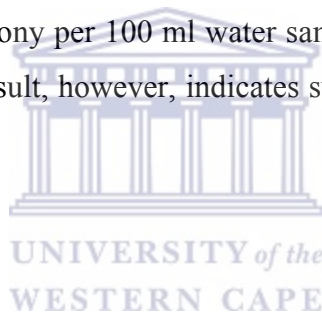
**ANALYSIS OF THE EFFECTIVENESS OF Fe/O<sub>2</sub> BATTERY  
ELECTROLYTE FOR THE DISINFECTION OF *ESCHERICHIA  
COLI (E.coli)* IN WATER**

WESTERN CAPE

## 5.1 INTRODUCTION

Waterborne disease is common in all parts of the world where there is poor hygiene and sanitation. The most common source of exposure to disease-causing organisms for human being is ingestion of contaminated drinking water and food. The emergency situations involving accidental contamination of drinking water supply systems continue to demonstrate the need for strong disinfectant agents as an ultimate defense against waterborne microbiological infection such as *E.coli* (coliform group bacteria). The presence of coliform bacteria in water indicates pathogenic contamination [126].

The allowed level of coliform bacteria in drinking water is less than 1 coliform colony per 100 milliliter of samples (<1/100 ml). Communal water systems are required to test frequently for coliform bacteria [126]. The test involves screening techniques, and a positive result (more than 1 colony per 100 ml water sample) means the water should be retested. A high positive test result, however, indicates substantial contamination and the water must be purified.



## 5.2 AIM

The aim of this work was to investigate the effectiveness of OH<sup>·</sup> radicals produced by Fenton's reaction and the electrolyte from the Fe/O<sub>2</sub> battery of inactivation of *E.coli* in water compared to chlorine disinfection treatment [127,128].



### 5.3 APPROACH

The approach used was as follows:

- To test three different concentrations of disinfecting solution (1/10<sup>th</sup>, 1/100<sup>th</sup> and 1/1000<sup>th</sup> dilutions) on different concentrations of *E.coli* in water (10<sup>-1</sup>-10<sup>-6</sup>)
- To stop disinfecting activity using Neutralizing Broth (NB)
- To count *E.coli* using the pour-plate method.

### 5.4 EXPERIMENTAL SECTION

#### 5.4.1 Materials and Preparation of reagents

All reagents were prepared with distilled water. Tryptone Soya Agar (TSA), Tryptone Soya Broth (TSB), and Neutralizing Broth (NB) were purchased from Oxoid (UK), Tap water used in all experiments was obtained from Chemistry laboratory (Chemistry Department, University of the Western Cape/ UWC). All solutions used were stored in a refrigerator after being autoclaved at 121<sup>o</sup>C for 20 min along with all glassware used. All glassware was washed with distilled water.

##### 5.4.1a *Preparation of Tryptone Soya Broth (TSB)*

A stock solution was prepared by diluting 7.5 g of TSB to the desired volume (250 ml) with distilled water in 500 ml sealed bottle. 10 ml of the solution was used in each test tube after the solution was autoclave at 121<sup>o</sup>C for 20 min.

#### 5.4.1b *Preparation of Neutralizing Broth (NB)*

9.75 g of stock solution NB was mixed with 250 ml of distilled water in 500 ml sealed bottle and then, followed by autoclave at 121°C for 20 min. 0.9 ml of the solution was used to each 1.5 ml eppendorf test tube for different concentration. The remaining solution was kept in the fridge for further experiment.

#### 5.4.1c *Preparation of Tryptone Soya Agar (TSA)*

10 g of stock solution of TSA were dissolved into 500 ml bottle with distilled water to the desired volume (250 ml). The solution was autoclave at 121°C for 20 min. The solution was then poured to each plate after 1 ml of each *E.coli* treatment was added into a plate.



#### 5.4.2 **Preparation and analysis of *E.coli* growth in water**

The method of preparation and analysis of *E.coli* growth in water has been proposed by Jeyong *et al* [129]. The *E.coli* culture was grown on a shaker for 24 h at 37 °C to reach the stationary growth phase. 10 ml of fresh *E.coli* culture was ready for the disinfection experimental. 1 ml of fresh *E.coli* was then centrifuged in 1.5 ml sterile eppendorf test tube at 13000 x g for 3 min [130]. 1 ml re-suspension of bacteria culture was aseptically transferred into sterile test tube containing 9 ml tap water dilution ( $10^{-1}$ - $10^{-6}$ ).

However, when *E.coli* is grown in batch culture with TSB, there is no decrease in the growth rate during this stage, and a quick cessation of growth occurs immediate with the reduction of TSB. In addition, a full-grown *E.coli* colony on an agar plate is a relatively stable entity, thus a hundred or better-isolated colonies can be used simply as an inoculum for further work only by touching the colony with a sterile toothpick or inoculating loop.

### 5.4.3 Methodology

The methodology used in this experiment was as follows:

- ✓ A 10 ml *E.coli* was grown for 24 hours to reach exponential phase.
- ✓ Transfer 1 ml bacteria culture to a 1.5 ml eppendorf test tube, after using vortex mixer.
- ✓ Spin down by using a centrifuge (13 000 x g for 3 min)
- ✓ Discard supernatant by keeping the pellet upside down
- ✓ Resuspend in 1 ml tap water, using vortex mixer (first time)
- ✓ Spin down by using a centrifuge (13 000 x g for 3 min)
- ✓ Discard supernatant (same procedure).
- ✓ Resuspend in 1 ml tap water, using vortex mixer (second time).
- ✓ Transfer 1 ml bacteria culture to  $10^{-1}$ - $10^{-6}$  of tap water dilution (always discarding tips and using vortex mixer).
- ✓ Remove and discard 1 ml of  $10^{-6}$  dilution series as the volume reached 10 ml.
- ✓ Transfer 4.5 ml of  $10^{-6}$  to 3 other test tubes.
- ✓  $1/10^{\text{th}}$  and  $1/100^{\text{th}}$  dilutions of disinfectant in distilled water will be used to make  $1/100^{\text{th}}$  and  $1/1000^{\text{th}}$  treatments.
- ✓ Add 0.5 ml of 100%,  $1/10^{\text{th}}$  and  $1/100^{\text{th}}$  disinfectant to 4.5 ml culture volumes of  $10^{-1}$ - $10^{-6}$ , however only the volume of  $10^{-6}$  will be used as experiments (fewer colonies to count). Each experiment has to be completed separately in duplication.
- ✓ Add 0.5 ml tap water to 4.5 ml culture volumes of  $10^{-6}$  for control (negative control).
- ✓ 0.1 ml of treatment will be removed after contact time and added to 0.9 ml NB solution.
- ✓ 1 ml of each solution will be pour plated with certain quantity of agar. Similar condition will be used for control (negative control) and NB (positive control).
- ✓ Incubate plates at 37 °C and count after 24 hours.

