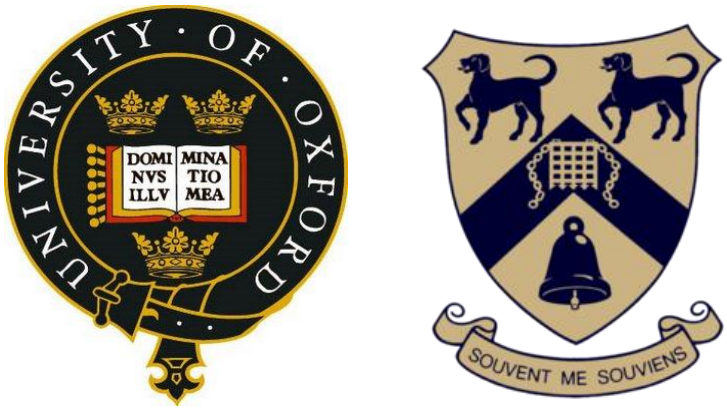
# Hybrid Technologies for Remediation of Recalcitrant Industrial Wastewater

Thesis submitted for the degree of Doctor of Philosophy

Department of Engineering Science

University of Oxford, Lady Margaret Hall




**Sheeja Jagadevan**

Michaelmas Term, 2011

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**Sheeja Jagadevan, Lady Margaret Hall, University of Oxford.**
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## ABSTRACT

In metal machining processes, the regulation of heat generation and lubrication at the contact point are achieved by application of a fluid referred to as metalworking fluid (MWF). This has the combined features of the cooling properties of water and lubricity of oil. MWFs inevitably become operationally exhausted with age and intensive use, which leads to compromised properties, thereby necessitating their safe disposal. Disposal of this waste through a biological route is an increasingly attractive option, since it is effective with relatively low energy demands when compared to current physical and chemical options. However, biological treatment is challenging since MWF are chemically complex, including the addition of toxic biocides which are added specifically to retard microbial deterioration whilst the fluids are operational. This makes bacterial treatment exceptionally challenging and has stimulated the search and need to assess technologies which complement biological treatment. In this study the remediation, specifically of the recalcitrant component of a semi-synthetic MWF, employing a novel hybrid treatment approach consisting of both bacteriological and chemical treatment, was investigated. Three chemical pre-treatment methods (Fenton's oxidation, nano-zerovalent iron (nZVI) oxidation and ozonation) of the recalcitrant components followed by bacterial degradation were examined. The synergistic interaction of Fenton's-biological oxidation and nZVI-biodegradation led to an overall COD reduction of 92% and 95.5% respectively, whereas pre-treatment with ozone reduced the total pollution load by 70% after a post-biological step. An enhancement in biodegradability was observed after each of the chemical treatments, thus facilitating the overall treatment process. The findings from this study established that the use of non-pathogenic microorganisms to remediate organic materials present in MWF wastewater is a favourable alternative to energy demanding physical and chemical treatment options. However, optimal performance of this biological process may require chemical enhancement, particularly for those components that are resistant to biological transformation.

## Peer-Reviewed Papers

Jagadevan, S., Jayamurthy, M., Dobson, P., Thompson, I. (2012) "A novel hybrid nano-zerovalent iron initiated oxidation- biological degradation for remediation of recalcitrant waste metalworking fluids." *Water Research* **46** 2395-2404.

Jagadevan, S., Dobson, P., Thompson, I. (2011) "Harmonisation of chemical and biological process in development of a hybrid technology for treatment of recalcitrant metalworking fluid."*Bioresource Technology* **102** 8783-8789.

Jagadevan, S., Graham, N., Thompson, I. (2012) "Treatment of waste metalworking fluid by a hybrid ozone-biological process" (In preparation).

## Conferences

Jagadevan, S., Jayamurthy, M., Bhattacharya, A., Dobson, P., Thompson, I. "Nano-Catalysts in Remediation of Recalcitrant Industrial Wastewater*"* Platform presentation in the Second IJAS Conference, Harvard University, Boston, May 29-June 2, 2011.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| AOP | Advanced Oxidation Processes |
| AOS | Average Oxidation State |
| BAT | Best Available Technology |
| BCC | Body Centered Cubic |
| BDD | Boron-Doped Diamond |
| BOD | Biochemical Oxygen Demand |
| $BOD_5$ | Biochemical Oxygen Demand of the sample after 5 days of incubation |
| BZT | Benzotriazole |
| CCD | Central Composite Design |
| COD | Chemical Oxygen Demand |
| DPD | N,N-diethyl-p-phenylenediamine |
| EA | Environmental Agency |
| EC | Electro-Coagulation |
| FID | Flame Ionisation detector |
| HP | Hypersensitivity Pneumonitis |
| HPLC | High Performance Liquid Chromatography |
| IPPC | Integrated Pollution Prevention and Control |
| MBR | Membrane Bioreactor |
| MEA | Monoethanolamine |
| MPa | Mega Pascal |
| MWF | Metalworking Fluid |
| NDIR | Non Dispersive Infrared |
| nNi-ZVI | Nanoscale Nickel Zero Valent Ion |
| nZVI | Nanoscale ZeroValent Iron |
| OD | Optical Density |

| | |
|---|---|
| pKa | Dissociation Constant |
| POD | Horseradish Peroxidase |
| RSM | Response Surface Methodology |
| SBR | Sequencing Batch Reactor |
| SRT | Solids Retention Time |
| SS | Suspended Solids |
| TCC | Tri Chloro Ethylene |
| TEA | Triethanolamine |
| TKN | Total Kjeldahl Nitrogen |
| TOC | Total Organic Carbon |
| TSB | Tryptone Soya Broth |
| WAO | Wet Air Oxidation |
| WWTP | Waste Water Treatment Process |
| XRD | X-Ray Diffraction |
| ZVI | Zero Valent Ion |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Metalworking fluids (MWFs) are employed in a variety of metal cutting and processing operations, primarily to reduce the heat produced and the friction caused by contact of the tool on the work piece. They are generally composed of oil and organic compounds mixed with water. In-use MWFs become operationally exhausted process waste when their functional properties are compromised, thereby necessitating their safe disposal. The treatment and disposal of these operationally exhausted fluids is challenging due to their high pollution load (chemical oxygen demand) and toxicity. In addition, MWF consists of a large array of different chemicals in the mixture. The exact compositions of these chemicals are proprietary information, seldom divulged by the manufacturers. This makes the efficient treatment and disposal of such wastes increasingly difficult.

Different methods have been used to treat waste metalworking fluids. The commonly employed methods consist primarily of physical-chemical solutions such as coagulation, membrane separation, incineration or disposal into landfills. Chemical methods such as coagulation invariably necessitate the use of large quantities of chemicals. In addition, the inorganic coagulants precipitate and cause the production of a substantial amount of excess oily sludge which increases disposal costs and slow the rate of separation. Membrane separation is a pressure-driven process which uses molecular size pores for removal of emulsified oil and other dispersed components found in MWF. The main disadvantage of membrane processes is fouling of membrane elements leading to a huge reduction in efficiency. Thermal based treatment options such as incineration are not a sustainable remediation method. Incineration of such wastes is associated with evolution of nitrogen oxides, sulfur dioxide and hydrogen chloride, hence leading to air pollution and resulting in

stack corrosion and odours. Furthermore, high energy input and high costs of investment and operation prevent commercialisation of this method. Disposal of waste MWF into landfills result in contamination of the soil and potential leaching of toxic compounds to the underground water table. The 2000/76/EC and EA 2004 Directives issued by the European Union and Environmental Agency in UK (EA) impose a strict framework for the disposal of these wastes into landfills (Cheng *et al.,* 2005). These regulations have created a growing interest in the development of environmentally benign and sustainable disposal methods of MWF wastewater.

There is a clear need to develop a cost effective and reliable system for treating MWFs. Disposal of this waste through a biological route is an attractive option, since it is effective with relatively low energy demands. However, it is enormously challenging since these fluids are chemically complex, including the addition of toxic biocides which are added specifically to retard bio-deterioration whilst the fluids are operational. This thesis develops methods for remediation of recalcitrant MWF by employing a combination of chemical and biological oxidation. Three chemical pre-treatment methods (Fenton's oxidation, nano zerovalent iron (nZVI) oxidation and ozonation) of the recalcitrant components followed by bacterial degradation are examined. The chemical pre-treatment methods presented here enable biological degradation of those compounds that are resistant to biological transformation.

## 1.2 Chapter Summary

This thesis is composed of 6 chapters including this introduction chapter.

**Chapter 2: Literature review**

This chapter gives a literature review on different parts of the study. The review starts with a brief overview on industrial waste effluent treatment. Then, metalworking fluids (MWF) are reviewed with their history, types and nature and clarifying the importance of safe disposal of operationally exhausted fluids. The central part of the chapter deals with microbiology of metalworking fluids and biological treatment of the MWF waste. This is followed by concepts and principles of advanced oxidation process and combinative/hybrid treatment approach. The last part of the chapter reviews nanotechnology in environmental applications.

**Chapter 3: Fenton's oxidation of recalcitrant metalworking fluid**

This chapter investigates the feasibility of employing Fenton oxidation for remediation of recalcitrant MWF and to check the possibility of combining this chemical treatment with a biological oxidation step to provide an environmentally benign and economically feasible treatment option. A statistical analysis employing central composite design was employed to determine optimum Fenton reagent concentrations required to enhance the biodegradability of the recalcitrant wastewater. (This work has been published: Jagadevan, S., Dobson, P., Thompson, I. (2011) "*Harmonisation of chemical and biological process in development of a hybrid technology for*

*treatment of recalcitrant metalworking fluid*". Bioresource Technology 102, 8783-8789).

**Chapter 4: Remediation of recalcitrant metalworking fluid wastewater employing nano-scale iron particles**

This chapter describes a hybrid nano-scale zerovalent iron (ZVI) induced oxidation of recalcitrant MWF wastewater. This treatment option follows a modified Fenton's reaction examined in chapter 3. The possibilities of integrating this chemical pre-treatment method with biological oxidation and recycling the spent iron oxides have also been investigated. (This study has been published in Water Research: Jagadevan, S., Jayamurthy, M., Dobson, P., Thompson, I. (2012) "*A novel hybrid nano-zerovalent iron initiated oxidation- biological degradation for remediation of recalcitrant waste metalworking fluids"*

**Chapter 5: Oxidation of recalcitrant metalworking fluid employing ozone gas**

This chapter applies gas-induced ozone treatment for highly recalcitrant MWF wastewater. A hybrid treatment combining ozonation with a robust biological treatment has also been studied. This chapter further investigates the rate and extent of degradation of three toxic non-biodegradable MWF constituents (monoethanolamine, triethanolamine and benzotriazole) when subjected to ozonation.

**Chapter 6: Conclusions and recommendations**

This final chapter gives general conclusions and recommendations for future work.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Overview of Industrial Waste Effluent

Every community produces liquid, solid and air emissions. Contaminated water (wastewater) is one of the oldest environmental problems confronting people throughout the world. Wastewater is a complex mixture of natural inorganic and organic material mixed with synthetic substances discharged from modern life style. Three categories of waste waters can be discharged from commercial premises;

**Domestic/sanitary wastewater** (sewage): Wastewater discharged from residences and from commercial, institutional and recreational facilities constitutes domestic sewage.

**Storm-water/infiltration**: Runoff resulting from rainfall, snowmelt and extraneous water that enters the collection system through joints, cracks and breaks, or porous walls constitutes storm-water.

**Industrial/trade effluent:** Most industrial processes emit wastewater during one or more stages of production. Trade effluents are liquid streams from all processes on the site, including all rinse water, washing water and any other discharge related to the process. The composition of this type of water can vary dramatically as it is determined both by the products and processes of production.

### 2.1.1 Charges for trade effluents

All industrial wastewaters are subject to a discharge consent system under either the UK Water Resources (1991) or the Water Industry (1991) Acts. These state that if industrial wastewater is sent to public wastewater network/foul sewer there will be a "Trade Effluent Charge" levied by the water company to the industries. The most widely used charging

system in Europe is the Mogden Formula. The analysis of sewage is normally based on polluting strength of wastewater. Chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids and ammonia are the commonly used parameters used to measure the polluting strength of wastewater. In environmental chemistry, the COD test is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidised chemically using dichromate in an acid solution. It is expressed in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution. BOD is a measure of the quantity of oxygen used by aerobic biological organisms to break down organic material present in a given water sample at a certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample during 5 days of incubation (hence called $BOD_5$) at 20°C and is often used as a robust surrogate of the degree of biodegradable organic pollution of water.

From the above mentioned parameters, the polluting strengths of wastewaters can be assessed and charges for treatment are levied as per the Mogden Formula. The formula takes into account the volume and strength of the waste and the type of treatment given at the waste water treatment work (Gray 2010). The formula was mutually agreed by the water industry and the Confederation of British Industry.

The Mogden Formula is stated as:

$$C = R + V + V_b + B \times O_t / O_s + S \times S_t / S_s \text{ Pence m}^{-3}$$

Where:

C = Total charge rate for disposal (pence/cubic metre)

R = Unit cost for conveyance (pence/cubic metre)

V = Unit cost for volumetric treatment (pence/cubic metre)

$V_b$ = Additional volume charge if there is no biological treatment

B = Unit cost for biological treatment (pence/cubic metre)

$O_t$ = COD of trade effluent (mg/L)

Os = COD settled sewage (mg/L)

S = Unit cost for sludge disposal (pence/cubic metre)

$S_t$ = Solids value trade effluent (mg/L)

Ss = Solids value settled sewage (mg/L)

## 2.1.2 Aims of industrial wastewater treatment plant

Wastewater treatment plants consists of separate treatment processes or units, designed to produce an effluent of specified quality from a wastewater (influent) of known composition and flow rate (Figure 2.1). The aims of wastewater treatment are:

(a) To convert the waste materials present in wastewaters into stable oxidised end products that can be safely disposed of to inland waters without any adverse ecological effects.

(b) To protect public health.

(c) To ensure wastewater is effectively disposed of on a regular and reliable basis without nuisance or offence.

(d) To recycle and recover the valuable components of wastewater.

(e) To provide an economic method of disposal.

(f) To comply with legal standards and consent conditions placed on discharges (Gray 2010, Metcalf and Eddy, 2002).

### 2.1.3 Pre-treatment of industrial wastewater

Industrial wastewaters vary considerably in their nature, toxicity and treatability and normally require pre-treatment before discharge to sewer. Excessive amounts of oil or fats, acidic or alkaline wastes, suspended solids, explosive or flammable materials, volatile and odorous compounds, and corrosive gases all need to be controlled on-site before further treatment is considered. While toxicity is a major factor associated with the treatment of industrial wastewater, excessive temperatures or variable hydraulic discharge rates are also important (Davis and Cornwell 1998, Gray 2010).

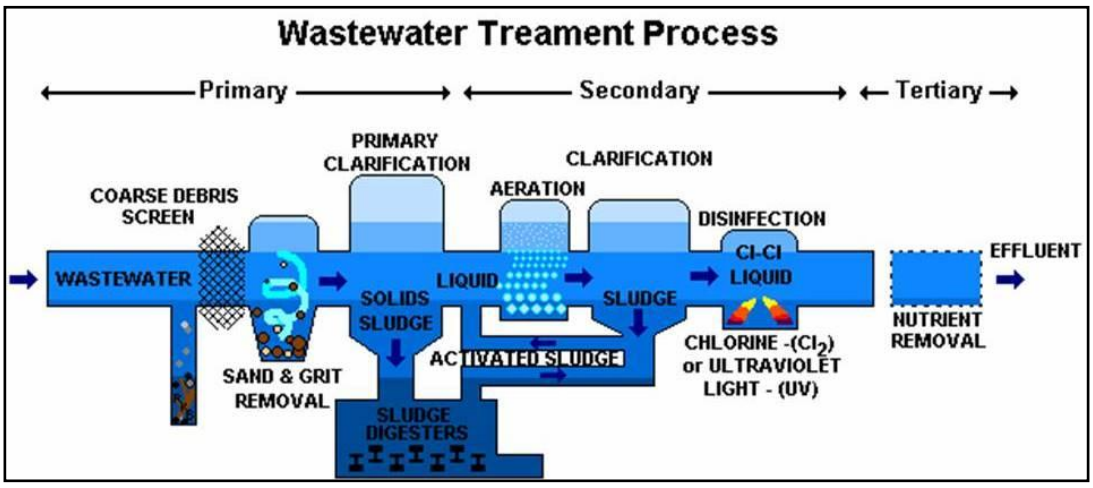



**Figure 2.1:** Schematics of conventional wastewater treatment process (Adapted from *http://www.businessprocessidea.com/wastewater-treatment-process/*)

Specific objectives of the pre-treatment program are:

1. To prevent the introduction of pollutants that will interfere with the operation of wastewater treatment plants (WWTPs).

2. To prevent the introduction of pollutants into WWTPs that will pass through the treatment works or otherwise be incompatible with such works.
3. To improve opportunities to recycle and reclaim municipal and industrial wastewaters and sludge.

### 2.1.4 Regulations

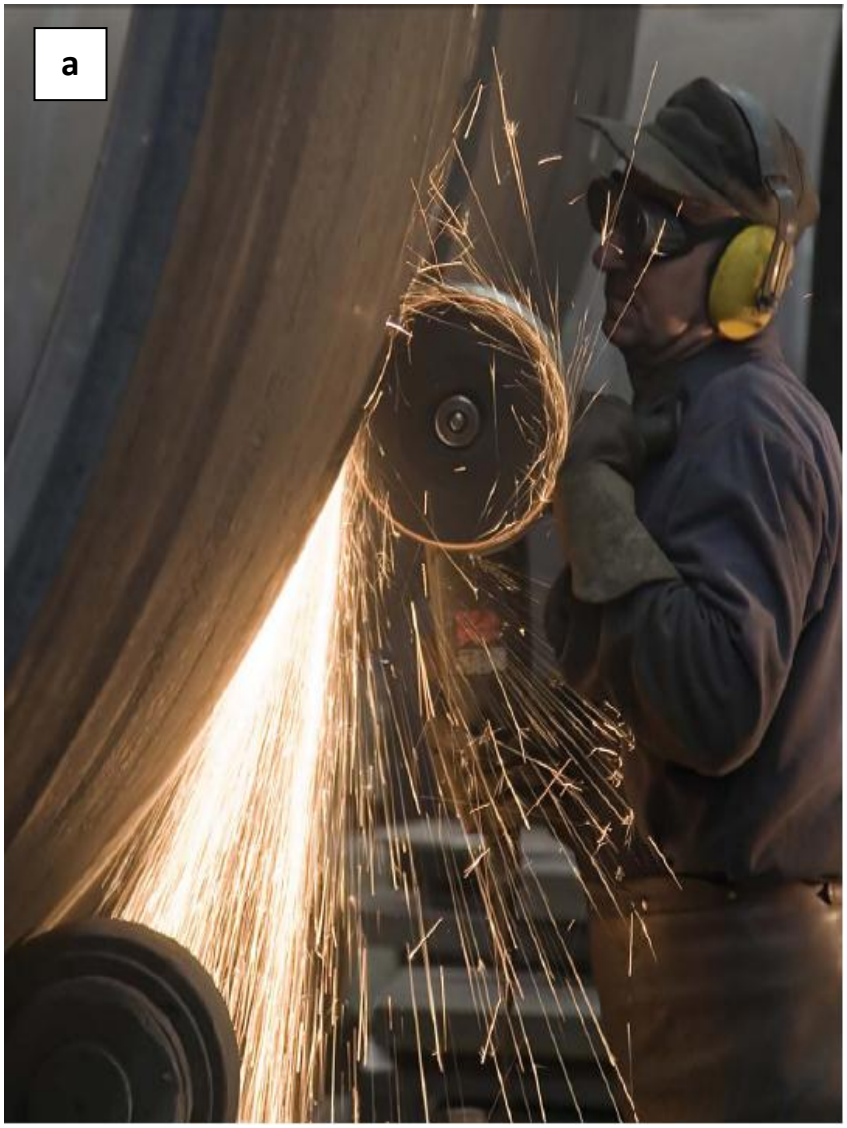
Environmental legislation focused on water quality is based largely on quality standards relating to suitability for a specific use, the protection of receiving waters or emission limits on discharges. Standards are usually mandatory with maximum permissible concentrations based on heath criteria or environmental quality standards. The Urban Wastewater Treatment EU Directive 91/271/EEC, regulates the collection and treatment of wastewater from homes and industries. In the UK, the Directive is implemented through the Urban Waste Water Treatment Regulations 1994 (Gray 2010).

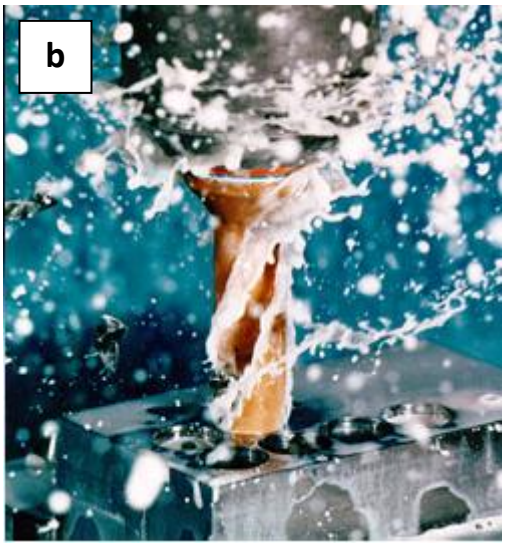
The establishment of discharge limit values is dictated by Directive (96/61/EC) on Integrated Pollution Prevention and Control (IPPC). This prefigures more drastic conditions for the discharge of industrial water into the environment and therefore to the discharge of sewage. The philosophy of the European (and USA) legislations are based on the "Best Available Technology (BAT) principal, but this has of course moderated the economics of the technology and its practical viability. In Europe, the IPPC Directive (96/61) impresses to member states that they have to improve environmental performances on the BAT principle and must focus on waste minimisation. Recycling and therefore, in the case of water treatment, close-loop options for industrial water use are ultimately implicated (Nazaroff and Cohen, 2001, Gray, 2010).

## 2.2 Metal Working Fluids

### 2.2.1 Brief history of metalworking fluid

Metal cutting operations have been performed as far back as the Greek and Roman era, and perhaps even before. In manufacturing and machining processes such as broaching, tapping, threading, gear shaping, reaming, drilling, milling, turning and in particular when the metal removal operations are conducted at high speeds and low pressures, the regulation of heat generation and lubrication of the contact point are achieved by pouring a fluid over the cutting surface called metalworking fluid (MWF) or cutting fluid (Fig 2.2) (Bataller *et al.*, 2004, Irani *et al.*, 2005). It was not until the industrial revolution had taken place, roughly between the years 1760 and 1850, that metal cutting was academically researched and studied. In mid 20$^{th}$ century, MWFs had acquired sufficient sophistication and proved to be a necessary adjunct in high speed machining and in the machining of exotic steels and specially alloyed nonferrous metals. Over the later part of the twentieth century, many MWF production companies have come into existence offering a multitude of metalworking formulations to ameliorate machining problems and increase rates of production (Mc Coy, 1994).



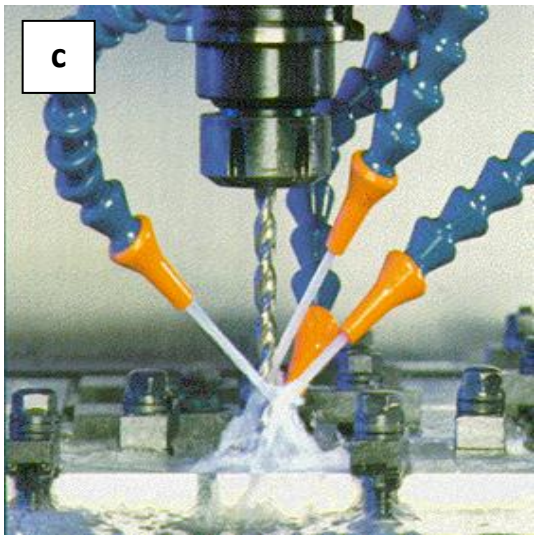




**Figure 2.2** (a) The occurrence of metal working fluids is widespread throughout the mechanical engineering industry, in the machinery of all kinds. Metal-working fluids used in (b) drilling and (c) grinding operations.

## 2.2.2 Role of metalworking fluids in machining processes

Metal working fluids are a complex mixture of chemicals, composed of base oil and additives, which together impart lubrication and cooling properties. They are usually constituted by diluting the cutting fluid concentrate with mains waters at a volume ratio ranging in the region of 98:2 to 95:5 depending on the machining operations (Bataller et al., 2004).

Metal working fluids are employed in machining industries for a number of reasons:

(i) Lubrication: The fluid reduces friction in the grinding zone.

(ii) Cooling: Water is the best coolant with a high heat capacity, and possesses latent heat of evaporation of an order of magnitude greater than straight oil. The fluid bathes the work-piece, the fixtures and the machine tool and establishes thermal stability of the system by conducting some of the heat energy into the fluid instead of the work-piece, thus bringing the components and the machine tool to one temperature.

(iii) Transportation of the chips: MWF transports the chips away from the cutting zone. If the chips are not removed, they can clog the wheel, thus greatly increasing the heat input to the work piece.

(iv) Corrosion protection: The "just machined" nascent metal surface is chemically active and would readily oxidise or react with the surroundings if not protected.

(v) To arrest re-welding: At high temperatures, especially in slower speed machining, there is a tendency for the material to stick back onto itself at the tool edges and surfaces called re-welding. The cutting fluid helps to prevent this reaction.

(vi) Power reduction: Metalworking fluids reduce friction, and in doing so reducing the power required to machine a given material.

(vi) Extend tool life and increase productivity: Surface active fluids with enhanced wetting agent chemistry will penetrate the surface of the work-piece and chemically react with the surface, and subsurface to reduce shear stress, thus reducing power consumption and heat generation. (Salmon 1994, Irani et al., 2005., Bataller et al., 2004).

## 2.2.3 Types of metalworking fluids

Initially, cutting fluids were simple oils applied with a brush to cool and lubricate the cutting tool. As the cutting operations became more elaborate, formulations of the fluids became more complex. Today, a spectrum of different formulations is available to cater to the needs of the industry. There are several types of cutting fluids that can be classified broadly as neat oils, synthetic fluids, and water-soluble oils.

**2.2.3.1 Neat oils/Straight oils** contain lubricant base oils without water. These MWFs are composed of petroleum oil or other animal, marine, vegetable, or synthetic oils used singly or in combination, and with or without additives.

**2.2.3.2 Soluble oils** are fluids based on mineral or synthetic oils that contain emulsifiers to allow for the dilution of the product into water. These are composed of an aqueous oil emulsion with oil in high concentration (50%-70%). The greatest challenge with soluble

oils is maintaining the emulsion. Binding of the emulsifying agent by minerals in hard water and biological degradation of the emulsifier are typically responsible for breaking the emulsion (Anderson et al., 2003). Soluble oils as a class provide good lubrication as well as improved cooling (as compared to straight oils). In contrast, soluble oils have a tendency to have poor corrosion control and generate smoke (because of insufficient ability to cool).

**2.2.3.3 Semi-synthetic MWF**: fluids contain less mineral oil than soluble oils. Usually less than 30% of the total concentrate volume is oil. Soluble and semi-synthetic MWFs contain naphthenic mineral oil. Semi-synthetics offer good lubrication, heat reduction, rust control, have longer sump life and are cleaner than soluble oils. Conversely, this class of products has a greater tendency to foam in softer water and can be unstable in hard water.

**2.2.3.4 Synthetic fluid**: formulations do not contain petroleum oils, but are composed of chemically synthesised organic compounds such as polybutene, polyethylene or polyglycol, as an "oily" replacement for mineral oil. Synthetic metalworking formulations are the cleanest, offer the best heat reduction, have excellent rust control and long sump life, are transparent, and are largely unaffected by hard water. In contrast, synthetics offer poor physical lubrication, can be more difficult to waste-treat, and can foam in some applications.

Water-soluble oils are used widely in approximately 80–90% of applications of the estimated 2 billion litres of MWFs consumed worldwide every year (Rao and Srikant, 2006, Cheng *et al.*, 2005). Water-based synthetic fluids have gained an increasing share of

the metalworking coolant market in response to growing demands with regards to improved occupational health standards, forecasted scarcity and the increased cost of straight oil products combined with greater demands for cooling capacity and due to their better and efficient performance and less severe environmental problems (Benito *et al.*, 2001, Mahdi and Skold, 1991). A common disadvantage of soluble oils however is their poor emulsion stability, their susceptibility to microbial degradation and requirement for high-quality water and tendency to foam very easily. Foam can inhibit heat transfer because it limits the amount of fluid in contact with the wheel and work-piece (Irani *et al.*, 2005, Mattsby *et al.*, 1989).

## 2.2.4 Chemistry of Metalworking fluids

The identity and proportion of chemical species in MWF formulations is dependent on the cooling and lubrication requirements of the machining process. A generic MWF formulation is composed of base oil and several additives added to enhance their operational lifespan. However, the exact formulations are proprietary information seldom divulged by the manufacturers.

### 2.2.4.1 Base oil

The base oil employed in MWF formulations have several functions, but primarily it serves as the lubricant, providing a fluid layer separating moving surfaces and removing heat and wear particles whilst minimising friction. In addition, they also provide a base for other additive molecules to attach themselves to refine and hone specific characteristics of the fluid. Base oils are most commonly mineral oils, which are hydrotreated (hydrogenated)

petroleum distillates and are of naphthenic or paraffinic nature (Irani *et al.*, 2005, Mortier and Orszulik, 1994, Tucker, 1994).

#### 2.2.4.2 Emulsifiers

Emulsifiers and surfactants are added to water-based fluids to disperse oil drops in the fluid and to reduce the fluid surface tension. Most commonly employed emulsifiers are petroleum sulphonates and ethoxylate products which constitute a significant fraction (upto 30%) of most soluble oil MWF (Anderson *et al.*, 2003).

#### 2.2.4.3 Corrosion inhibitors

The corrosion inhibitors (e.g., fatty acid salts, sulphonates, amines, amides, borates, silicates, phosphates and nitrates) operate by creating a physical barrier, in the form of a dense hydrophobic, monolayer of chemisorbed surfactant molecules, which prevent access of water and oxygen and attack by acids, and peroxides on the metal surface (Rasberger, 2010, Anderson *et al.*, 2003).

#### 2.2.4.4 Biocides

Biocides are intended to control the proliferation of bacteria, fungi, yeast, and mould in the fluid.. Target regions for antibacterial agents are the cell wall, cytoplasmic membrane and cytoplasm (Denyer, 1990). The biocides may include phenolic derivatives (chlorinated phenols), aliphatic derivatives (hydrazine), aldehydes, formaldehyde release biocides (triazine, isotiazolines) and organosulphur nitrogen compounds (Carvalhinha *et al.*, 2010, Shennan 1983).

#### 2.2.4.5 Alkaline reserve compounds

Sodium hydroxide and ethanolamines are added to keep a MWF alkaline (pH between 8.5 and 9) and this is specifically to control bacterial growth, maintain rust protection and retain emulsion stability (Anderson *et al.,* 2003).

#### 2.2.4.6 Extreme pressure agents and anti-weld agents

These are used to guard against welding of the work piece with the metal tool in heavy duty machining. They react with metal surfaces at high temperature and pressure to create a protective layer. Sulphur, chlorine, and phosphorus chemical compounds are commonly employed as pressure additives (Anderson *et al.,* 2003, Cheng *et al.,* 2005).

### 2.2.5 Deterioration and contamination of MWFs during use

Physical, chemical, and microbial effects can cause the chemical components of in-use metal working fluids to deteriorate. These contaminants can include wear debris, rust, weld spatter, lint, metal chips, abrasives, as well as contaminants entering through broken seals, dirty oil filter pipes, chemical residue on metal parts, tramp oils, reaction products such as nitrosamines, and microbial agents such as bacteria and fungi. Additionally, oil may degrade from excessive temperatures. The oxidation of metal working fluid oils and other constituents can lead to the formation of acids, resins, varnishes, sludges, and carbonaceous deposits. Additives such as biocides and anticorrosives may be depleted with use, requiring routine product addition or supplemental additions to maintain metal working fluid performance. Microbial contamination of metal working fluids is undesirable primarily

because of detrimental health effects, changes in physical characteristics, fluid performance, odour and slime accumulation, as discussed below:

### 2.2.5.1 Health effects

Workers can be exposed to metal working fluids through skin contact by exposure to splashes during immersion of the machine tool and while handling parts, tools, and equipment covered with metal working fluids. Workers also receive a significant exposure to metal working fluids by inhalation of aerosols composed of diverse chemicals, microbial organisms and their respective toxins; thermal degradation products caused by heat generated during the machining process; tramp oil from the machines themselves and the pumps, metallic particles created during the machining operations; and small amounts of dissolved metals from the tools and work pieces (Eisen *et al.,* 2001, Mattsby *et al.,* 1989).

Skin disorders such as dermitis, nose and throat irritations and serious respiratory effects like acute impairment of lung function, asthma, hypersensitivity pneumonitis (HP), chronic bronchitis and airway irritation are the most commonly noted occupational diseases among operators (Khan *et al.,* 2009, Eisen *et al.,* 2001, Mattsby *et al.,* 1989, Irani *et al.,* 2005, Shashidhara and Jayaram, 2010). HP cases have been reported where responses have been attributed to the presence of specific bacterial and fungal populations including *Bacillus, Mycobacterium*, *Alternaria* and *Penicillium*. Recent study suggests possible role of *Mycobacteria* ($10^6$-$10^7$ per ml MWF) in the diagnosis of HP in workers (Thompson and van der Gast, 2010). Various microbial toxins have been routinely recovered from contaminated MWF. These toxins include thousands of different chemicals produced by bacteria and

fungi (mycotoxins), posing serious threat to human health. Certain species of Clostridium genera isolated from MWF can generate up to seven different toxins. (Passman, 1994, Thompson and van der Gast, 2010).

#### 2.2.5.2 Change in physical characteristics

Fluid degradation from microorganisms may result in changes in fluid viscosity, and the acid products of fermentation lowers the pH of the fluids, causing corrosion and leaks in the metal working fluid system, posing a problem for its application in the machining of various ferrous and non-ferrous metals (Bakalova *et al.*, 2007, Rao and Srikant, 2006).