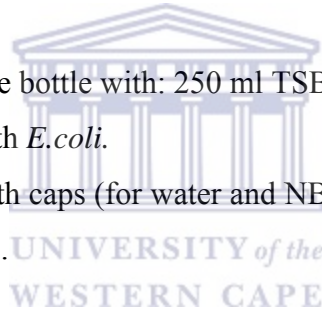
## 5.5 EXPERIMENTAL PROCEDURES AND METHODS

The experiment was carried out in the microbiology laboratory, at room temperature. Tap water contaminated by *E.coli* culture was used for the experimental analysis. The test water was prepared by diluting a pure culture of *E.coli* to a complete dilution series  $10^{-1}$ - $10^{-6}$ . The *E.coli* culture was cultivated by using the method proposed previously by Jeyong *et al* [129] and followed by the membrane filtration method in accordance with the standard methods [131]. A fresh culture was then utilized to create the reproduction water for the disinfection experiment.

The methodology (section 5.4.3) has shown the experimental procedures, as this includes planning and preparation techniques (see day 1-3).

### DAY 1

- Prepare media (autoclave bottle with: 250 ml TSB, 250 ml NB and 250 ml TSA).
- Inoculate 10 ml TSB with *E.coli*.
- Autoclave: test tubes with caps (for water and NB dilutions), blue tips box, 1L tap water and 20 ml pipettes.



### DAY 2

- Prepare disinfectant solutions 100%-1% (to make  $1/10^{\text{th}}$  – $1/1000^{\text{th}}$ ). Method: make 50 ml 100% disinfectant, prepare 2 sterile test tubes with 18ml distilled water (i.e. each test tube contains 9 ml of distilled water), add 2 ml of 100% disinfectant to make 10% and 1% of dilution series (1 ml disinfectant solution to each test tube).
- Place agar at 50°C.
- Prepare test tubes with tap water (each test tube contains 9 ml of tap water) and 1.5 ml eppendorf test tubes with NB (each eppendorf test tube contains 0.9 ml of NB solution).
- Label test tubes and eppendorf test tubes.
- Repeat the methodology (see section 5.4.3) for entire experiment.

### DAY 3

- Count plates.

Same procedures and techniques were used for the *E.coli* cells treatment with chlorine disinfection [132]. The results obtained have been discussed below.

## 5.6 RESULTS

The results that are tabulated below are the average killing efficiency (%) for the three readings with standard deviation. All analyse involving different disinfectant (oxidant) concentrations were conducted after 15, 30 and 60 min of contact time between the oxidant and the solutions containing *E.coli*.

The final concentration ( $\mu\text{M}$ ) of the disinfectant (oxidant) was calculated by using the equation  $y = 0.0213x - 0.0202$ , stated previously in the section 3.4.4.2 (figure 3.5), as  $y$  represented the absorbance ( $\lambda_{\text{max}}$  420 nm) and  $x$ , the concentration ( $\mu\text{M}$ ).

### 5.6.1 *Effect of OH<sup>•</sup> radicals produced from the Fenton reaction on E.coli in water at 25°C.*

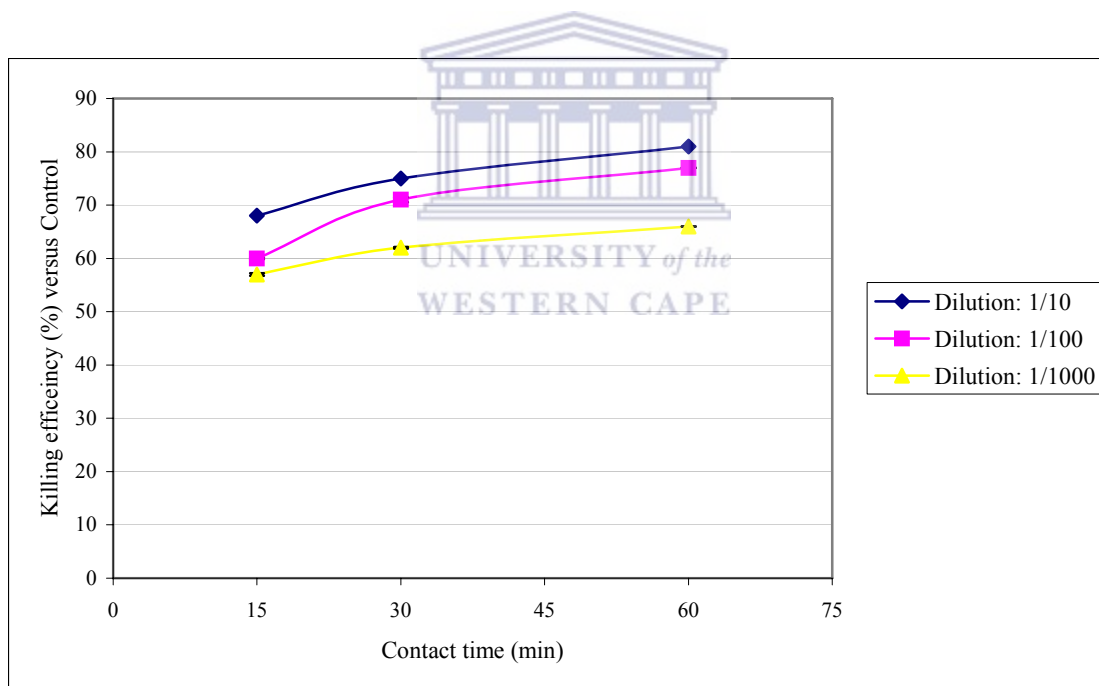
Three solutions (A, B and C) were prepared using Fenton reagent under similar conditions. The absorbance ( $\lambda_{\text{max}}$  420 nm) for OH<sup>•</sup> radical production was (i) solution **A**: 0.5924 with final concentration of 29  $\mu\text{M}$ , (ii) solution **B**: 0.6084 with final concentration of 30  $\mu\text{M}$  and (iii) solution **C**: 0.6446 with final concentration of 31  $\mu\text{M}$ . These solutions were followed by dilution series: 1/10, 1/100 and 1/1000.