#### 2.2.5.3 Disruption of fluid performance

Microbial growth results in biodeterioration of MWF due to degradation of various additives and eventually renders the fluid ineffective for metalworking operations. Direct microbial attack on petroleum sulphonates degrades the emulsion stability. Removal of phosphates from their associated esters defunctionalises extreme pressure additive. Selective depletion of neutralising amines results in microbially induced corrosion. Strong inorganic acids formed when weak organic acids react with inorganic salts, results in attack of metal surfaces directly. Furthermore, Gram positive rods (*Clostridium sp*.) possess the enzyme hydrogenase which accelerates galvanic corrosion process. Excessive microbial growth also results in clogged filters and ports, and may interfere with the piped metalworking operation.

#### 2.2.5.4 Odour and slime accumulation

The most common aesthetic concerns associated with the use of water-miscible MWF are odour and slime accumulation. "Monday morning odour" is a very common phenomenon, resulting from accumulation of several odouriferous volatile excretion organics as a result of normal microbial metabolism. Anaerobic bacteria, specifically the sulphate reducers, may produce hydrogen sulphide and other disagreeable and toxic gases like methyl mercaptan especially after a weekend shutdown. Ammonia flush caused due to release of ammonia from MWF occurs when microbes attack MWF amines oxidatively (Theaker and Thompson, 2010, Passman, 1994).

#### 2.2.5.5 Adverse effects on the environment

Disposal of operationally exhausted MWF places a significant burden on the environment, leading to surface water, soil and groundwater pollution, air pollution and consequently, agricultural product and food contamination.

### 2.2.6 Microbiology of metalworking fluids

Fluid recirculation during machining operations aerates and thoroughly agitates the bulk fluid while microbial biofilm consortia and quiescent zones provide niches for anaerobic metabolism and other microbial activities. These conditions when maintained at a warm temperature in an open system, particularly when the machine stands still (most frequently at the weekend) provide the perfect habitat for colonisation, proliferation and activity of microbes. In addition, the presence of an array of organic MWFs components, which are susceptible to biodegradation in part or as a whole, render an ideal environment for some

microorganisms to multiply leading to MWF biodeteriotation (Muszynski *et al.,* 2007, Rasberger, 2010). Suspended metal fines such as swarf, chips and tailings provide surface area for microbial colonisation. Furthermore, tap water used to dilute metal-working fluids can contain a source of inoculum and provides a balanced blend for the growth of a variety of microorganisms. In addition, contamination can also originate from sources like machine operator's hands, sweat or saliva, from the atmosphere, tramp oil, parts, and residues of the previous fluid in the machine sump or coolant plumes (van der Gast *et al.,* 2001, Connolly *et al.,* 2006). Two kinds of biodeterioration have been reported to occur in MWFs during machining.

(i) Primary biodeterioration occurs when microbes assimilate one or more MWF components as sole source of carbon. Some molecules are consumed completely; converted to biomass, carbon dioxide and energy while others are only partially degraded.

(ii) Secondary biodeterioration occurs when microbial metabolites react with MWF components. Most microbes excrete a variety of weak organic acids as metabolic waste, which may react with inorganic ions, such as chloride, sulphate and nitrate that are present in the MWF, leading to the formation of fatty acid salts plus strong inorganic acids (hydrochloric, sulphuric, and nitric acids respectively). These resulting inorganic acids then react with any neutralising amines that are present in the MWF, thereby bringing about destabilisation of the formulation (Childers 1994).

Several studies on bacterial community have demonstrated that diversity within MWF is relatively low, which is reflective of the harsh conditions prevailing in the MWF (Mattsby *et al*., 1989, van der Gast *et al*., 2001, 2003). The populations that form bacterial

communities within oil-based and synthetic MWF are quite distinct. The oil-based MWF bacterial communities are predominately comprised of Gamma proteobacteria (Enterobacteriaceae and Pseudomonadaceae) and facultative anaerobes. Populations detected in synthetic MWF are typically aerobic (Thompson and van der Gast, 2010). Once spoilage is actively under way, the facultative anaerobic fermentative *Enterobacteria* may dominate the community with sulphide producing bacteria present at lower levels. During the final stages of biodeterioration, characterised by decline of pH and incipient splitting of the emulsion, a broad diversity of Gram-negative bacteria can be isolated, including species of *Acinetobacter, Achromobacter* and *Alcaligenes*. Yeasts and filamentous fungi are also often present in spoiling soluble oil emulsions at low counts (Shennan, 1983). Attempts to manage microbial growth by the incorporation of biocides have resulted in the emergence of biocide-resistant strains like *Pseudomonas spp.* because the biocide concentrations in MWF are often too low to prevent microbial growth (Bakalova *et al.,* 2007, Mattsby *et al.,* 1989).

Manufacturers and users of MWF are faced by contradictory challenges, in terms of the microbiology of the product. As MWFs are vulnerable to microbial attack, the initial aim is to develop a product with a long working life which is achieved by the addition of biocides and maintaining a high pH. In contrast, personnel responsible for disposal of operationally-exhausted MWF need to develop methods that promote the biodegradation of the used product. Consequently, the life cycle of MWF is one that requires the initial suppression of bio deterioration, followed by procedures that promote biodegradation (van der Gast *et al.,* 2003).

## 2.2.7 Disposal of operationally exhausted MWFs

MWFs operate in an extreme environment with high alkalinity (pH 8-10), excessive temperatures and become less effective with use because of their thermal degradation, microbial contamination and routine periodic factory shutdown, generating a waste stream called spent MWF. Apart from the discharge of used metalworking fluids, parts of washing operations also lead to the release of wastewaters containing metalworking fluids. Bell *et al.,* 1999 identified three mechanisms through which waste fluid enters into shop floor environment: splash (the result of momentum transfer due to impact of fluid particles on a solid surface), spin-off (related to the centrifugal force at the part surface in rotation motion) and evaporation (due to the cutting temperature that brings the contacting fluid to a vapour state). In-use MWFs therefore become process waste when functional properties such as lubrication or rust prevention are compromised, hence necessitating periodic replacement of the waste (Benito *et al.,* 2001, Deluhery and Rajagopalan, 2005).

The amount of waste generated from metal working operations increases every year, constituting a serious danger to the environment due to their high surface-active and organic pollutant loads. Waste metal cutting fluid wastewaters are of very high strength containing exceptionally high chemical oxygen demand (COD), total organic carbon (TOC) and turbidity. Over 400 million litres of waste MWF is disposed off annually in the UK with estimated yearly disposal costs of between £8 and £16 million, and the world-wide figures are estimated to be $22.4 \times 10^9$ litres annually (Connolly *et al.,* 2006, van der Gast *et al.,* 2003, Chen *et al.,* 2005, Knoblock *et al.,* 1994). These fluids require treatment prior to disposal to meet local sewer discharge standards, which are subject to local and state laws.

On-site treatment is increasingly promoted and is an efficient and cost-effective alternative to off-site disposal which has significantly reduced the volume and strength of the effluent discharged from the site. The on-site treated effluent can then either be discharged to a sewer at a much reduced cost, or reused on-site if the quality allows (Bio-wise, 2001). Many of the synthetic components in MWFs often pass untreated and remain as pollutants in the final effluent. The organic nitrogen compounds are also essentially unaffected by chemical de-emulsification and ultrafiltration, resulting in greater effluent concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total Kjeldahl nitrogen (TKN) (Anderson *et al.,* 2009, Knoblock *et al.,* 1994).

Various remediation tools such as chemical treatment, electrochemical treatment, membrane filtration (microfiltration, ultrafiltration and nanofiltration), thermal treatment and biological methods have been investigated by several researchers for treatment and safe disposal of used MWFs.

#### 2.2.7.1 Chemical treatment

In the chemical treatment method, electrolytes capable of splitting the emulsion (mineral salt, acid or organic demulsifier) are added to neutralise the stable emulsion, following which the emulsion breaks down, and the water and oil phases are separated in a settling tank (Muszynski and Lebkowska 2005, Rios *et al.*, 1998).

### 2.2.7.2 Electrochemical treatment

The electro-coagulation (EC) technique is applied for the de-emulsification of waste MWFs (Muszynski and Lebkowska 2005, Chen *et al.,* 2008, Kobya *et al.,* 2008, Muszynski *et al.,* 2007). When direct current is applied to water through a pair of electrodes, water molecules are broken down into hydrogen and oxygen gases. However, when the anode is made of metals that have lower oxidation potentials than water (such as iron and aluminium), the anode is dissolved to produce metal ions. The metal ions react with hydroxyl ions, (which are the by-products of hydrogen generation), to produce metal hydroxides. As the electrochemical reaction progresses, the ionic strength of the wastewater increases, the pH rises and causes the destabilisation of the stern ionic layer surrounding oil droplets in the emulsion. The dispersed oil droplets begin to coalesce, resulting in the disintegration of the emulsion into water and oil phases. Ultimately, the destabilised oil droplets are adsorbed into the highly dispersed metal hydroxide particles. The oil-rich sludge floats to the top of the solution. In this method, adsorption and electro-coagulation phenomena occur concurrently.

### 2.2.7.3 Membrane filtration

Membrane filtration is a pressure-driven process which uses molecular size pores for removal of emulsified oil and other dispersed components found in MWF. This brings about partitioning of emulsions and macromolecules into two phases: a clean permeate phase and a concentrated retentate phase (Skerlos *et al.,* 2000). Several researchers have investigated the feasibility of employing membrane filtration for treatment of milky cutting oil emulsions referred to as macro-emulsions (Lin and Gomez, 2005, Benito *et al.,* 2001, Benito *et al.,* 2002, Chang *et al.,* 2001, Hilal *et al.,* 2004, Hu *et al.,* 2002, Mahdi and Skold,

1991). Polymeric membranes used for the separation of oil from aqueous solutions can be defined as materials for microfiltration, ultrafiltration or nanofiltration membranes, depending on size of the pores. Previous studies in MWF waste have established oil rejection rates between 99 and 99.9% and it was noted that the resulting final effluent was acceptable for discharge into the sewage system in order to be treated in a conventional waste water treatment plant (Benito *et al.,* 2000, 2001). Although membrane filtration has been found to be effective in treatment of MWF wastewater, it has not been widely employed in remediation of waste MWF as this is an energy intensive and expensive method. In addition, concentration polarisation (due to accumulation of oil droplets on the membrane surface) and membrane fouling (caused by plugging of the pore openings by suspended solids in the feed) leads to reduced efficiencies (Lin and Gomez, 2005, Balkacem *et al.,* 1995, Benito *et al.,* 2001).

#### 2.2.7.4 Thermal treatment options

Incineration and hydrothermal oxidation of MWF involves thermal treatment of the waste solution, causing it to boil. The steam/distillate vapours are vented to the atmosphere by way of an exhaust stack. The 2000/76/EC and European Cooperation for Accreditation (EA) 2004 directives issued by the European Union and Environmental Agency in UK (EA) impose a stricter framework for the disposal of these wastes by prohibiting incineration of spent MWF (Cheng *et al*., 2005). Hydrothermal oxidation is carried out at high temperature and pressure, above the critical point for pure water, usually ranging from 400 to $650^0$ C and from 25 to 35MPa, respectively, leading to oxidation of organic and inorganic compounds (Portela *et al.,* 2003). However, the limitations preventing the commercialisation of these methods are high energy input, corrosion of reactors and

preheaters, precipitation of salts in the super critical phase, is a fire hazard, high costs of investment and operation and possible air pollution source (volatile organics) resulting in stack corrosion and odours. Furthermore, a thermal based treatment option is not a sustainable remediation method. Water is a limited resource and the key driver in today's world should be water conservation and recovery.

## 2.2.8 Biological treatment of MWF wastewater

There is an increasing interest in biological treatment systems for processing industrial wastes, such as metal working fluids (MWF), due to in-practicability, high energy demand and carbon foot-print associated with physico-chemical methods. Treatment of such wastewaters biologically represents a cost-effective alternative for achieving compliance with effluent regulations. Biological treatment of waste MWF by microorganisms in bioreactor systems has been investigated by several researchers. Specialised micro-organisms enriched from spent MWF have the ability to degrade almost all components of MWF effluent, including synthetic ones. They are also much more resilient than the micro-organisms used in sewage treatment works, because they are adapted to the highly polluting and toxic nature of the MWF effluent.

### 2.2.8.1 Bioaugmentation

In developing a biological treatment strategy, one way to optimise bioreactor performance is by the addition of specialised microorganisms (bioaugmentation) or bacterial consortia isolated from a range of sources. Bioaugmentation is the addition of microorganisms to enhance the treatment efficiency when the existing indigenous populations are not

degrading the target contaminant at a satisfactory rate. It has been shown to be effective for enhancing rates of water treatment when indigenous degradative activity is suboptimal or even absent, significantly improving biotransformation rates (van der Gast *et al.,* 2002, Muszynski and Lebkowska, 2005).

One area where there is considerable promise for bioaugmentation is its application in bioreactors for the treatment of chemically mixed industrial wastes where biotransformation processes often depend upon co-metabolism. The same bacteria associated with the biodeterioration of MWF could be the dominant species in the biodegradation process (van der Gast *et al.,* 2002, Muszynski and Łebkowska 2005). These studies indicate that bioaugmentation with selected strains, based upon an understanding of the diversity of indigenous populations in operationally exhausted MWF waste, can lead to more effective processing of wastes than applying uncharacterised communities (Thompson *et al.,* 2005).

#### 2.2.8.2 Aerobic biodegradation

All chemical constituents of an operationally exhausted synthetic MWF formulation, exhibited 60% reduction in concentration when subjected to an aerobic bioreactor system augmented with specifically selected indigenous strains (van der Gast *et al.,* 2004). Reduction in the COD by the selected consortium was approximately 85% of the total pollution load and was reported to be 30–40% more effective than an activated sludge treatment, wherein both reactors were operated at the same hydraulic retention times. Maximum degradation took place within the first 200 hours after the reactor start-up. In a

similar recent study, Bakalova and co-workers, 2007 demonstrated that the predominant bacterial species isolated from contaminated metalworking fluids are able to utilise all the individual components of MWFs as a sole source of carbon and energy, and hence demonstrating its ability to act as a nutrient source and support microbial growth.

Muszynski and Łebkowska (2005) developed a biotechnological method for operationally exhausted full strength MWF wastewater treatment. Their research demonstrated that multiple re-inoculation of 5 bacterial species, isolated from used MWF, into a sequencing batch reactor (SBR) immobilised with microorganisms on PVC foam carrier was effective for biotreatment. The process was carried out in anoxic/aerobic conditions and resulted in elimination of 87% of the COD and 97% of $BOD_5$.

Connolly *et al.,* 2006 adopted a hybrid approach to enhance the potential for biological degradation of spent MWF in a bioreactor system, by identification and removal of a synthetic polymer by physical approaches. Their study demonstrated that polymer removal was found to have little effect on the degradation efficiency of the remaining components, but resulted in a waste product with 14% lower chemical oxygen demand. Their work further established that the toxicity of the MWF declined to zero during the first 10 days of the bioreactor treatment, which is the duration corresponding to the degradation of four of the organic acids and the benzene derivative in the MWF. Similarly, 87% reduction in toxicity was observed by Muszynski *et al.,* 2007 after biological treatment of waste MWF.

Activated sludge can also be employed for biological treatment of the waste MWF (Baker *et al.,* 1983). They observed that none of the predominant bacteria present in an activated sludge reactor established for the degradation of cutting fluids were classified to be strict pathogens. The reactor was supplemented with enough potassium phosphate to maintain phosphate levels at 20 mg/L. whereas nitrogen and sulphur were found to be supplied by organics in the coolant concentrate, and other minerals were supplied by the dilution water.

The fate of individual MWF components on biodegradation has been investigated by several researchers (Prince, 1994, Buers *et al* 1997). These studies indicated that growth on these components was due to utilisation of the components themselves rather than to the presence of degradable contaminants.

### 2.2.8.3 Membrane bioreactor

Membrane Bioreactor (MBR) systems are conventional activated sludge process systems consisting of aerobic, suspended-growth biological treatment that however utilises membrane filtration instead of gravity sedimentation for solids separation. Two types of MBR configurations exist: internal/submerged, where the membranes are immersed in and is an integral part of the bioreactor; and external/sidestream, where membranes are a separate unit process requiring an intermediate pumping step. The ultrafiltration unit in the system retains oil and grease whilst they are biologically degraded in the aerobic reactor (Knoblock *et al.,* 1994, Sutton *et al.,* 1994). Membrane bioreactors have been used as primary treatment processes to remove both emulsified oil and hydrophilic organic compounds, and been demonstrated. to achieve almost complete removal of COD and total

nitrogen of a simulated semi-synthetic MWF waste (Anderson *et al.,* 2009, Knoblock *et al*., 1994). However, this process necessitates high capital and energy costs, the need to control membrane fouling, and uncertainty about membrane life (Anderson *et al.,* 2009).

#### 2.2.8.4 Anaerobic biodegradation

Many toxic and recalcitrant organic compounds are degraded under anaerobic conditions, with the compound serving as a growth substrate with fermentation and ultimate methane production. For treating high-strength industrial wastewaters, anaerobic treatment has been shown to provide a very cost effective alternative to aerobic processes with savings in energy, nutrient addition, and reactor volume. Anaerobic processes are advantages because of the lower biomass yields and because energy in the form of methane can be recovered from the biological conversion of organic substrates (Metcalf and Eddy, 2003). Several researchers have investigated the application of anaerobic biological treatment for waste MWF. Kim *et al*., 1989, 1992, Carvalhinha *et al., (*2010), Perez *et al.,* 2006, Perez *et al., 2007* employed anaerobic fluidised bed process to treat MWF wastewater. Their study indicated that the bioreactor removed biodegradable COD effectively and efficiently.