Table 5.6.1 (figure 5.1-5.3) shows the results of the effect of the OH<sup>•</sup> radical production from the Fenton reaction for solution: A, B and C on *E.coli* in water at 25 °C (pH 7).

**Table 5.6.1** Effect of OH<sup>•</sup> radicals produced from the Fenton reaction on *E.coli* in water at 25°C (pH 7)

(i) **Solution: A.** Absorbance: 0.5924; OH<sup>•</sup> radicals final concentration= 29 μM

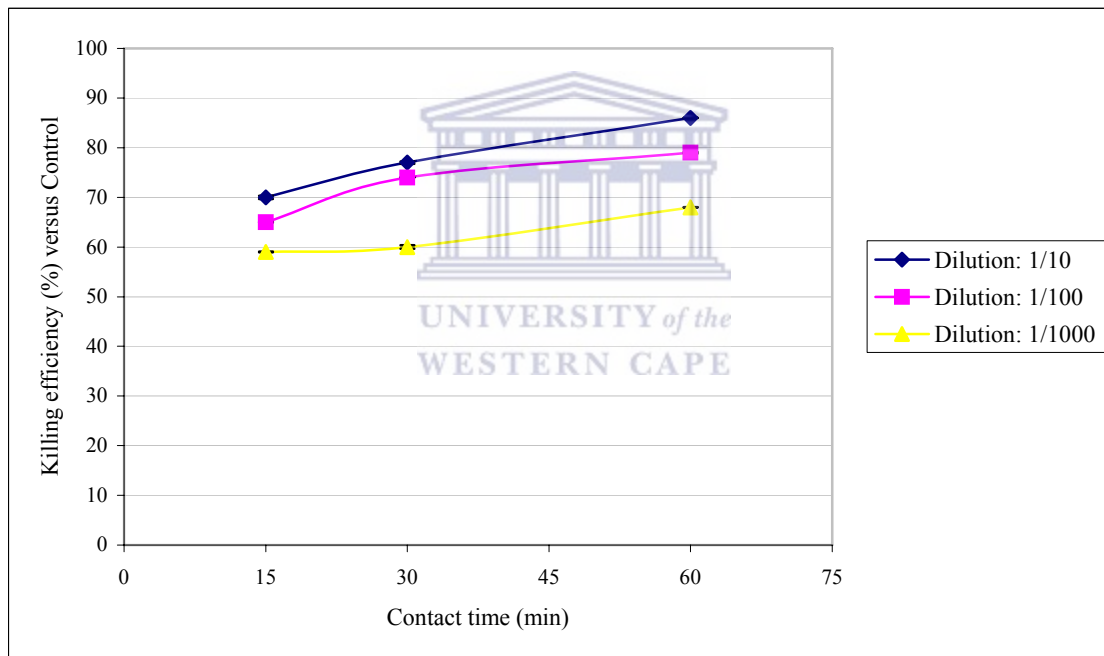
Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [OH <sup>•</sup> ] = (2.9 μM)	1/100 [OH <sup>•</sup> ] = (0.29 μM)	1/1000 [OH <sup>•</sup> ] = (0.029 μM)
Fenton's reaction	15	68 ± 0.33	60 ± 0.11	57 ± 0.24
	30	75 ± 0.08	71 ± 0.44	62 ± 0.13
	60	81 ± 0.12	77 ± 0.04	66 ± 0.00



**Figure 5.1** Killing efficiency (%) of OH<sup>•</sup> radicals produced from the Fenton reaction (solution A) on *E.coli* in water.

(ii) **Solution: B.** Absorbance: 0.6084; OH<sup>•</sup> radicals final concentration= 30 μM

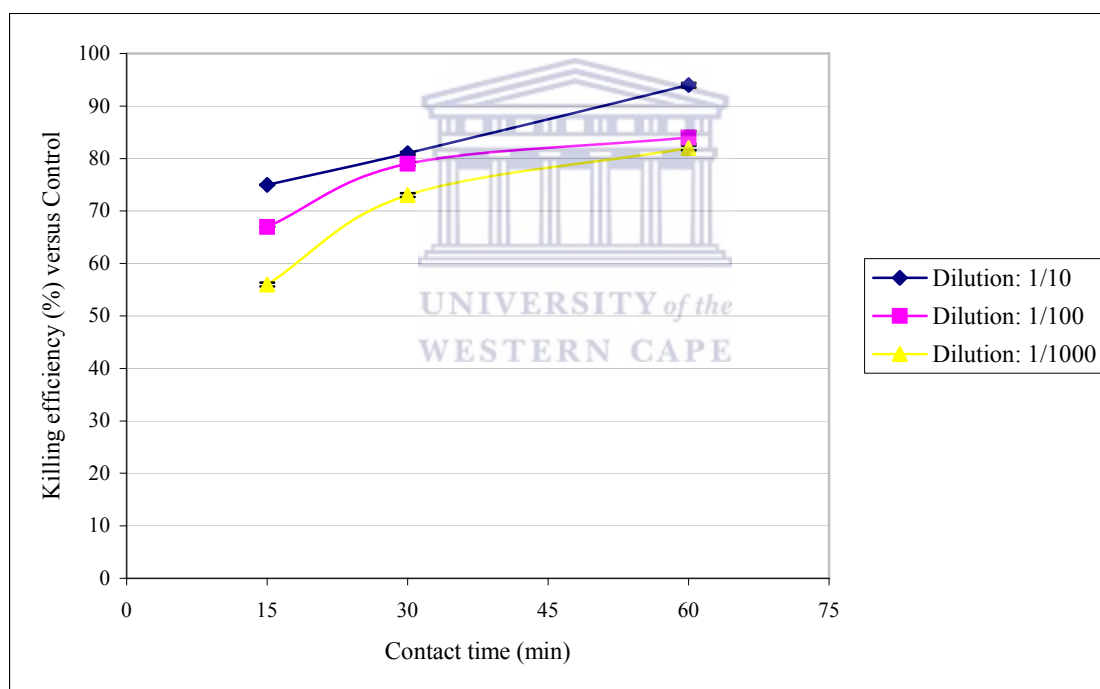
Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [OH <sup>•</sup> ]=3μM	1/100 [OH <sup>•</sup> ]=0.3 μM	1/1000 [OH <sup>•</sup> ]=0.03 μM
Fenton's reaction	15	70 ± 0.31	65 ± 0.04	59 ± 0.04
	30	77 ± 0.22	74 ± 0.04	64 ± 0.36
	60	86 ± 0.00	79 ± 0.18	68 ± 0.04