In general, all of the treatment schemes were demonstrated to efficiently remove biodegradable organics, but leave a substantial amount of residual organics in treated effluents. These residuals appeared to be either non-biodegradable or difficult to degrade organics. Approximately 10 to 15% of organic compounds in metalworking fluid wastewater are typically not biodegraded (Anderson *et al.,* 2009, Carvalhinha *et al.,* 2010).

#### 2.2.8.5 Fixed-film bioreactors

Fixed film treatment processes are also called attached growth processes and include trickling filters and rotating biological contactors. These bioreactors consist of a bed of highly permeable medium to which microorganisms are attached, and through which wastewater is percolated or trickled. The filter media usually consists of rock or a variety of plastic media. The organic material in the wastewater is degraded by aerobic microorganisms attached to the media, and forms a biological film or slime layer. The treated wastewater is then clarified to remove the sludge, disinfected and discharged.

## 2.3 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are methods that accelerate the oxidation and degradation of a wide range of organic and inorganic substances that are resistant to conventional treatment methods. AOP has been defined (Glaze *et al.,* 1987) as "near ambient temperature and pressure water treatment processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification". The hydroxyl radical ($OH^{\bullet}$) is a powerful, non-selective chemical oxidant. They are reactive electrophiles, which act very rapidly with most electron-rich organic compounds, thereby transforming the organic molecules to more oxidised intermediates; carbon dioxide and water. The oxidation potential of the hydroxyl radical is second only to the strongest oxidising agent, namely fluorine (Table 2.1). Depending upon the nature of the organic species, three types of initial attack of $OH^{\bullet}$ on the organic compound are possible (Munter, 2001, Sarria *et al.,* 2002, Stasinakis, 2008):

(i) The hydroxyl radical attacks organic molecules by abstracting a hydrogen atom (Eq. 2.1) as with alkanes or alcohols, or

(ii) It can add itself to the double bonds of the contaminant (Eq. 2.2), as in the case of olefins or aromatic compounds or

(iii) By electron transfer (Eq.2.3)

$$R + OH^{\bullet} \rightarrow R^{\bullet} + H_2O \qquad (2.1)$$

$$R + OH^{\bullet} \rightarrow ROH \qquad (2.2)$$

$$R_n + OH^{\bullet} \rightarrow R_{n-1} + OH^{-} \qquad (2.3)$$

(R is used to describe the reacting organic compound).

**Table: 2.1 Relative oxidising power of some oxidising species (***Adapted from Munter 2001*)

| Species | Oxidation potential |
|---|---|
| Fluorine | 3.03 |
| Hydroxyl radical | 2.80 |
| Atomic oxygen | 2.42 |
| Ozone | 2.07 |
| Hydrogen peroxide | 1.78 |
| Perhydroxyl radical | 1.70 |
| Hypochlorous acid | 1.49 |
| Chlorine | 1.36 |

Because of its fast reactions with practically all organic substrates, second order radical-radical recombination reactions (e.g., $OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2$) can be neglected unless the substrate concentration is very low or in cases with very high radical concentrations, such as those generated upon intense or prolonged electron beam irradiation or sonication (Alaton *et al.*,2009).

AOPs include processes like gas induced ozonation, Fenton's oxidation, ultrasonic irradiation, heterogeneous and homogeneous photocatalysis based on near ultraviolet or solar visible irradiation, electrolysis, and wet air oxidation (WAO). Furthermore, less conventional but evolving processes include ionising radiation, microwaves, pulsed plasma and the ferrate reagent. It is very common to see coupling of various AOPs for pollutant remediation or to achieve better biodegradability of the wastewater stream. Examples of such processes are integration of ozone with hydrogen peroxide (peroxone), Fenton with UV irradiation (Photo-Fenton), ozone-$H_2O_2$ process, ozone-UV, ultrasound-photocatalyst (Sonophotocatalysis), ultrasound-Fenton. The feasibility of an adequate combination treatment depends upon the composition and concentration of wastewater and researchers the world over continue to experiment various new combinations to optimise efficiency.

## 2.3.1 Fenton's oxidation

The oxidation system based on the Fenton's reagent (hydrogen peroxide in the presence of a ferrous salt) has been used for the treatment of both organic and inorganic substances, under laboratory conditions, as well as real effluents from different sources (Gogate and Pandit 2004). Fenton reactions encompass reactions of peroxides (usually $H_2O_2$) with iron ions, to form active oxygen species (Eq. 2.4) that oxidise organic or inorganic compounds when they are present. The by-product, ferric iron, in turn reacts with peroxide or superoxide $(O_2^-)$ radical to regenerate ferrous iron. The iron cycles between the ferrous and ferric oxidation states until the $H_2O_2$ is fully consumed, producing $OH^\bullet$ in the process.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \qquad (2.4)$$

The use of Fe(II)/$H_2O_2$ as an oxidant for wastewater treatment is attractive due to the fact that: (1) iron is a highly abundant and non-toxic element, and (2) hydrogen peroxide is easy to handle and environmentally benign (Munter, 2001, Pignatello *et al.*, 2006).

## 2.3.2 Ozonation

Gas-induced ozonation has proven to be effective in degrading a number of recalcitrant organic pollutants in water and wastewater (Rice, 1997). Ozone is a very powerful oxidising agent (oxidation potential of 2.07 V) that can react with most species containing multiple bonds (such as $C=C, C=N, N=N$) but not with singly bonded functionality such as ($C-C, C-O, O-H$) at high rates. These oxidations are simple and the mechanism only requires contact of ozone with the organic pollutant (Gogate and Pandit, 2004). When applied in aqueous solution, substrate reactions occur partly by direct interaction with molecular ozone, and partly by indirect interactions with hydroxyl radicals, produced by the decomposition of ozone. The type of oxidant generated depends on reaction conditions, such as pH. The combination of molecular ozone and the more powerful, nonselective $OH^{\bullet}$ allows for the degradation of more recalcitrant compounds and acceleration of the overall treatment process (Gerrity and Snyder 2011). The hydroxyl radicals produced have a higher oxidation potential (2.8 V) than molecular ozone and can attack organic and inorganic molecules non-selectively with very high reaction rates.

## 2.3.3 Photocatalytic oxidation

The basis of photocatalysis is the photo-excitation of a semiconductor catalyst, so that it can catalyse redox reactions with chemicals in aqueous solution. The irradiation of a

suitable semi-conductor material (eg. $TiO_2$), with photons (hv) possessing energies of magnitude equal to or greater than its band gap results in migration of electrons from the valance band (VB) to the conduction band (CB) of the particle (Eq. 2.5). This process generates regions of positive charges termed 'hole ($h^+$)' in the valence band, and free electrons ($e_{cb}$) in the conduction band (Bolduc and Anderson 1997, Munter 2001).

$$TiO_2 + h\nu \rightarrow e_{cb} + h^+ \qquad (2.5)$$

The surface of the catalyst is readily hydroxylated when in contact with moisture, hence both dissociated ($OH^-$ and $H^+$) and molecular ($H_2O$) water is bound to it. The valence band holes interact with adsorbed hydroxide ions and molecular water to produce reactive hydroxyl radicals ($OH^\bullet$) (Eq. 2.6).

$$h^+ + H_2O \rightarrow H^+ + OH^\bullet \qquad (2.6)$$

Although hydroxyl radicals are believed to be primarily responsible for organic contaminant destruction, some researchers believe that valence band holes and excited-state electrons are also capable of initiating a wide range of chemical reactions (Cooper *et al.,* 2009, Munter, 2001, Stasinakis 2008). Titanium dioxide is one of the most widely used photocatalysts in industry (Rizzo *et al.,* 2009, Bolduc and Anderson, 1997, Westerhoff *et al.,* 2009).

## 2.3.4 Ultrasonic irradiation

Among the various AOPs, considerable interest has been devoted to the application of ultrasonic irradiation for the treatment of contaminants in water and wastewater (Mason *et al.,* 2003; Mason *et al.,* 2007; Naddeo *et al.,* 2007). When a liquid is irradiated with

ultrasound (usually in the range of 20–1000 kHz), the waves pass through the medium in a series of alternate compression and rarefaction cycles. When the acoustic amplitude is large enough to stretch the liquid molecules, during its rarefaction cycle, to a distance that is greater than the critical molecular distance to hold the liquid intact, microbubbles or cavitation bubbles are created. These cavitation bubbles then collapse violently and adiabatically in the subsequent compression cycle, giving rise to extremes of temperature (greater than $5000^0$ C) and pressure (1000 atm). This bubble collapse decomposes water to create both oxidizing ($OH^{\bullet}$) and reducing ($H^{\bullet}$) radical species (Beckett and Hua, 2003, Joseph *et al.*, 2000, Shimizu *et al.*, 2008, Neppolian *et al., 2002,* Adewuyi, 2005). The sonochemical degradation in aqueous phase involves several reaction pathways and creates three regions for high energy chemical reaction to take place; the region inside the bubble cavity, the interfacial region and the liquid bulk (Joseph *et al.*, 2009, Adewuyi, 2005).

### 2.3.5 Electrochemical mineralisation

The electrochemical mineralisation of organic pollutants is a relatively new technology for the treatment of wastewaters of moderate concentration (COD < 5g $L^{-1}$) (Comninellis *et al.*, 2008, Chatzisymeon *et al.*, 2009). Water is initially discharged at the anode active sites (M), producing adsorbed hydroxyl radicals ($M(OH^{\bullet})_{ads}$) which are involved in the mineralisation of organic pollutants in aqueous solutions. An ideal anode for this type of treatment is the boron-doped diamond (BDD).

## 2.4 Combinative/Hybrid Treatment Approach

Among a variety of physical, chemical and biological processes developed for water treatment, each has inherent limitations in applicability, effectiveness and cost. Physical processes such as precipitation, incineration, adsorption or air stripping, for example, transfer pollutants from the aqueous to a second phase, but the pollutant is not destroyed. Chemical oxidation may be slow to moderate in rate and selectivity, or rapid but non-selective, thus generating appreciable reactor or oxidant costs. Biological oxidation techniques are conventionally the most popular and economic treatment strategies. However, their efficiencies are often compromised when the feed is either recalcitrant to biodegradation, inhibitory or toxic to the bio-culture. Effective treatment of a particular wastewater, given these limitations, may require a combination of available processes, in such a way as to exploit their individual strengths, reduce waste treatment costs substantially over single-step processes and thus attain the desired water characteristics. The design key to such coupled or hybrid systems lies in choosing processes that complement each other and lead to a synergistic effect (Adewuyi, 2005, Scott and Ollis, 1995).

Advanced oxidation processes (AOPs) efficiently degrade recalcitrant organic pollutants. In terms of the economic perspective, the use of advanced oxidation processes as a stand alone treatment procedure may not look lucrative owing to huge energy and chemical consumptions. Therefore, cutting down treatment costs will be essential in order to make AOPs attractive to the water industry; a step in this direction would be the use of AOPs solely as a pre-treatment to improve the biodegradability of the wastewater. Thus, a hybrid method consisting of employing advanced oxidation processes to reduce the toxicity of the effluent down to a desired level, followed by less costly and more robust biological

oxidation seems an obvious way forward. These oxidative end-products, obtained by AOP treatment, typically represented by short carboxylic acids, can be degraded easily biologically (Gogate and Pandit, 2004b, Comninellis *et al.,* 2008).

The combination of various AOPs with other technologies appears to be an effective strategy to improve the rate and extent of mineralisation and biodegradability of recalcitrant pollutants (Ikehata and El-Din, 2004, Arriaga *et al.,* 2009, Lin and Ma 2000, Neppolian *et al., 2002*, Rivas *et al.,* 2008).

## 2.4.1 Factors affecting AOP-microbial combinative treatment process

### 2.4.1.1 Choice of Chemical Oxidant

The chemical oxidant has to be chosen based on its ability to oxidise the target of interest. The chemical oxidant used in integrated chemical-biological treatment schemes will determine the oxidation intermediates and thus affects both the subsequent biodegradability of the reaction products and the overall effectiveness of the system. Different intermediates may result due to the oxidising potentials, selectivity or reaction mechanism which is characteristic of each oxidant. Care should be taken to choose those oxidants which will not lead to the generation of toxic intermediates, thus ensuring the procedure is compatible with subsequent post-biological steps (Scott and Ollis, 1995, Martin *et al.,* 2009).

### 2.4.1.2 Effect on Fate of pollutants

Oxidants can affect the fate of chemicals found in the environment. Organics that are sparingly soluble in aqueous medium will become more soluble as chemical oxidation leads to hydroxylated intermediates. Likewise, some volatile organics may also remain in aqueous solution more readily upon oxidation (Scott and Ollis, 1995, Martin *et al.*, 2009).

### 2.4.1.3 Choice of Biological Agent

Wastewater composed of multiple biodegradable organics may be more efficiently treated with a robust activated sludge community or bioaugmented with specialist microbial cultures able to effectively biodegrade the chemical constituents. Conversely highly acclimated pure cultures may be appropriate for biotreatment of individual, bio-resistant pollutants. An interesting aspect of the biological processes employed in combined treatment is the effect of acclimation on the degradation ability. Acclimatisation of microorganisms to the substrate of interest is widely used to maximise the removal efficiency of specific compounds, which are difficult to biodegrade. In a combined chemical and biological treatment, however, the substrate degraded by the culture may not be the original compound to which it was acclimated, but one or more breakdown intermediates. Degradation of these intermediates will depend if specific populations with in the community have the necessary enzymes and ability to successfully biodegrade them. In some instances, the acclimated cultures may have competitive advantage in degrading the intermediates due to their specialised metabolic pathways. Conversely, a culture not specifically acclimated will have to compete with a large number of species which are better adapted to degrading the intermediates (Martin *et al.*, 2009).

#### 2.4.1.4 Practical aspects of combining processes

Specific steps have been taken by some researchers to prevent the introduction of chemical oxidants into subsequent biological processes. Ozone, for example, is used as a disinfectant and would be detrimental to the biological culture if toxic residual traces remained from pre-treatment steps. High concentrations of residual hydrogen peroxide from Fenton treatment can also prove detrimental to exposed microbial cells although relatively low concentrations do not pose a serious problem. (Pignatello, 2006, Jagadevan *et al.*, 2011).

#### 2.4.1.5 Effect of reaction time

The reaction time of the chemical oxidation step is an important consideration when assembling a combined process treatment. Longer oxidation times lead to a greater degree of oxidation and greater removal when this is the sole treatment. Large oxidant doses can be therefore be wasted on easily biodegradable reaction intermediates resulting in decreased system efficiency (Bolduc and Anderson, 1997). Short oxidation times give rise to chemical compounds structurally similar to the parent pesticides that are still highly toxic and recalcitrant. Thus, optimal treatment time is related to the biodegradability and toxicity of the intermediates generated, and consequently the chemical reaction proceeds must be evaluated as a function of treatment time (Martin *et al.*, 2009).

## 2.5 Nanotechnology

Nanotechnology is the science of manipulating matter on an atomic and molecular scale that has cut across such disciplines as chemistry, physics, biology and engineering.

Nanotechnology refers broadly to using materials and structures with nano-scale dimensions, usually ranging from 1 to 100 nanometers (nm). A nanometer is one billionth of a meter ($10^9$ m), and is derived from the Greek word, *nanos*, meaning 'Dwarf'. The particle dimensions in this range results in materials and systems that often exhibit novel and significantly changed physical, chemical and biological properties due to their size and structure (Masciangioli and Zhang, 2003). These new properties include improved catalysis, tunable wavelength sensing ability and increased mechanical strength (Masciangioli and Zhang, 2003). Nanoscale iron particles represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems (Matheson and Tratnyek, 1994, Agrawal and Tratnyek, 1996, Farrell *et al.*, 2000, Lee *et al*., 2004). Environmental applications of metallic iron have been enthusiastically accepted by many users and regulatory agencies, largely due to the low costs and absence of any known toxicity induced by the use of iron. Elemental iron itself has no known toxic effect, bearing in mind it is one of the most abundant metals on Earth (Tratnyek, 1996), and is indeed an essential trace element in our diet. ZVI has been successfully employed for the degradation of a wide range of contaminant organics in recent years, including chlorinated and nitro-substituted entities.

## 2.6 Research Objectives

Safe disposal of operationally exhausted metalworking fluids is very challenging because they have variable chemical composition, are particularly toxic due to the inclusion of biocides in the formulations and typically have very high chemical oxygen demand. The

complex and unknown chemical content of the different formulations further complicates the treatment of these wastes.

The overall aim of this research was to improve understanding, development and implementation of environmentally friendly novel hybrid treatment (biological and chemical) approaches for remediation of the recalcitrant chemically-mixed industrial wastewaters, and specifically operationally exhausted metal working fluids. The major reason for selection of Fenton reaction, ozonation and zerovalent iron induced oxidation as treatment methods are the following: (a) all three processes have demonstrated capabilities for destruction of various hazardous compounds, (b) all have full-scale applications, reflecting their technical and economic feasibility, (c) all three processes can convert refractory and inhibitory organic compounds to biogenic and therefore biodegradable compounds with low probability of formation of toxic intermediate products, and (d) the reaction mechanisms of these processes are fairly well established in terms of the primary oxidants involved. Hence, it was assumed that each oxidation method would produce different by-products or different ratios of by-products, thus demonstrating varying degrees of biodegradability. An additional aim was to find the most appropriate way of combining chemical oxidation processes with biological degradation. More specifically to harmonise the potential toxic chemical step with the subsequent biological procedure. To this end, the research objectives were as follows:

1. To investigate the oxidation of recalcitrant metalworking fluids with Fenton's reagent, ozone and nano-sized zerovalent iron.
2. To optimise the chemical oxidation treatment steps.