**Figure 5.2** Killing efficiency (%) of OH<sup>•</sup> radicals produced from the Fenton reaction (solution **B**) on *E.coli* in water.

(iii) **Solution: C.** Absorbance: 0.6446; OH<sup>•</sup> radicals final concentration= 31 μM

Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [OH <sup>•</sup> ]=31μM	1/100 [OH <sup>•</sup> ]=0.31μM	1/1000 [OH <sup>•</sup> ]=0.031μM
Fenton's reaction	15	75 ± 0.33	67 ± 0.11	60 ± 0.24
	30	81 ± 0.08	79 ± 0.44	73 ± 0.13
	60	94 ± 0.12	84 ± 0.04	82 ± 0.00



**Figure 5.3** Killing efficiency (%) of OH<sup>•</sup> radicals produced from the Fenton reaction (solution C) on *E.coli* in water.



5.6.2 *Effect of OH<sup>•</sup> radicals produced from the Fe/O<sub>2</sub> battery (sample) on E.coli in water at 25°C.*

The OH<sup>•</sup> radicals were generated at 15 min intervals for a period of 60 min in the Fe/O<sub>2</sub> battery under similar conditions to those described in section 4.4. The amount of absorbance ( $\lambda_{\text{max}}$  420 nm) for OH<sup>•</sup> radicals production was (i) production time 15 min: 0.5289 with final concentration of 26  $\mu\text{M}$ , (ii) production time 30 min: 0.6027 with final concentration of 29  $\mu\text{M}$  and (iii) production time 60 min: 0.6386 with final concentration of 31  $\mu\text{M}$ . These solutions were followed by dilution series: 1/10, 1/100 and 1/1000. Current (I) = 21 mA.

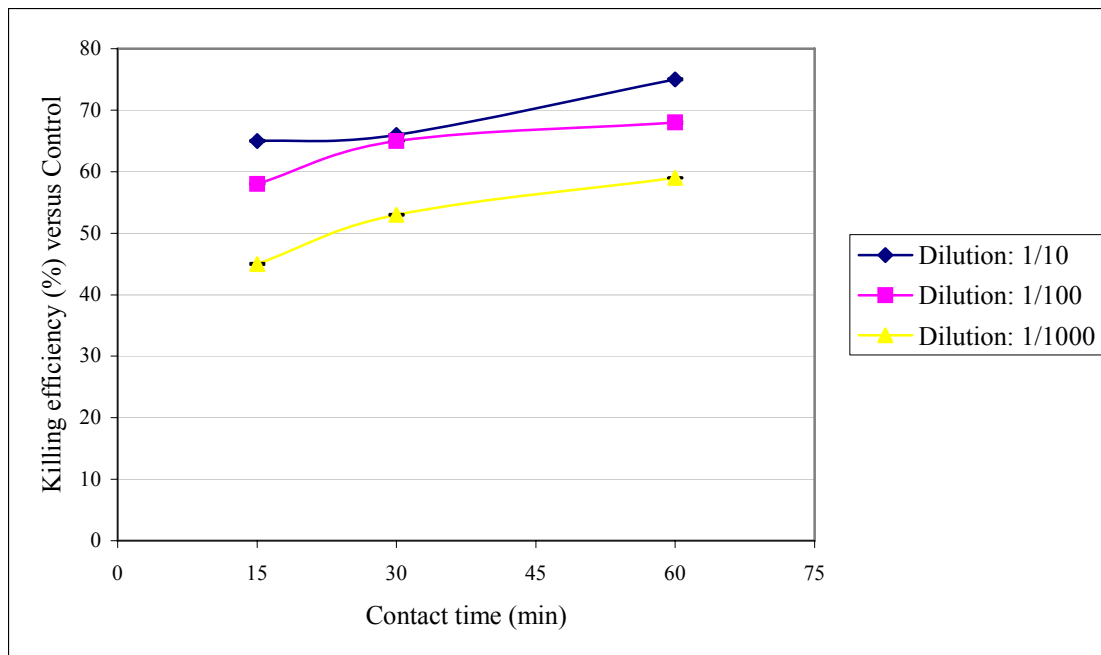
Table 5.6.2 (figure 5.4-5.6) shows the results of the effect of the OH<sup>•</sup> radicals production from the Fe/O<sub>2</sub> battery (various production time: 15, 30 and 60 min) on *E.coli* in water at 25 °C (pH 7).



**Table 5.6.2** Effect of OH<sup>•</sup> radicals produced from the Fe/O<sub>2</sub> battery (sample) on *E.coli* in water at 25 °C (pH 7).

(i) **Production time (PT): 15 min.** Absorbance: 0.5289; OH<sup>•</sup> radicals final concentration= 26  $\mu\text{M}$

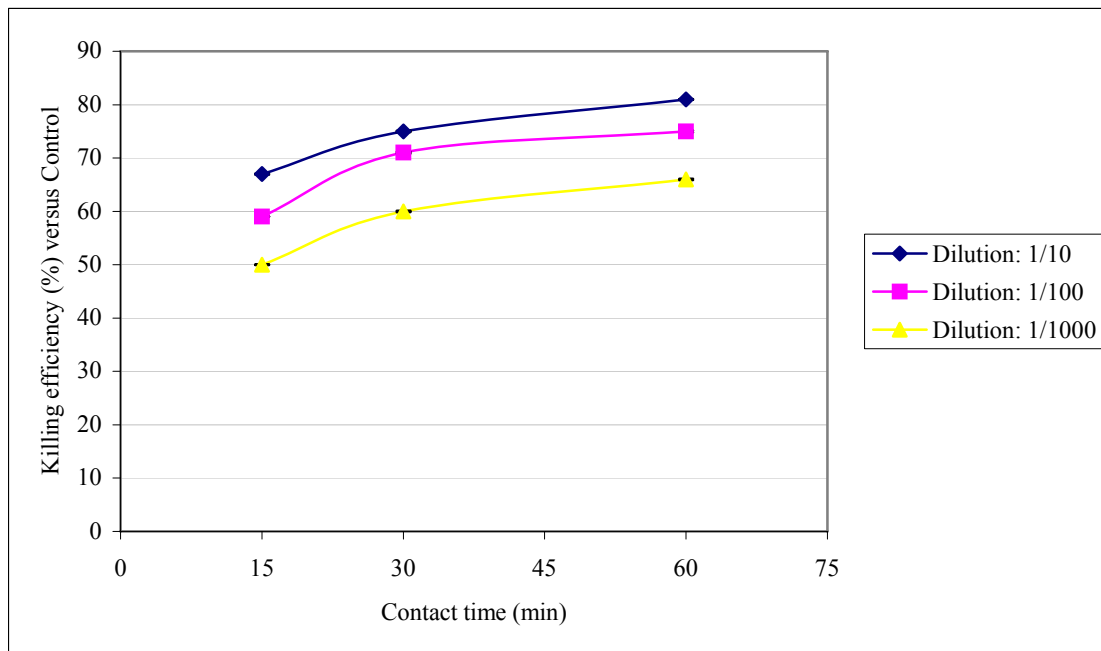
Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [OH <sup>•</sup> ]=2.6 $\mu\text{M}$	1/100 [OH <sup>•</sup> ]=0.26 $\mu\text{M}$	1/1000 [OH <sup>•</sup> ]=0.026 $\mu\text{M}$
Fenton's reaction (sample from Fe/O <sub>2</sub> battery)	15	65 ± 0.13	58 ± 0.11	45 ± 0.13
	30	66 ± 0.11	65 ± 0.04	53 ± 0.09
	60	75 ± 0.13	68 ± 0.18	59 ± 0.04



**Figure 5.4** Killing efficiency (%) of  $\text{OH}^\bullet$  radicals produced from the  $\text{Fe}/\text{O}_2$  battery (sample) on *E.coli* in water. Production time: 15 min.

(ii) **Production time (PT): 30min.** Absorbance: 0.6027;  $\text{OH}^\bullet$  radicals final concentration =  $29 \mu\text{M}$

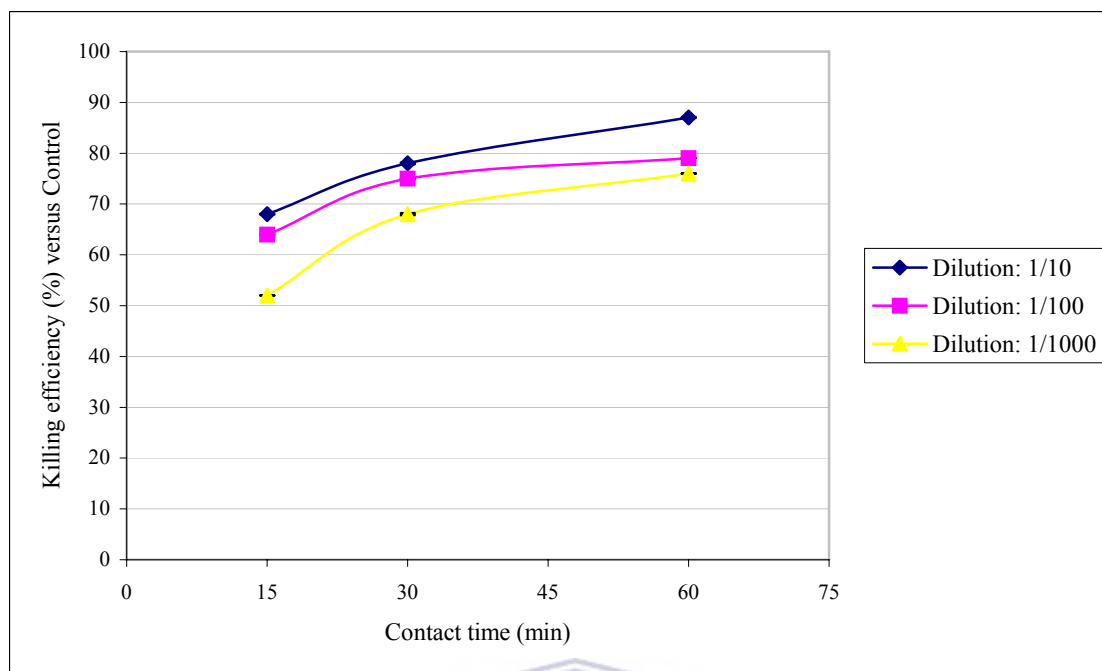
Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [ $\text{OH}^\bullet$ ]=( $2.9 \mu\text{M}$ )	1/100 [ $\text{OH}^\bullet$ ]=( $0.29 \mu\text{M}$ )	1/1000 [ $\text{OH}^\bullet$ ]=( $0.029 \mu\text{M}$ )
Fenton's reaction (sample from $\text{Fe}/\text{O}_2$ battery)	15	$67 \pm 0.08$	$59 \pm 0.00$	$50 \pm 0.04$
	30	$75 \pm 0.11$	$71 \pm 0.13$	$60 \pm 0.11$
	60	$81 \pm 0.07$	$75 \pm 0.18$	$68 \pm 0.11$