3. To investigate the feasibility of a hybrid treatment scheme, combining the aforementioned oxidation steps with biological degradation.
4. To determine the most efficient type of integrated treatment for recalcitrant metalworking fluids.
5. To gain an insight into the reaction and kinetics for various oxidation methodologies.

# CHAPTER 3

# FENTON'S OXIDATION OF RECALCITRANT METALWORKING FLUID

## 3.1 Overview of Fenton's Chemistry

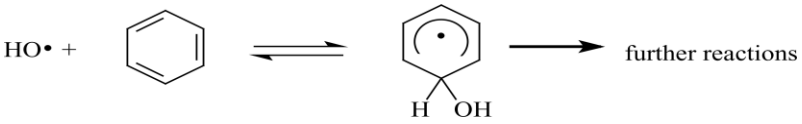
Fenton's reagent is recognised as one of the most powerful oxidising reactions available that can be used to destroy a wide variety of water-miscible and bio-refractory organic compounds in aqueous waste, soils, and ground water. The key Fenton reaction centres on the generation of hydroxyl radicals by the decomposition of hydrogen peroxide catalysed by ferrous ions (Eq. 3.1), in acid conditions, under ambient temperature and pressure. Fenton reagent ($H_2O_2$ + $Fe^{2+}$) reacts to form an unstable iron-oxide complex that subsequently gives rise to hydroxyl radicals. However, this produces stoichiometric amounts of Fe(III) which later precipitates to amorphous ferric oxyhydroxides as the pH is increased from strongly acidic to neutral, hence generating undesirable sludge in technological applications. The iron cycles between the ferrous and ferric oxidation states until $H_2O_2$ is fully consumed, producing hydroxyl free radicals ( $HO^{\bullet}$ ) in the process (Pignatello *et al.,* 2006).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \qquad (3.1)$$

The highly reactive hydroxyl radical ( $HO^{\bullet}$ ) generated in Eq.1 reacts with organic compounds principally by abstracting H from C-H, N-H, or O-H bonds (Eq.3.2), adding to C-C bonds (Eq.3.3) or to aromatic rings (Eq.3.4) (Pignatello *et al.,* 2006). These reactions of the hydroxyl radical with organic compounds results in the oxidation of the later (Kitis *et al.,* 1999, Tekin *et al.,* 2006, Pignatello *et al.,* 2006), leading to the formation of carbon-centered radicals.

$$RH + HO^{\bullet} \rightarrow R^{\bullet} + H_2O \qquad (3.2)$$

$$HO^{\bullet} + C = C \rightarrow HO - C - C^{\bullet} \qquad (3.3)$$




(3.4)

The catalyst ($Fe^{2+}$) is regenerated through the reaction summarised in Equation (3.5) or from reaction of $Fe^{3+}$, with intermediate organic radicals, Equations (3.6) (Pignatello *et al.*, 2006; Bautista *et al.*, 2008; Kitis *et al.*, 1999).

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \quad (3.5)$$

$$R^{\bullet} + Fe^{3+} \rightarrow R^+ + Fe^{2+} \quad (3.6)$$

The oxidation potential of Fenton reaction depends on the rate and amount of hydroxyl radical generated in the reaction. Pathways leading to scavenging of these free radicals occurs when excess $H_2O_2$ and Fe(II) is present in the system (Eqs. 3.7 and 3.8). In addition, the iron catalysed conversion of $H_2O_2$ to molecular oxygen and water (Eq. 3.9) further contributes to a waste of bulk oxidants.

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \quad (3.7)$$

$$HO^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (3.8)$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (3.9)$$

In the Fenton treatment, oxidation proceeds by two steps: (i) Reaction of organic compounds with hydroxyl radicals (predominates at a low pH) and (ii) coagulation by precipitation of ferric-hydroxo complex (takes place at a high pH of 7–8) (Goi *et al.*, 2010, Tekin *et al.*, 2006).

### 3.1.1 Fenton treatment of waste metalworking fluid

Fenton oxidation has been previously employed for treatment of metalworking (MWF) wastewater in combination with ultrasonication (Seo *et al.,* 2007), whereby 98% removal in chemical oxygen demand (COD) was obtained by the integrated treatment. Although very good COD removal efficiency was achieved by Seo *et al.,* high quantities of Fenton reagents ($H_2O_2$=10% v/v; $FeSO_4$=3 $gL^{-1}$) were employed in their study. Such high quantities of reagents would inadvertently lead to greater treatment costs and is hence not practically a viable solution. This chapter investigates the feasibility of employing Fenton oxidation for remediation of recalcitrant MWF and to check the possibility of combining this chemical treatment with a post-biological oxidation to provide an environment-friendly and economically feasible treatment option. The main costs of the Fenton treatment are the chemicals; hence for harmonisation of chemical process with biological step and for economical reasons, Fenton's oxidation process at lower doses was used to achieve oxidation of waste MWF components. A rigorous statistical analysis, using central composite design, was employed to determine the minimum Fenton reactant concentration that is effective at increasing the bioavailability of the recalcitrant components of MWF wastewaters, and coupling this step to a microbiological reactor, so providing maximum organic load removal.

## 3.2 Response Surface Methodology (RSM)

Conventionally, wastewater treatments are optimised by adopting a one-factor-at-a-time approach (where the effect of each variable was studied independently, with the other variables held constant). However, the process parameters may involve synergistic effects, as a result of complex interactions between process variables. The one-factor-at-a-time

approach is time-consuming and inefficient as it fails to recognise any possible interactions between independent factors and does not necessarily allow a precise process optimization. Consequently, the application of conventional optimisation techniques is not adequate for process optimisation. In order to overcome these drawbacks, experimental process optimisation is based on statistical design tools. In the design and statistical evaluation of experiments, response surface methodology (RSM) can be used for process optimisation and prediction of the interaction between process variables, reducing the numbers, thus the time and associated costs spent for conducting these experiments (Montgomery, 2001, Myers and Montgomery, 2009, Alaton *et al.,* 2010).

### 3.2.1 Central composite design

The most successful of the RSM approaches is central composite design (CCD) which has been used to optimise experimental parameters owing to its simple procedure (Guaraccho *et al.,* 2009, Torrades *et al.,* 2011), fewer experimental runs and demonstrates a full evaluation on interactions amongst factors. It determines which independent variables, and cross-interactions, have statistical meaning in terms of the response of the system (dependent variable), by performing a small number of experiments. CCD is widely used for fitting a second-order model and it is not imperative in the modelling procedure to know the detailed reaction mechanism, since the mathematical model is empirical (Rodrigues *et al.,* 2009, Sahu *et al.,* 2010). CCD is a sequential process involving three steps:

(i) Performing the statistically designed experiments;

(ii) Estimating the coefficients in a mathematical model and predicting the response through regression and;

(iii) Optimisation and checking the adequacy of the model.

CCD contains a fractional factorial design with centre points that is augmented with a group of `star points' that allow estimation of curvature (Figure 3.1). If the distance from the centre of the design space to a factorial point is ±1 unit for each factor, the distance from the centre of the design space to a star point is ± α with $|\alpha|>1$. The precise value of α depends on certain properties desired for the design and on the number of factors involved. The star points represent new extreme values (low and high) for each factor in the design.

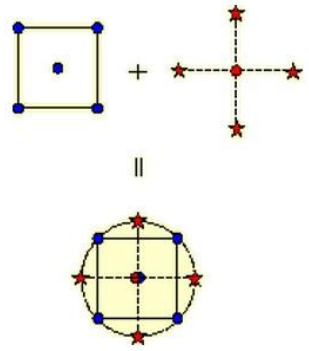


**Figure 3.1**: Generation of a central composite design for two factors

The number of tests required for the CCD includes

(i) the standard $2^n$ factorial design in the factors studied, each having two levels;

(ii) A set of centre point experimental runs; values of which are the medians of each factor used in the factorial portion. Replicates of the test at the centre are very important, as they provide an estimate of the experimental error and the reproducibility of the data (Montgomery, 2001; Myers and Montgomery, 2009, Zavareh *et al*., 2010).

(iii) A set of axial points experimental runs are identical to the centre points except for one factor, which will take on values below and above the median of the two factorial levels, and typically outside their range.

For two variables, the recommended number of tests at the centre is 5 (Montgomery, 2001). Hence the total number of tests (N) (Eq. 3.10) required for two independent variables is:

$$N = 2^n + 2n + n_c \qquad (3.10)$$

$$= 2^2 + (2 \times 2) + 5 = 13$$

After running the experiments, the responses and the corresponding parameters are modelled and optimised using ANOVA to estimate the statistical parameters by means of response surface methods. The goal is to optimise the response variable (Y). It is assumed that the independent variables are continuous and controllable by experiments with negligible errors and a suitable approximation for the true functional relationship between independent variables (X) and the response surface is necessitated (Gunaraj and Murugan, 1999, Sahu *et al.,* 2010).

The experiments under this chapter were conducted in three parts. The first part consisted of performing statistically designed experiments and analysis of the corresponding response variables to determine optimum Fenton dose. The second part consisted of running Fenton reaction at the optimised conditions and checking the biodegradability index and toxicity profile. The last part dealt with combining the chemical pre-treated effluent with aerobic biodegradation.

## 3.3 Aims and Objectives

The main aim of this part of the study was to investigate the feasibility of a sequential Fenton's-biological treatment for metalworking fluid (MWF) waste and to optimise the process. The aims of the studies described within this chapter were:

- To determine optimum Fenton reagent dose leading to maximum reduction in chemical oxygen demand (COD), total organic carbon (TOC) and toxicity by employing a statistical tool (central composite design).
- To determine the effect of reaction conditions (pH, reaction time and temperature) on Fenton oxidation of recalcitrant MWF.
- To investigate the feasibility of integrating Fenton pre-treatment with a biological post treatment under aerobic conditions.

## 3.4 Materials and Methods

### 3.4.1 Wastewater characteristics

The recalcitrant MWF wastewater samples (Hocut 795-B, Houghton International Inc.) were obtained from Microbial Solutions Limited, Upper Heyford, Oxfordshire, a company which employs a repository of non-pathogenic bacteria to treat contaminants in metal working fluid wastewater. The as-received operationally exhausted semi-synthetic MWF wastewater was characterised to have a high COD ranging from 100,000-150,000 mg $L^{-1}$. These high strength MWF samples could not be treated by aerobic bacteria because of their high COD (100,000-150,000 mg $L^{-1}$), concentration effects, the presence of biocides, other toxic chemicals and alkaline pH (8.5-9.5), none of which are conducive for growth of bacterial population (Anderson *et al*., 2003, van der Gast *et al*., 2001, 2002). The MWF were then diluted with tap water (COD~45,000 mg $L^{-1}$) and treated in an aerobic fixed film batch bioreactor in Microbial Solutions Ltd, employing a previously described bacterial consortium which were assembled on the basis of their apparent ubiquity in waste MWF, degradation ability and tolerance to fluctuating chemistry of the waste (van der Gast *et al.,*

2003). Initial pH of the as-received operationally exhausted MWF was 8.5-9.0, which was lowered to near neutral range by microbial activity after the aerobic biological treatment.

The exact chemical composition of the wastewater was difficult to ascertain, due to the large number of unknown chemicals in the formulation and the fact that manufacturers of MWF never disclose the exact composition since this is proprietary information. Furthermore, the composition of the formulation changes temporally and spatially with use. Although effective in removing the labile components of the effluent, bacterial populations are not always able to degrade the recalcitrant components, which consequently accumulate. The specific focus of this study was the recalcitrant components of MWF wastewater that persisted after a primary bacterial treatment step conducted in Microbial Solutions Limited. The physico-chemical parameters of the recalcitrant wastewater received from Microbial Solutions are summarised in Table 3.1.

**Table 3.1: Wastewater characteristics**

| | pH | SS (mg/L | COD (mg/L) | $BOD_5$ (mg/L) | $BOD_5$/COD | TOC (mg/L) | T-N (mg/L) |
|---|---|---|---|---|---|---|---|
| **Wastewater characteristics** | 7.4 | 2,350 | 11,500 | 1,840 | 0.160 | 2,100 | 810 |

## 3.4.2 Chemicals

All reagents used in this work were of analytical grade and employed without any further purification. Ferrous sulphate ($FeSO_4.7H_2O$) and xylenol orange were obtained from Acros Organics and Tryptone Soya Broth (TSB) (Oxoid Ltd, England). All other chemicals; hydrogen peroxide ($H_2O_2$ 30% w/v), sodium hydroxide (NaOH), ammonium ferrous

sulphate ($[NH_4]_2[Fe][SO_4]_2 \cdot 6H_2O$), sorbitol and sulphuric acid ($H_2SO_4$) were supplied by Fisher Scientific, UK.

### 3.4.3 Analytical techniques

Chemical oxygen demand (COD) was analysed using dichromate solution as the oxidant in a strongly acidic media, employing a Hach reactor HT200S and spectrophotometer (Hach DR 2800). All COD measurements were performed after the Fenton's coagulation stage (pH >7.5), to avert any interference of the peroxide ion with the COD measurements, as hydrogen peroxide is unstable in basic solutions and decomposes to give $O_2$ and $H_2O$. $BOD_5$ measurements were carried out in Oxitop (WTW), according to the supplier's operating instructions. Total organic carbon (TOC) was measured by direct injection of the sample into a Shimadzu TOC-$V_{CPH}$ analyser, provided with a NDIR detector and calibrated with standard solutions of potassium phthalate. pH measurements were made using a pH meter (Jenway 3345) and probe (model 924005, Keison Products, England). Other analyses were undertaken according to Standard Methods 2005.

Residual hydrogen peroxide concentration was assayed by the FOX method, using FOX reagent (xylenol orange, ammonium ferrous sulphate, sorbitol and sulphuric acid) (Wolff and Lester, 1994). Absorbance was read at 560nm and the hydrogen peroxide concentrations of the samples were compared against a linear standard curve (0-100mM). The toxicity of the Fenton treated effluent was assayed by *Acinetobacter baylyi* ADP1_recA_lux biosensor, as described by Song *et al.*, 2009. This chromosomally based bioreporter expresses bioluminescence when exposed to any DNA damaging toxicants and act as a proxy of the response of other bacterial species with in the treatment system. The

toxicity was quantified in terms of relative bioluminescence (absolute intensity of bioluminescence divided by $OD_{600}$).

### 3.4.4 Fenton's oxidation experiment

Fenton's oxidation experiments for the optimisation studies were carried out in 50 ml batch reactors, containing 25 ml of wastewater at room temperature. The pH of the wastewater was adjusted to $3.0 \pm 0.2$ with $H_2SO_4$ (2M). Pre-determined measures of $Fe^{2+}$ catalyst and $H_2O_2$ (30% w/v) were added in succession. The oxidation reaction proceeded under conditions of perfect mixing using a magnetic stirrer for 30 minutes. Thereafter, the reaction was stopped by spiking the sample with NaOH (2M), which adjusted the pH to $\sim 7.5 \pm 0.3$. The treated effluent was then allowed to stand overnight. The iron precipitate was separated from the reactor and COD, TOC, $BOD_5$ and toxicity analysis were carried out. For biological degradation experiments, the Fenton pre-treatment was performed in glass beakers with an operating capacity of 1.5L and the clear supernatant fed as influent to an attached biofilm reactor.

### 3.4.5 Biological treatment experiments

Aerobic biodegradation studies were carried out in 2 L batch reactors, with a working volume of 1.5 L, where air flow was maintained at 150 L $min^{-1}$, using aquarium air pumps and air spargers (Fisher Scientific, UK). Two replicate bioreactors were run. The reactor consisted of a glass column of inner diameter 8cm and height 55 cm. Plastic matrices with 1cm pore size served as the packing medium for microbial attachment. The bioreactors were fed with dilute (1:5 v/v MWF: tap water) operationally exhausted semi-synthetic

MWF (Hocut-795B) and a 5-membered bacterial consortia (grown in 1/10 w/v Tryptose Soya Broth, (TSB) and incubated at 28°C for 12h on an orbital shaker at 120rpm). When the COD stabilised, fresh medium and inocula were added to the reactor. By the end of 60 days, a thick and actively growing biofilm had developed on the matrix. The bacterial consortia consisted of *Agrobacterium radiobacter*, *Comamonas testosteroni*, *Methylobacterium mesophilicum, Microbacterium esteraromaticum* and *Microbacterium saperdae*, all of which was assembled specifically for the task and has previously been reported to be effective for bio-treatment of operationally exhausted MWF (van der Gast *et al*., 2003). During the acclimation phase, reactors were fed with increasing feed concentration of the Fenton treated effluent over a period of time to allow for the growth of microorganisms which can utilise the compound as a carbon or nutrient source. On alternate days 0.5 L supernatant was withdrawn and replaced by 0.5 L "fresh" waste metal working fluid. After acclimation, 1.5 L Fenton's treated effluents were fed to the reactors.

## 3.4.6 Optimisation of Fenton's reagents by central composite design

### 3.4.6.1 Statistically designed experiments

The present study adopted a response surface methodology, based on a five-level-two-variable central composite rotatable design (Montgomery, 2001), to optimise the concentrations of hydrogen peroxide and ferrous sulphate. A central composite design (CCD) consisting of 13 runs (4 factorial design, 4 axial points and 5 central points), with $\alpha$ value (distance from centre of the design space to an axial point) of $\pm 1.414$ was applied. Table 3.2 summarises the selected experimental ranges and values of the process independent variables (concentration of hydrogen peroxide and iron sulphate). The experimental sequence was randomised in order to minimise the effects of the uncontrolled

factors. Preliminary experiments were performed to determine the extreme values of the variables $H_2O_2$ [10-50mM] and $Fe^{2+}$ [2-10mM], the effect of temperature, pH and time of reaction.