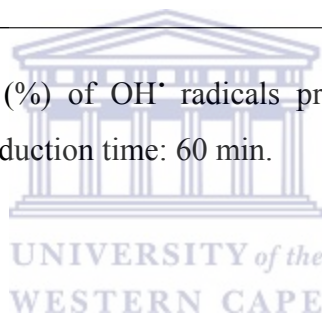
**Figure 5.5** Killing efficiency (%) of  $\text{OH}^{\bullet}$  radicals produced from the  $\text{Fe}/\text{O}_2$  battery (sample) on *E.coli* in water. Production time: 30 min.

(iii) **Production time (PT): 60 min.** Absorbance: 0.6386;  $\text{OH}^{\bullet}$  radicals final concentration = 31  $\mu\text{M}$

Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [ $\text{OH}^{\bullet}$ ]=3.1 $\mu\text{M}$ )	1/100 [ $\text{OH}^{\bullet}$ ]=0.31 $\mu\text{M}$ )	1/1000 [ $\text{OH}^{\bullet}$ ]=0.031 $\mu\text{M}$ )
Fenton's reaction (sample from $\text{Fe}/\text{O}_2$ battery)	15	68 ± 0.04	64 ± 0.07	52 ± 0.00
	30	78 ± 0.16	75 ± 0.11	68 ± 0.18
	60	87 ± 0.11	79 ± 0.00	76 ± 0.00



**Figure 5.6** Killing efficiency (%) of  $\text{OH}^\bullet$  radicals produced from the Fe/ $\text{O}_2$  battery (sample) on *E.coli* in water. Production time: 60 min.



### 5.6.3 *Effect of chlorine on E.coli growth in water at 25°C.*

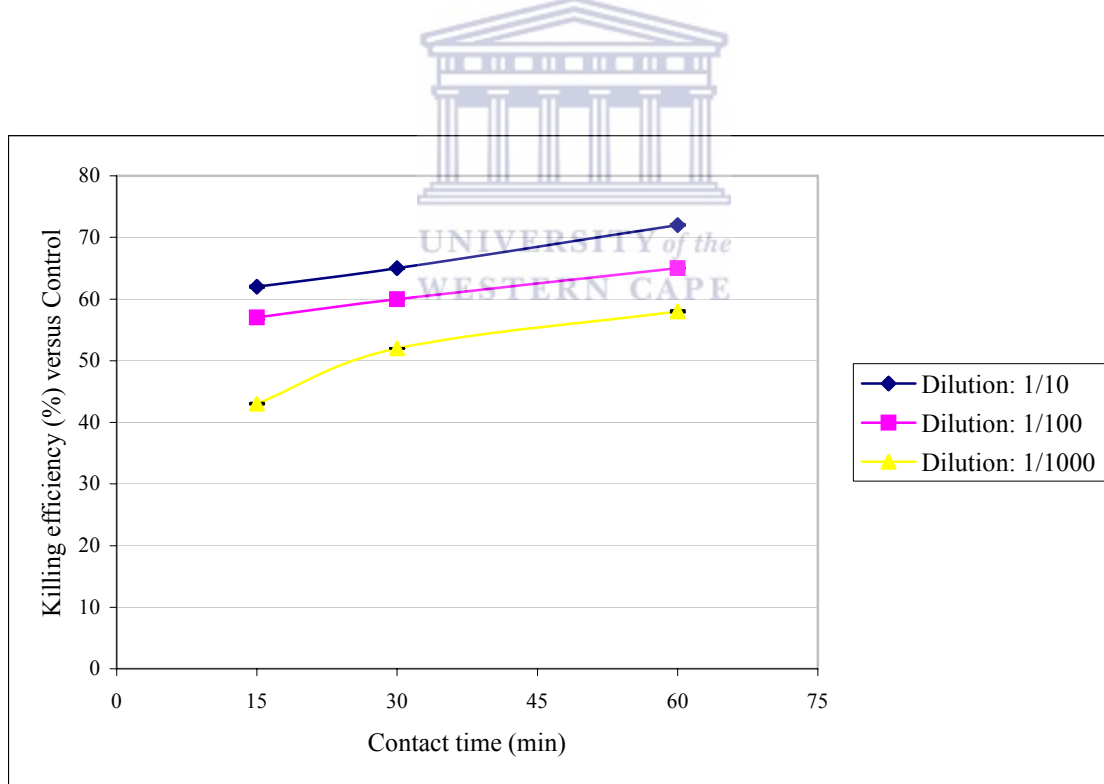
Bleach, (NaOCl) solution, was used to prepare the chlorine solutions. The procedure was as follows: 1 ml of NaOCl solution was diluted to 1000 ml of volumetric flask filled up to the mark with distilled water to make a 1000 ml of solution. The final concentration of chlorine of 10 mg/L (equivalent to 134 $\mu\text{M}$ ) was measured using DPD photometric method.

Three solutions (A, B and C) were prepared under similar conditions conventionally concentration for chlorine. These solutions were followed by dilution series: 1/10, 1/100 and 1/1000. Table 5.6.3 (figure 5.7) shows the triplicate results of the effect of chlorine on *E.coli* in water at 25 °C (pH 7).