**Table 3.2:** Levels of the parameters selected for the factorial design.

| **Independent variables** | ***Level*** | | | | |
|---|---|---|---|---|---|
| | **-α** | **-1** | **0** | **+1** | **+α** |
| $[H_2O_2]$ (mM) $X_1$ | 1.72 | 10 | 30 | 50 | 58.28 |
| $[Fe^{2+}]$ (mM) $X_2$ | 0.34 | 2 | 6 | 10 | 11.66 |

### 3.4.6.2 Selection of response variables

The exact composition and formulation of the MWF wastewater is unknown and hence conventionally employed gross parameters of pollution such as COD and TOC were selected as response variables. In addition, toxicity of the treated effluent was selected as the third response variable as it is imperative to conduct a detailed analysis of the toxicity contributed due to the oxidation by-products, recalcitrant components, biocides and of any residual hydrogen peroxide, as sometimes it may happen that the intermediates formed might be toxic towards the microorganisms. Three responses were therefore selected in the design: percentage removal of COD, percentage removal of TOC and toxicity to bacterial cells (measured in terms of relative bioluminescence) denoted by $Y_1$, $Y_2$ and $Y_3$. Thirteen experiments were carried out in random order.

## 3.5 Results

### 3.5.1 $Fe^{2+}$:$H_2O_2$ molar ratios

The dosage of $FeSO_4$ and $H_2O_2$ are important operating parameters affecting the performance of Fenton oxidation. Figure 3.2 depicts the influence of $FeSO_4$ dosage on the COD removal at pH 3. Experiments were carried out for a duration of 1 hour with initial COD concentration of 11,500 mg $L^{-1}$. The reaction was conducted at pH 3 with $Fe^{2+}$:$H_2O_2$ molar ratios varying from 1:20 to 1:1. Majority of ferrous ions exist in the hexa-aquo $(Fe(H_2O)_6^{2+})$ state, in acidic solution. As the pH increases, this ion undergoes extensive hydrolysis leading to precipitation of ferric oxyhydroxides $(Fe_2O_3.nH_2O)_{(s)}$, which subsequently form $(Fe(OH)_4)$ at higher pH. The formation of this ferric hydroxide complex at pH's greater than 3.0 leads to a drop in the Fe(II) concentration and loss in their catalytic activity, thereby impeding the Fenton reaction. Limited decrease in COD was observed at lower ratios (1:20, 1:15 and 1:10), due to the greater carbon content of MWF investigated in these studies.

From the data shown in Figure 3.2, it can be seen that maximum COD reduction occurred at $Fe^{2+}$:$H_2O_2$ molar ratio of 1:5 and 1:2 and there was no appreciable difference in COD reduction between these two ratios ($F=23.764$, $\alpha=0.05$ based on one-way ANOVA). Hence, in the present study, a $Fe^{2+}$:$H_2O_2$ molar ratio of 1:5 ($Fe^{2+}$ (10mM), and $H_2O_2$ (50 mM)) was chosen for all further experiments. However, when a higher molar ratio of 1:1 was used, a decrease in the COD removal was observed. This is because when the catalyst concentrations relative to the oxidant are set excessively high, ferrous iron concentrations will reach levels typically applied during coagulation, and hence the contribution of

coagulation takes prominence relative to Fenton's oxidation. Furthermore, scavenging of free radicals will occur in presence of excessive $Fe^{2+}$ (Eq. 8).

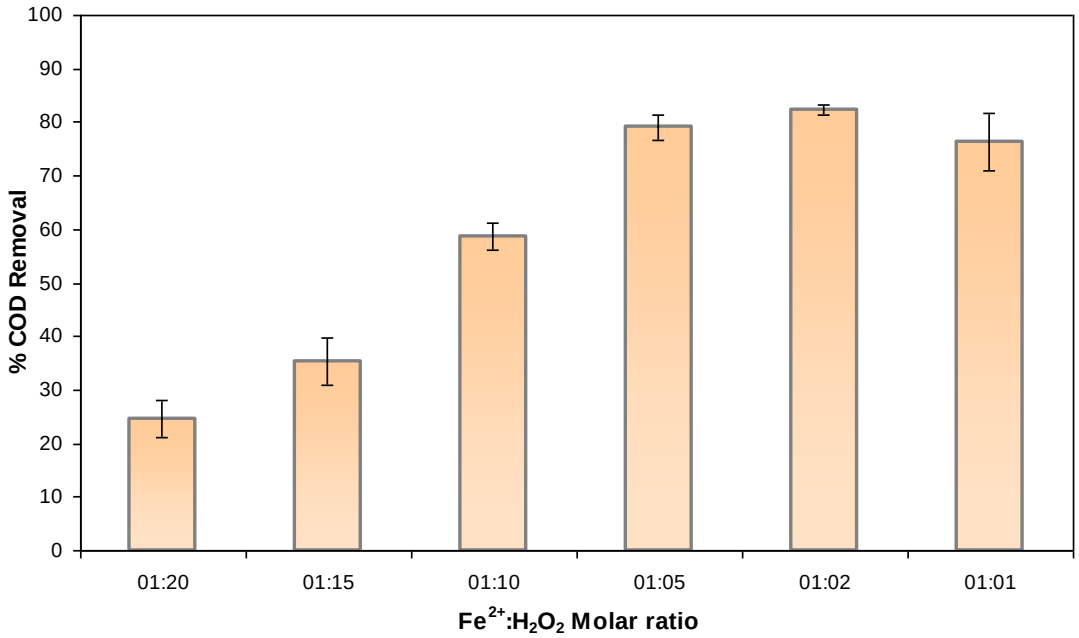



**Figure 3.2**: Effect of $Fe^{2+}$:$H_2O_2$ molar ratio on COD removal ($COD_0$=11,500 mg $L^{-1}$, pH=3.0, Reaction time=60 minutes, Temperature=$20^0$ C) (Mean ± SD of triplicates shown).

## 3.5.2 Effect of pH

Several previous investigations have indicated that the treatment efficiency of Fenton oxidation is highly pH dependent **(**Torrades *et al.,* 2011, Pignatello *et al.,* 2006, Elmolla and Chaudhuri, 2009, Tantak and Chaudhari, 2006**)**. Figure 3.3 illustrates the effect of pH on the COD reduction of recalcitrant MWF wastewater of COD 11,500 $mgL^{-1}$. All these experiments were run for 60 minutes, with pH varying from 2-7 under constant doses of $Fe^{2+}$ (10mM), and $H_2O_2$ (50 mM) at room temperature. It is apparent from the Figure that the extent of degradation decreases with increase in pH. Maximum COD reduction of 81% was seen at pH 3. The COD reduction at pH of 2, 4, 5, 6 and 7 were 47%, 73%, 65%, 42% and 29% respectively. It was also observed during the course of this study that at pH lower

than pH 3, the degradation efficiency decreased; which may be due to the scavenging of hydroxyl radical by $H^+$ ions (Neyens and Baeyens, 2003) or could be attributed to a greater stability of hydrogen peroxide at low pH, probably because it solvates a proton to form an oxonium ion ($H_3O^+$). An oxonium ion makes hydrogen peroxide electrophilic to enhance its stability and presumably to reduce substantially the reactivity with ferrous ion. Therefore, the quantity of hydroxyl radicals generated from the Fenton reaction would decrease at low pH (Chen *et al.*, 2007, Tekin *et al.*, 2006, Mandal *et al.*, 2010).

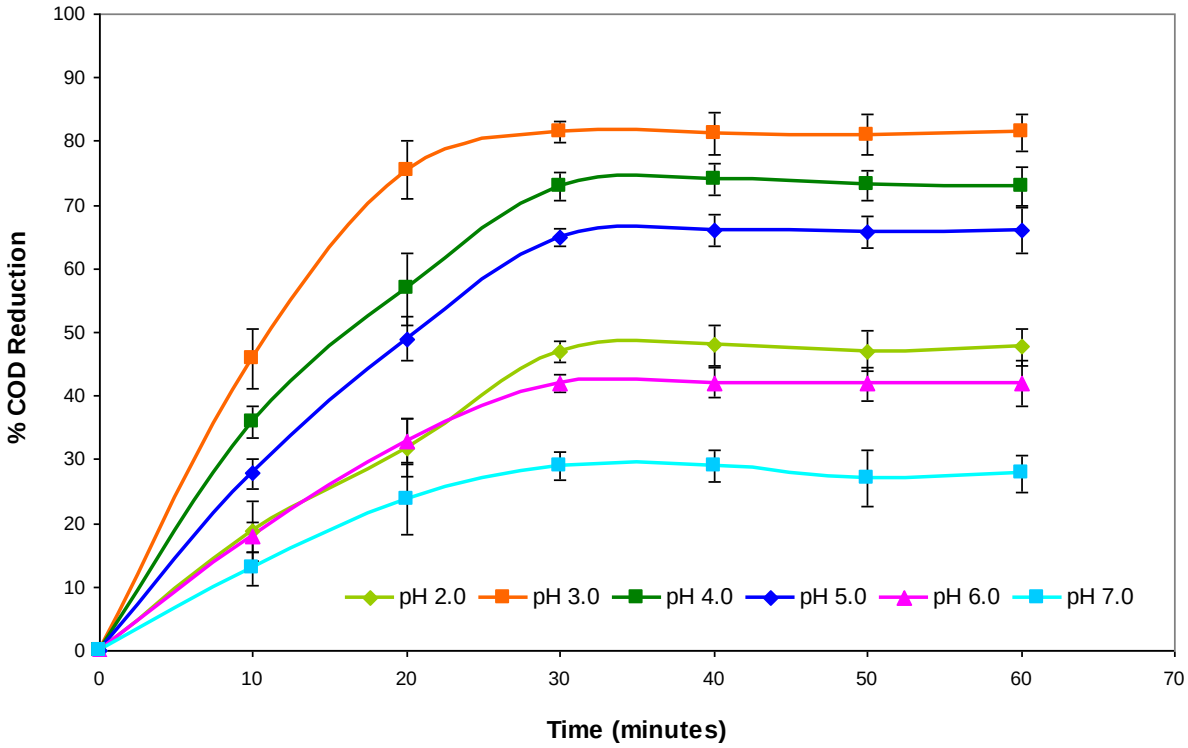



**Figure 3.3**: Effect of change in pH on COD removal ($COD_0$=11,500 mg $L^{-1}$, pH=3.0, Reaction time=60 minutes, $Fe^{2+}$ (10mM) and $H_2O_2$ (50 mM), Temperature=$20^0$ C) (Mean ± SD of triplicates shown).

The observed maximum degradation at pH 3 is in resonance with several previous studies (Lodha and Chaudhari 2007, Rodrigues *et al.*, 2009; Torrades *et al.*, 2011, Gogate and Pandit, 2004; Wang *et al.*, 2009; Wu *et al.*, 2010). Figure 3.3 also illustrates lack of a change in COD reduction profile after 30 minutes of treatment, and hence a reaction time of

30 minutes was fixed for all subsequent Fenton experiments. A pictorial representation of the Fenton treated effluent in lab-scale is illustrated in Figure 3.4.

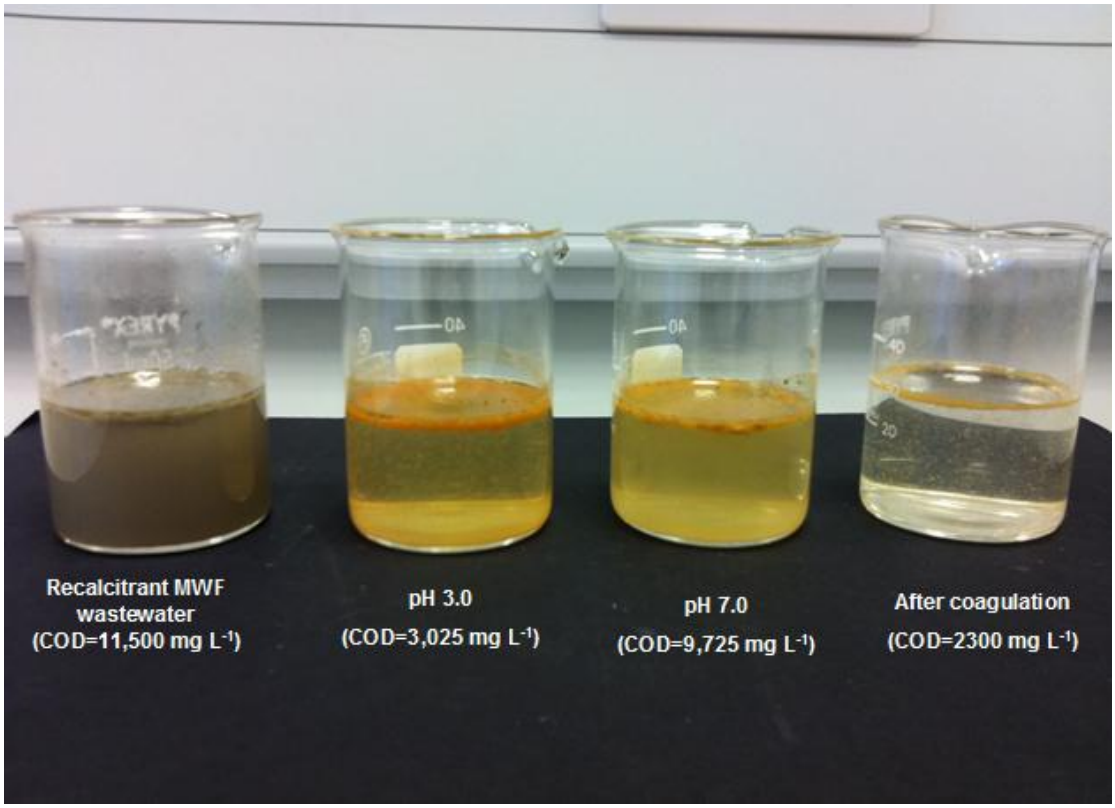



**Figure 3.4:** Pictorial representation of Fenton experiment.

### 3.5.3 Kinetic studies

Several authors (Tekin *et al*., 2006, Gogate and Pandit, 2004,) pointed out that the reactions of Fenton reaction with dissolved organic substances usually follow a first order kinetics with respect to the substrate. Kinetic studies for COD removal were performed under the following experimental conditions: wastewater COD=11,500 mg $L^{-1}$, Fenton's reagent [$Fe^{2+}$]=10mM, [$H_2O_2$]=50 mM with the pH varied from 2–7. The decrease in COD by Fenton's reaction was observed as a function of time.

The kinetic data of 30 minutes were fitted into the following equation (Eq. 3.11):

$$\ln\left[\frac{C_t}{C_0}\right] = -Kt \qquad (3.11)$$

where $C_0$ and $C_t$ are COD concentrations at time 0 and at time *t*. *K* is the first order rate constant in $min^{-1}$ and *t* is the time in minutes. The terms ln $C_t/C_0$ are plotted against time (Figure 3.5) and the pseudo-first order reaction rate constant, *K*, was calculated from the linear regression of the pseudo-first order kinetic model. The coefficient of correlation (*R*) was in the range of 91–99%. The rate constants at all pH are summarised in Table 2.3. Kinetic results from Table 3.3 and Figure 3.5 indicate that maximum COD reduction was observed at pH 3.0 and the rate of reduction decreased with increasing pH.

**Table 3.3**: Pseudo-first order rate constant (*K*) for MWF wastewater at varying pH

| pH | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| | 0.103 | 0.2913 | 0.2176 | 0.1745 | 0.0945 | 0.0582 |

In the later stages of the reaction, ferric ions produced and represented in Eq. 3.12, reacted with hydrogen peroxide to produce hydroperoxyl radicals ($HO_2^{\bullet}$) and ferrous ions (Eq. 3.13, 3.14). The oxidation capability of hydroxyl radical generated during earlier stages of the reaction was much greater than the hydroperoxyl radicals, hence leading to decreased rate of reaction (Tantak and Chaudhari 2006).

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+} \qquad (3.12)$$

$$H_2O_2 + Fe^{3+} \rightarrow H^{+} + FeOOH^{2+} \qquad (3.13)$$

$$FeOOH^{2+} \rightarrow HO_2^{\bullet} + Fe^{2+} \qquad (3.14)$$

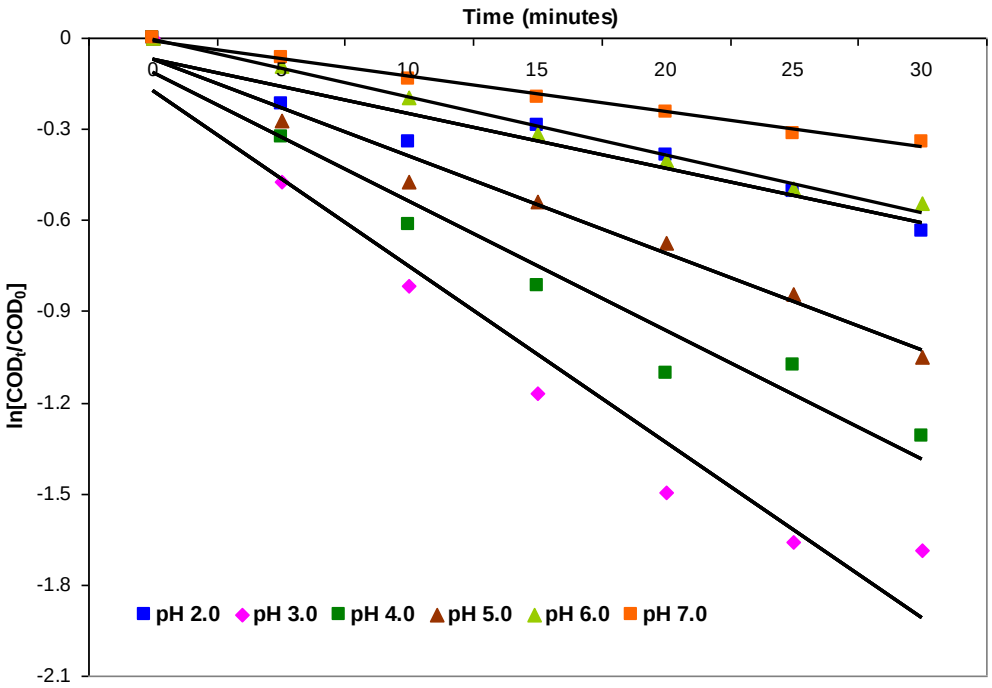



**Figure 3.5:** Pseudo first order plots (pH varying from 2-7.0, COD=mg $L^{-1}$, $[Fe^{2+}]$ = 10mM, $[H_2O_2]$=50 mM, Temperature=$20^0$ C).

## 3.5.4 Effect of temperature

Traditionally, Fenton oxidation was usually carried under room temperature. However, some studies have indicated that an increase in operating temperature has a favourable effect on the Fenton oxidation (Gogate and Pandit 2004, Lin and Kiang, 2005). Figure 3.6 (a) shows the temperature effect on COD removal of MWF wastewater. These experiments were run for 30 minutes with temperature varying from 20-$50^0$C, under constant doses of $Fe^{2+}$ (10mM) and $H_2O_2$ (50 mM). The activation energy was calculated to be 5.887 kJ $mol^{-1}$, assuming the COD reduction followed a pseudo-first order reaction (Figure 3.6 b). Statistical analysis revealed no significant effect (based on one-way ANOVA) on COD removal at various temperature profiles, ($F= 1.976$, $P<0.001$). The probable explanation for this may be at higher temperatures, though coagulation occurred more efficiently: decomposition of hydrogen peroxide into oxygen and water compromised the degradation.

Thus, all further experiments were carried out at room temperature ( $20 \pm 0.5^{0}$ C), for practical and economic reasons.

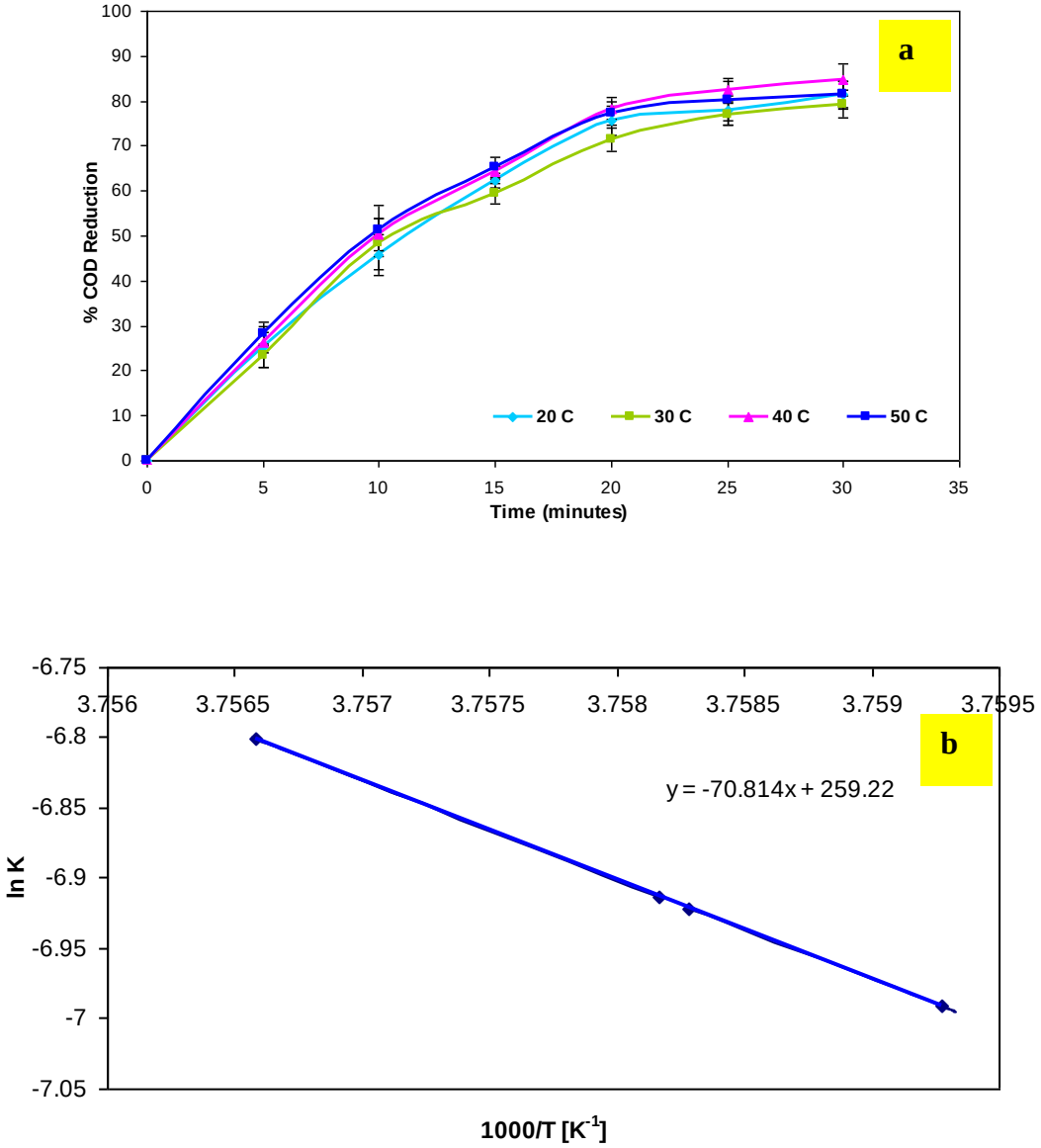



**Figure 3.6** (a) Effect of temperature on COD removal (COD=11,500 mg $L^{-1}$, $[Fe^{2+}]$= 10mM and $[H_2O_2]$=50 mM, pH=3.0, Time of reaction=30 minutes) (Mean ± SD of triplicates shown) (b) Arrhenius plot of Fenton oxidation conducted at temperatures between 293 K and 323 K.

## 3.5.5 Mathematical model

### 3.5.5.1 Performing the statistically designed experiments

A central composite design (CCD) consisting of 13 runs (4 factorial design, 4 axial points and 5 central points) was conducted. The responses obtained from the 13 designed experiments were used to develop an empirical model that correlated the responses to the process variables (concentration of Fenton reagents) using a second-degree polynomial equation. The statistical software package Design-Expert 7.1.3, Stat-Ease, Inc., was used for regression analysis of experimental data to fit the equations developed and also to plot the response surface. ANOVA was used to estimate the statistical parameters. The complete design matrix of the experiments carried out using the Design-Expert 7.1.3, together with the results obtained (percentage COD, TOC abatements and toxicity), are shown in Table 3.4.

**Table 3.4:** Central Composite Design layout and experimental results

| | | **Factors** | | **Response variables** | | |
|---|---|---|---|---|---|---|
| **Run** | **Type** | **[$H_2O_2$]** $X_1$ | **[$FeSO_4$]** $X_2$ | **%COD Reduction** $Y_1$ | **%TOC Reduction** $Y_2$ | **Toxicity (Relative Bioluminescence)** $Y_3$ |
| 1 | Centre | 30 | 6 | 76.81 | 69.21 | 2798 |
| 2 | Axial | 1.72 | 6 | 40.63 | 32.45 | 804 |
| 3 | Factorial | 10 | 2 | 29.43 | 23.04 | 891 |
| 4 | Factorial | 50 | 2 | 38.98 | 26.63 | 5015 |
| 5 | Centre | 30 | 6 | 79.24 | 67.34 | 2835 |
| 6 | Centre | 30 | 6 | 77.31 | 70.39 | 2883 |
| 7 | Centre | 30 | 6 | 78.43 | 69.04 | 2924 |
| 8 | Centre | 30 | 6 | 79.36 | 71.34 | 2792 |
| 9 | Axial | 30 | 11.66 | 92.46 | 80.34 | 730 |
| 10 | Factorial | 50 | 10 | 85.65 | 76.39 | 5345 |
| 11 | Axial | 30 | 0.34 | 9.03 | 3.32 | 729 |
| 12 | Axial | 58.28 | 6 | 52.5 | 45.63 | 4391 |
| 13 | Factorial | 10 | 10 | 68.03 | 55.38 | 819 |

### 3.5.5.2 Estimating the coefficients and ANOVA

Relationship between the responses ($Y_1, Y_2, Y_3$) and the independent process variables ($X_1$ and $X_2$) were calculated by a second-order polynomial equation (i.e. quadratic) using the least square method and expressed by semi-empirical quadratic polynomial equations (3.15-3.17):

$$Y_{1(\%CODremoval)} = 78.23 + 5.49X_1 + 25.41X_2 + 2.02X_1X_2 - 14.12X_1^2 - 12.03X_2^2 \qquad (3.15)$$

$$Y_{2(\%TOCremoval)} = 69.46 + 5.40X_1 + 23.88X_2 + 4.35X_1X_2 - 13.98X_1^2 - 12.59X_2^2 \qquad (3.16)$$

$$Y_{3(Toxicity)} = 2846.40 + 1715.35X_1 + 32.43X_2 + 100.50X_1X_2 + 214.05X_1^2 - 719.95X_2^2 \qquad (3.17)$$

The quality of the model developed was evaluated, based on the correlation coefficient value from the ANOVA for response surface quadratic model for COD reduction, TOC reduction and toxicity (Table 3.5-3.7). The model F-values of 42.78, 64.93 and 7.38 (from F distribution graph) at 5% significance level implied that all three models were significant at 5%. The $R^2$ value for equation 15, 16 and 17 were 0.9683, 0.9789 and 0.8405 respectively. This indicated that 96.83%, 97.89% and 84.05% of the total variation in removal of COD, TOC and toxicity was attributed to the experimental variables studied. The standard deviations for the three models were 5.94, 4.66 and 10.86 respectively. This indicated that the predicted value for $Y_2$ would be more accurate and closer to its actual value, compared to $Y_1$ and $Y_3$. The closer the $R^2$ value to unity and the smaller the standard deviation, the better the model is as it provides a predicted value which is closer to the actual value for the response.

**Table 3.5: ANOVA for Response Surface Quadratic Model for COD Reduction**

| Source | Sum of squares | Degrees of freedom | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Model | 7542.16 | 5 | 1508.43 | 42.78 | <0.0001 |
| $X_1$ | 241.52 | 1 | 241.52 | 6.85 | 0.0345 |
| $X_2$ | 5164.22 | 1 | 5164.22 | 146.47 | <0.0001 |
| $X_1X_2$ | 16.28 | 1 | 16.28 | 0.46 | 0.5186 |
| $X_1^2$ | 1386.09 | 1 | 1386.09 | 39.31 | 0.0004 |
| $X_2^2$ | 1006.02 | 1 | 1006.02 | 28.53 | 0.0011 |
| Residual | 246.81 | 7 | 35.26 | | |
| Lack of fit | 241.61 | 3 | 80.54 | 61.95 | 0.0008 |
| Pure error | 5.20 | 4 | 1.30 | | |
| Cor Total | 7788.97 | 12 | | | |
| Std dev = 5.94 | | $R^2$= 0.9683 | | C.V% = 9.56 | |

**Table 3.6: ANOVA for Response Surface Quadratic Model for TOC Reduction**

| Source | Sum of squares | Degrees of freedom | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Model | 7050.19 | 5 | 1410.04 | 64.93 | <0.0001 |
| $X_1$ | 233.71 | 1 | 233.71 | 10.76 | 0.0135 |
| $X_2$ | 4561.21 | 1 | 4561.21 | 210.03 | <0.0001 |
| $X_1X_2$ | 75.86 | 1 | 75.86 | 3.49 | 0.1038 |
| $X_1^2$ | 1359.73 | 1 | 1359.73 | 62.61 | <0.0001 |
| $X_2^2$ | 1101.92 | 1 | 1101.92 | 50.74 | 0.0002 |
| Residual | 152.02 | 7 | 21.72 | | |
| Lack of fit | 142.88 | 3 | 47.63 | 20.86 | 0.0066 |
| Pure error | 9.13 | 4 | 2.28 | | |
| Cor Total | 7202.21 | 12 | | | |
| Std dev = 4.66 | | $R^2$= 0.9789 | | C.V% = 8.77 | |

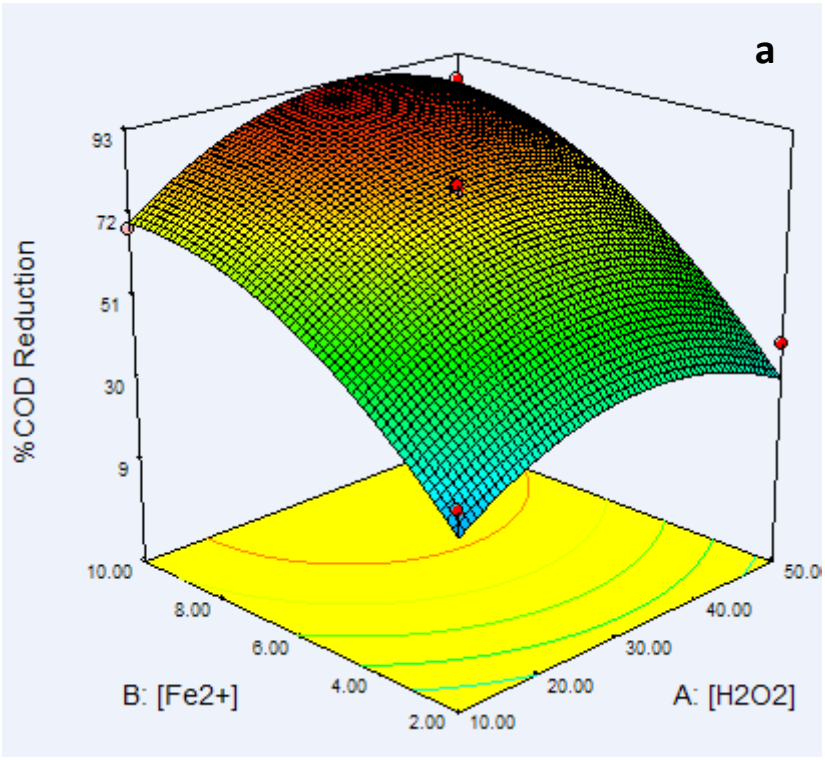
**Table 3.7: ANOVA for Response Surface Quadratic Model for Toxicity**

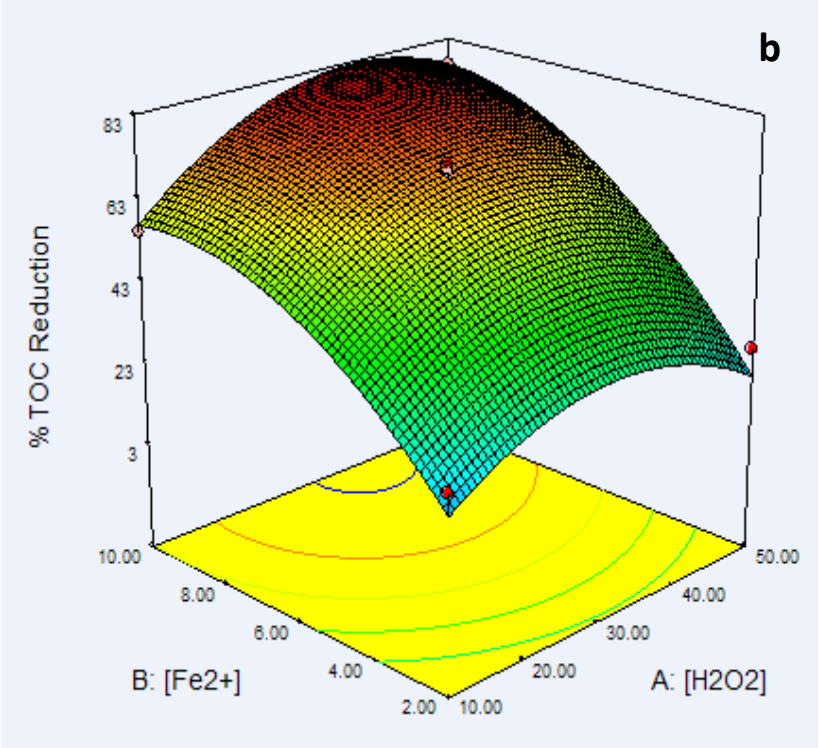
| Source | Sum of squares | Degrees of freedom | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Model | 4354.04 | 5 | 870.81 | 7.38 | 0.0103 |
| $X_1$ | 3678.07 | 1 | 3678.07 | 31.16 | 0.0008 |
| $X_2$ | 1.32 | 1 | 1.32 | 0.011 | 0.9189 |
| $X_1X_2$ | 6.31 | 1 | 6.31 | 0.053 | 0.8237 |
| $X_1^2$ | 49.84 | 1 | 49.84 | 0.42 | 0.5367 |
| $X_2^2$ | 563.42 | 1 | 563.42 | 4.77 | 0.0652 |
| Residual | 826.19 | 7 | 118.03 | | |
| Lack of fit | 824.18 | 3 | 274.73 | 547.65 | <0.0001 |
| Pure error | 2.01 | 4 | 0.50 | | |
| Cor Total | 5180.23 | 12 | | | |
| Std dev = 10.86 | | $R^2$= 0.8405 | | C.V% = 15.90 | |

Hydrogen peroxide concentration ( $X_1$ ) and ferrous ion concentration ( $X_2$ ) were significant model terms for COD and TOC reduction, whereas [$H_2O_2$] emerged as the significant term for toxicity. From the statistical results obtained, it was demonstrated that the above models were adequate to predict the variation in COD, TOC and toxicity within the range of variables studied.