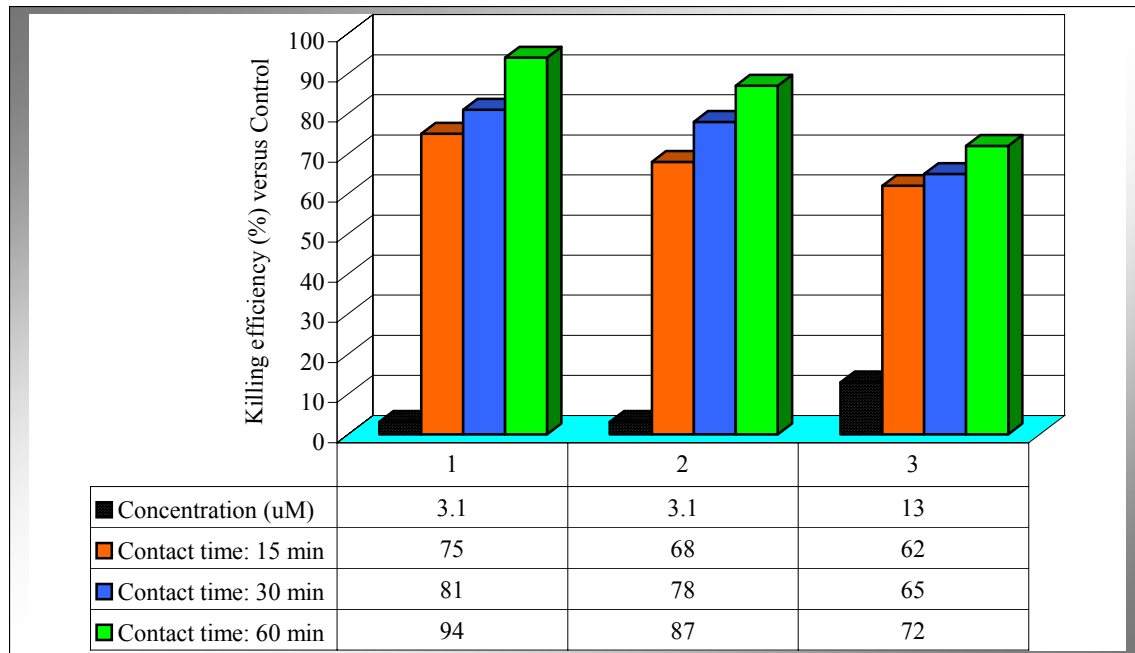
However, figure 5.8 illustrates the combined graph of inactivation efficacy of the *E.coli* using Fenton solution, sample from Fe/O<sub>2</sub> battery and chlorine.

**Table 5.6.3** Effect of chlorine on *E.coli* in water at 25 °C (pH 7). Final concentration chlorine = 10 mg/L (equivalent to 134 µM)

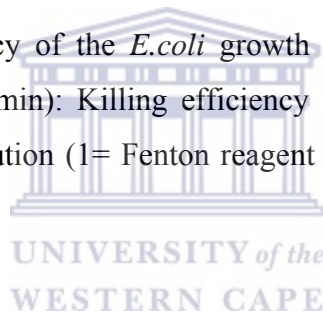
Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [Cl <sub>2</sub> ]=(13µM)	1/100 [Cl <sub>2</sub> ]=(1.3 µM)	1/1000 [Cl <sub>2</sub> ]=(0.13 µM)
Chlorination (chlorine)	15	62 ± 0.13	57 ± 0.29	43 ± 0.11
	30	65 ± 0.07	60 ± 0.16	52 ± 0.00
	60	72 ± 0.13	65 ± 0.04	58 ± 0.13



**Figure 5.7** Killing efficiency (%) of chlorine (Cl<sub>2</sub>) for each dilution on *E.coli* in water.



**Figure 5.8** Inactivation efficacy of the *E.coli* growth in water at 25 °C with various contact times (15, 30 and 60 min): Killing efficiency (%) versus concentration ( $\mu\text{M}$ ). Dilution: 1/10 of the stock solution (1= Fenton reagent solution; 2= sample from Fe/O<sub>2</sub> battery and 3= chlorine).



The data presented above (Table 5.6.1-5.6.3) are plotted as a killing percentage (%) against contact time (see Figure 5.1-5.7). The contact time is that period between introduction of the disinfectant and when the microorganisms in water (i.e. *E.coli* treatment) are estimated.

The results show that the killing efficiency (%) of OH<sup>•</sup> radical concentration ( $\mu\text{M}$ ) increases with effective contact time in all experiments (Figure 5.1- 5.6). A high killing efficiency has been shown for OH<sup>•</sup> radicals in both the standard Fenton solution (94 %) and the sample from the Fe/O<sub>2</sub> battery (87 %) when compared to chlorine (72 %) with dilution 1/10 of the stock solution (Figure 5.8). Therefore, a Fenton system is potentially a more efficient disinfectant than chlorination methods.

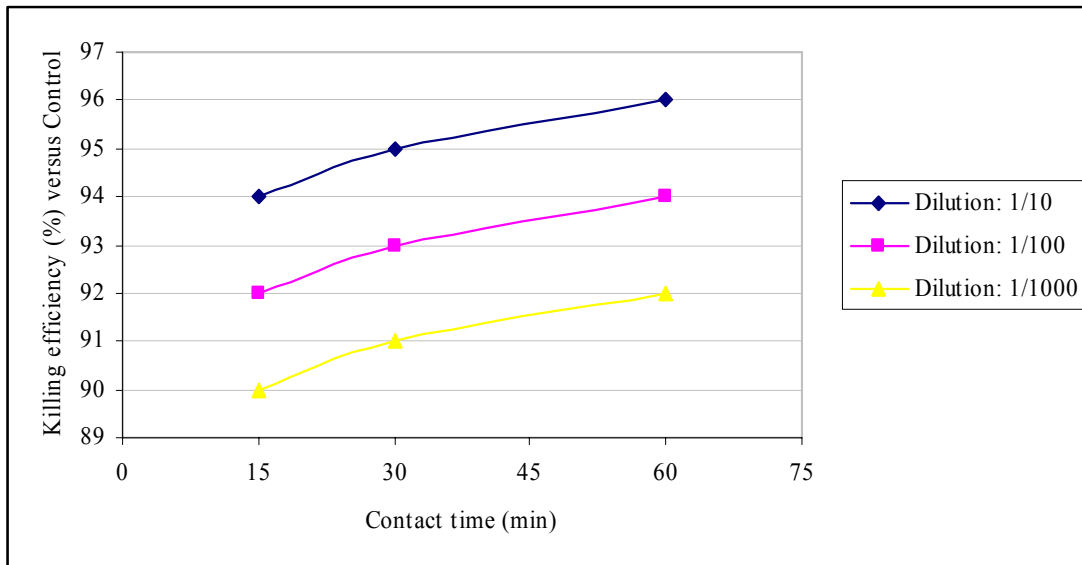
### 5.6.3 Effect of the electrolyte produced from the DiaCell<sup>®</sup> on *E.coli* in water at 25°C.

The DiaCell technology was tested against *E.coli* growth in water (see table 5.6.4). The methodology used for the *E.coli* inactivation was similar to that employed in this chapter 5 (see section 5.4.3). The OH<sup>•</sup> radicals were generated after 12 minutes in the DiaCell<sup>®</sup> under similar conditions. The current and voltage observed were respectively, 0.46 A and 10.01 V.

The flow rate of 42 ml/min was kept constant during the experiment. The average amount of absorbance ( $\lambda_{\max}$  420 nm) for OH<sup>•</sup> radical production was 0.5324 with the average final concentration of 26  $\mu$ M. These solutions were followed by dilution series: 1/10, 1/100 and 1/1000. Table 5.6.4 (figure 5.9) indicates the results of the effect of the electrolyte produced from the DiaCell<sup>®</sup> on *E.coli* in water at 25 °C (pH 7).

**Table 5.6.4** Effect of electrolyte produced from the DiaCell<sup>®</sup> on *E.coli* in water at 25 °C (pH 7). Production time (PT): 12 min. OH<sup>•</sup> radical final concentration = 26  $\mu$ M

Disinfection method	Contact time (CT) (min)	Killing efficiency (%) versus control		
		Dilution		
		1/10 [OH <sup>•</sup> ]=2.6 $\mu$ M)	1/100 [OH <sup>•</sup> ]=0.26 $\mu$ M)	1/1000 [OH <sup>•</sup> ]=0.026 $\mu$ M)
Electroanalysis	15	94 ± 0.33	92 ± 0.11	90 ± 0.24
(Sample from the DiaCell <sup>®</sup> )	30	95 ± 0.08	93 ± 0.44	91 ± 0.13
	60	96 ± 0.12	94 ± 0.04	92 ± 0.00



**Figure 5.9** Killing efficiency (%) of OH<sup>•</sup> radical production (sample from the DiaCell<sup>®</sup>) on *E.coli* in water. Production time: 12 min.

These set of experiments (Table 5.6.4) are similar with those discussed earlier (see Table 5.6.1 and 5.6.2) as compared to the data of the experiments in table 5.6.3. The results show that the disinfection ability of OH<sup>•</sup> radical production from Fenton reaction and DiaCell<sup>®</sup> on *E.coli* in water is higher than chlorination method.

It is worth noting that the DiaCell<sup>®</sup> appears to have the most effective disinfection ability of all the methods examined although the nature of the species which is referred to here as “OH<sup>•</sup> radical” may perhaps be another species yet to be identified [105].





**CHAPTER 6**

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**CONCLUSIONS AND RECOMMENDATION**

## 6.1 CONCLUSIONS

The factors affecting the electrogeneration of OH<sup>•</sup> radicals in the Fe/O<sub>2</sub> battery were investigated. The following conclusions can be drawn:

- A higher amount of OH<sup>•</sup> radicals was produced at initial pH=3.0 as compared with the initial pH=2.5.
- Both air and oxygen used in the gas diffusion electrode were investigated in the Fe/O<sub>2</sub> battery. The results show that air can be used as an alternative to pure O<sub>2</sub>.
- Higher currents and cell voltages occur with increasing running time of the battery which are consistent with the increasing pH and iron of the electrolyte with running time.
- Increasing electrolyte concentration (Na<sub>2</sub>SO<sub>4</sub>) increases OH<sup>•</sup> radical production. Na<sub>2</sub>SO<sub>4</sub> is a better electrolyte than NaCl for OH<sup>•</sup> radical production.
- The electrolyte solution continues to generate OH<sup>•</sup> radicals even after standing for several days.
- The higher the OH<sup>•</sup> radical concentration produced in the system, the more rapid is the *E.coli* inactivation after a certain period of contact time.
- The killing efficiency on *E.coli* has shown to be more efficacy in the Fenton reagents (94%) and the Fe/O<sub>2</sub> electrolyte (87%) than in the chlorination system (72%) after 60 min of contact time.
- The DiaCell<sup>®</sup> was found to be a more efficient means of disinfection (killing efficiency (96%)) on *E.coli* when compared to the Fenton reagent, the Fe/O<sub>2</sub> electrolyte and chlorine.

Since the treatment chemicals (H<sub>2</sub>O<sub>2</sub> and mainly OH<sup>•</sup> radicals) are produced in *in-situ* in the Fe/O<sub>2</sub> battery electro-Fenton application, no hazardous chemicals need be transported to the application point. The additional advantage of this electro-Fenton system [18,133,134] for drinking water treatment is that the OH<sup>•</sup> radicals (short life time) leave no residual as compared to other oxidants like chlorine. The DiaCell<sup>®</sup> technology has also shown to produce a powerful oxidant species for disinfection purposes.

## 6.2. RECOMMENDATION

The electro-Fenton system has potential for water disinfection in rural sites where surface water or groundwater is normally contaminated by microorganisms. This type of application is promising when considering the simplicity of the cell and procedures, (i) the ability to electrogenerate  $\text{OH}^\bullet$  radical in aqueous solution via Fenton's reaction, (ii) the safety of all chemicals in the system. The iron/oxygen battery should be further investigated for the production of on-site disinfectant solutions suitable for dosing raw water supplies. The DiaCell<sup>®</sup> system has potential as flow through continuous on-line electrochemical disinfection system and merits further investigation.





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