### 3.5.5.3 Response surface contours

Response surfaces can be visualised as three-dimensional plots that display the response as a function of two factors. Figures 3.7 (a, b, c) illustrates the three-dimensional response surface plots corresponding to equations 3.15-3.17 which were generated using the same software.


a
%COD Reduction
93
72
51
30
9
10.00
8.00
6.00
4.00
2.00
B: [Fe2+]
10.00
20.00
30.00
40.00
50.0
A: [H2O2]


b
% TOC Reduction
83
63
43
23
3
10.00
8.00
6.00
4.00
2.00
B: [Fe2+]
10.00
20.00
30.00
40.00
50.00
A: [H2O2]

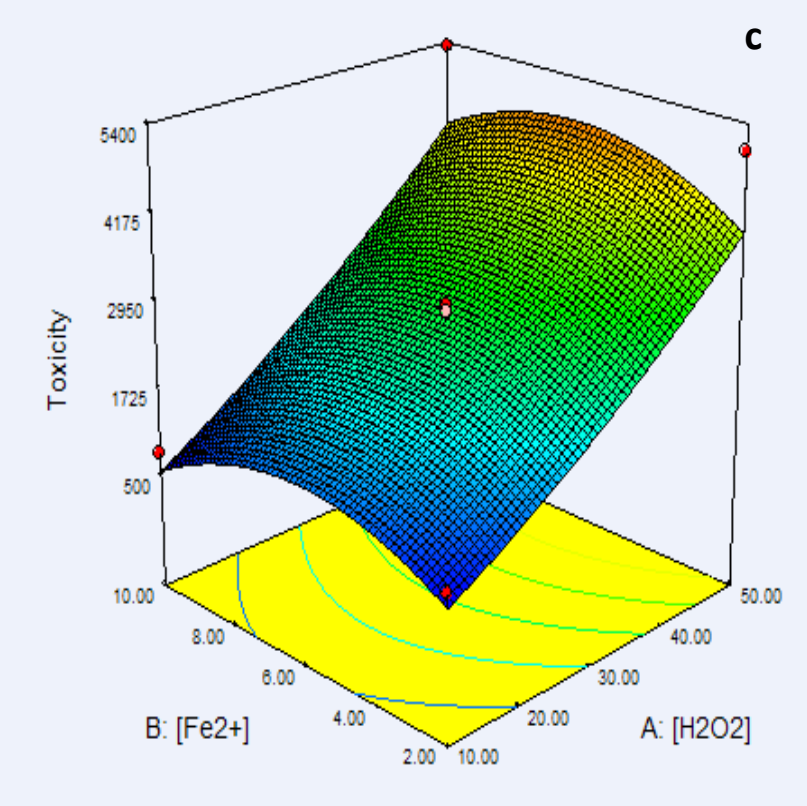



**Figure 3.7 (a,b,c)** Response surface plot of (a) %COD removal, (b) %TOC removal and (c) toxicity.

Fig 3.7(a) and (b) represents the response surface plots for an estimate of percent COD and TOC removal as a function of the two process variables: $H_2O_2$ concentration and initial $Fe^{2+}$ concentration. As can be understood from the plot, mono-tonic increases in the responses were observed with increased dosage of $H_2O_2$ up to 30mM, beyond which the COD and TOC removal gradually declined. This reduction in treatment efficiency at higher doses of H2O2 may be due to the auto-scavenging effect of peroxide on hydroxyl radicals. In contrast, as is evident from the same figures, an increase in $Fe^{2+}$ concentration markedly improved the COD and TOC removal efficiencies. Concentrations greater than 8.0mM however, did not enhance COD and TOC reduction significantly, rather slightly inhibiting COD reduction at higher $FeSO_4$concentrations. $Fe^{2+}$ affected COD and TOC removal to a greater extent than $H_2O_2$ concentration. This was evidenced by response surface plots and

the ANOVA Tables (Tables 3.5-3.7); thus indicating that Fe(II) concentration was the most important parameter for removal of COD and TOC in remediation of MWF wastewater. Hydrogen peroxide was however the most crucial variable affecting toxicity. The dose response plot (Fig 3.7c) illustrates clearly that toxicity increased steadily with rising hydrogen peroxide dose. This could be due to inhibitory action of excess un-reacted hydrogen peroxide to microorganisms. This plot illustrates that the residual toxicity in the Fenton treated effluent is owing to the hydrogen peroxide dose rather than the presence of biocides (due to the monotonic increment in toxicity with increase in concentration of hydrogen peroxide).

### 3.5.5.4 Process optimisation and validation experiments

After analysing the response surface plots and ANOVA tables, an optimal design point was achieved by adjusting the settings for input parameters in the model. The desired goals for each process (independent) variable and responses (dependent) were defined. In the present study, desired goals for the process variables (initial $Fe^{2+}$ and $H_2O_2$ concentrations) were selected to be "minimised" and responses (COD and TOC removals and toxicity) were chosen as to "maximise" and "minimise" respectively (Table 3.8). These individual goals were then combined into an overall desirability function, by the software, in order to identify the best local maximum, referred to as the point prediction. The $[H_2O_2]$ and $[Fe^{2+}]$ were set to a target of minimum since the lower $[H_2O_2]$ would minimise toxicity towards the microbial biomass and lower $[Fe^{2+}]$ would reduce the amount of sludge produced, hence potentially making saving on the disposal costs. In addition, minimising the concentration of reagents used would result in a lower cost of reagent.

**Table 3.8: Process optimisation parameters fed in Design-Expert software**

| Parameter | Goal | Lower limit | Upper limit |
|---|---|---|---|
| $[H_2O_2]$ | Minimise | 10 | 50 |
| $[Fe^{2+}]$ | Minimise | 2 | 10 |
| % COD Reduction | Maximise | | |
| % TOC Reduction | Maximise | | |
| Toxicity | Minimise | | |

In light of the above optimisation settings, the optimal conditions for removal of COD, TOC and toxicity were obtained as $[H_2O_2]$ = 14.91mM and $[Fe^{2+}]$ = 5.62mM, by Design-Expert software (Table 3.9). Under these conditions, additional experiments were performed to validate the predicted responses. Figure 3.8 illustrates the experimental responses obtained at the predicted point. The observed COD and TOC reduction values were estimated to be 64.73% and 55.93% respectively, with a concurrent 91% decrease in the toxicity, which was in good agreement with the predicted results.

**Table 3.9: Design-Expert optimum point prediction**

| $[H_2O_2]$ | $[Fe^{2+}]$ | %COD Redn | %TOC Redn | %Toxicity Redn |
|---|---|---|---|---|
| 14.91 | 5.62 | 63.6869 | 55.3718 | 88.74 |

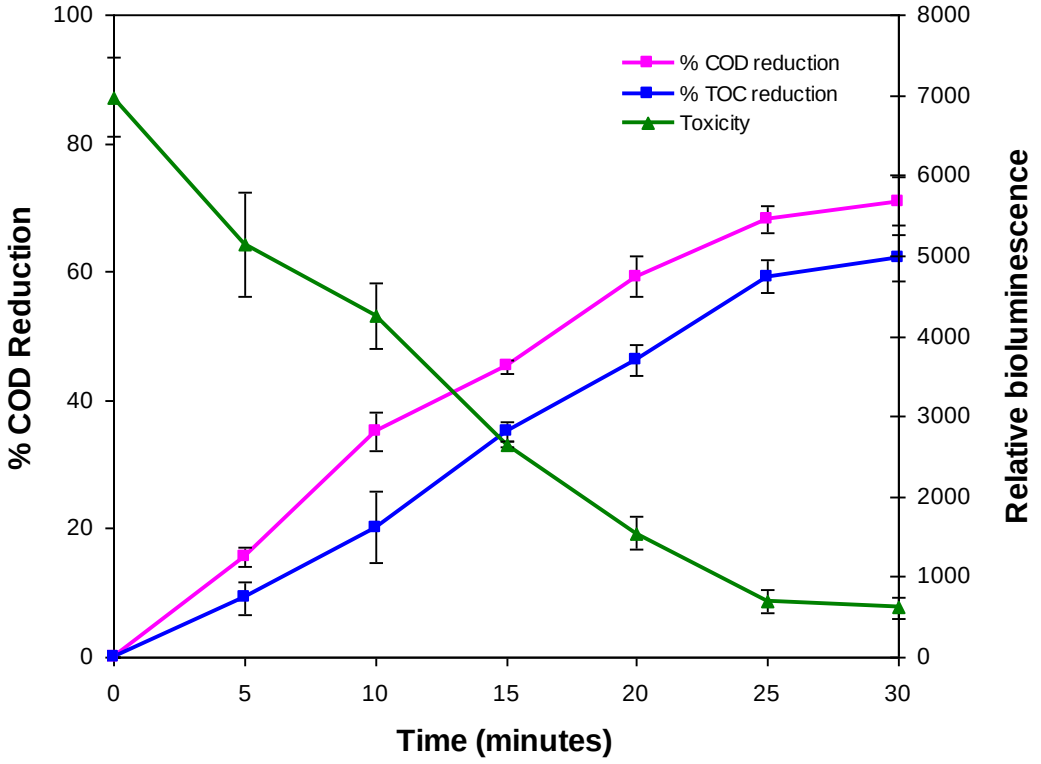



**Figure 3.8:** Degradation pattern and toxicity assessment during Fenton's oxidation process at optimum conditions (pH 3, $[H_2O_2]$ =14.9mM, $[Fe^{2+}]$ =5.6mM, Temperature=$20^0$C, Reaction time=30 min) (Mean ± SD of triplicates shown).

## 3.5.6 Biodegradation studies

To confirm that the Fenton solution treated (under optimised conditions) effluent was suitable for a final bio-treatment step, residual hydrogen peroxide concentration and $BOD_5$/COD analyses were performed. There was a positive correlation between hydrogen peroxide concentration and toxicity and it was found that the toxicity value could be decreased as long as $H_2O_2$ dosage was kept at lower levels. Toxicity assay was done by employing a bioluminescence sensor (Song *et al.* 2009). Figure 3.9 represents the bioluminescence response for different concentrations of hydrogen peroxide using bioreporter *Acinetobacter baylyi* ADP1_recA_lux, which displays luminescence only in the

presence of any DNA damaging toxicants. The figure clearly reflects the toxic effect of hydrogen peroxide on the biosensor at all concentrations investigated (50-250µM).

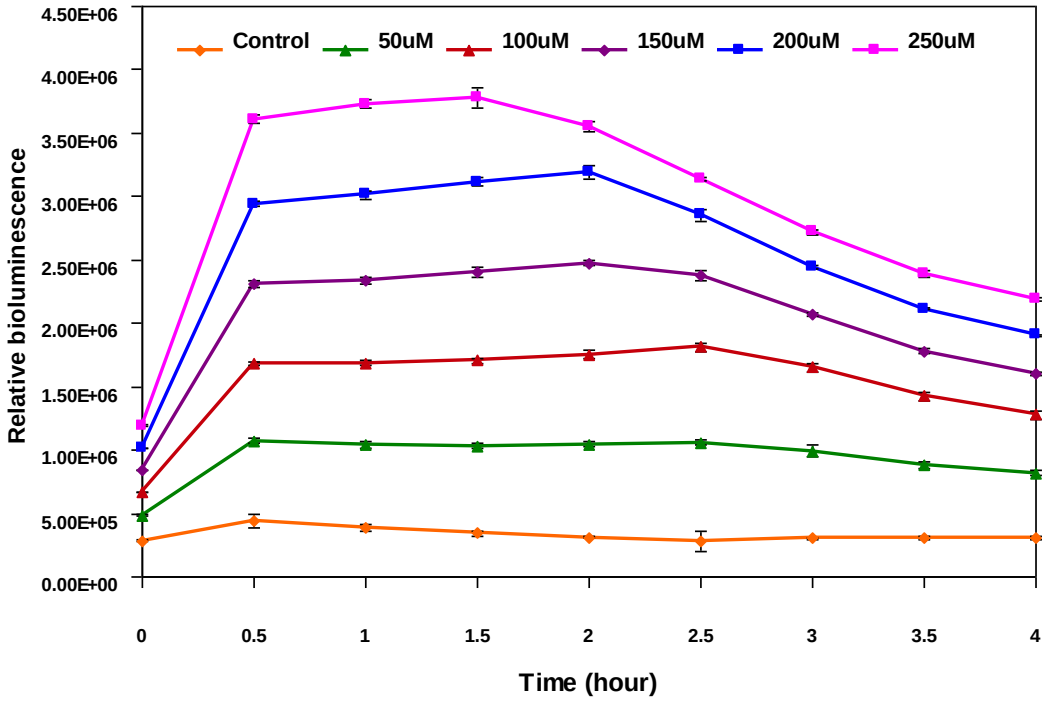



**Figure 3.9:** Quantitative response of the ADP1_recA_lux to different concentrations of hydrogen peroxide (Mean ± SD of 8 replicates shown).

The reduction in toxicity for the Fenton treated effluent was confirmed by employing a luminescence sensor, whose expression decreased by 91% from that of the untreated MWF wastewater control, indicating reduced toxicity (Figure 3.10). This reduction in toxicity is indicative of absence of toxic oxidation by-products, recalcitrant compounds, biocides and residual hydrogen peroxide in the effluent. This might have been due to the fact that at greater concentrations of hydrogen peroxide, the reagent might not have been completely utilised leading to residual $H_2O_2$ in the treated effluent. Exposure to hydrogen peroxide, almost inevitably would suppress exposed biological process rates. Hydrogen peroxide exposure would also alter DNA integrity to liberate all four bases, forming single strand

breaks, and apurinic-apyrimidinic sites. It also breaks the sugar-phosphate backbone sufficiently to inactivate transforming DNA (Ananthaswamy and Eisenstark 1977, Fiorenza and Ward, 1997, Watts *et al*., 2003). It was observed that maximum utilisation of $H_2O_2$ occurred at pH 3 with no residual $H_2O_2$ detected in the aqueous sample. The main reason for this was that at a low pH, more $Fe(OH)^+$ was formed, which had a much greater activity as compared to $Fe^{2+}$ in Fenton's oxidation (Tantak and Chaudhari, 2006, Pignatello *et al.,* 2006).

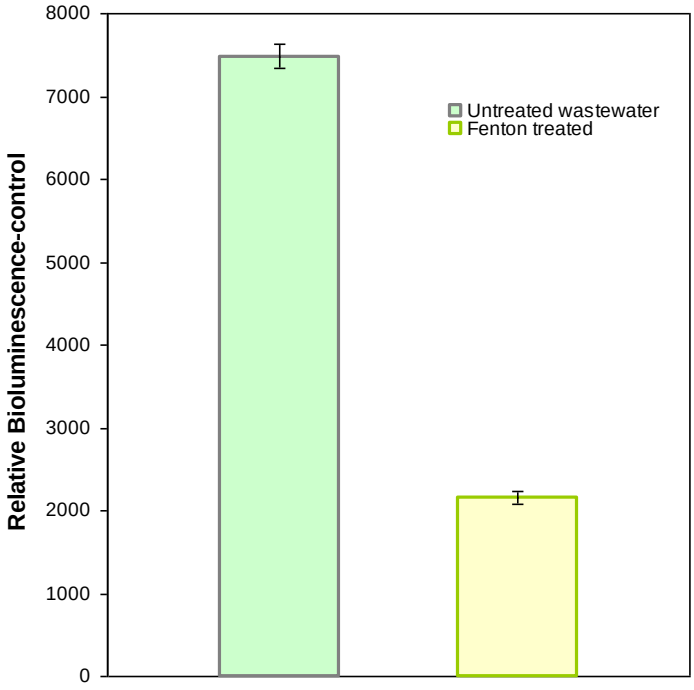



**Figure 3.10:** Toxicity analysis based on relative bioluminescence of bacterial biosensors of the untreated and Fenton treated effluent (Mean ± SD of triplicates shown).

The biodegradability index ($BOD_5$/COD) after the chemical treatment (which provides information regarding the ratio of readily biodegradable and recalcitrant substances) of the wastewater increased from 0.160 to 0.538 (Figure 3.11). The greater biodegradability of the Fenton treated wastewater may have been due to production of smaller, more oxidised and biodegradable by-products such as carboxylic acids, formic acid or formaldehyde. Similar

findings in increase of biodegradability from ~0 to 0.37 in 10 minutes (Elmolla 2009), 0.3 to 0.9 and 0.3 to 0.7, have been reported by other researchers (Wang *et al.*, 2008).

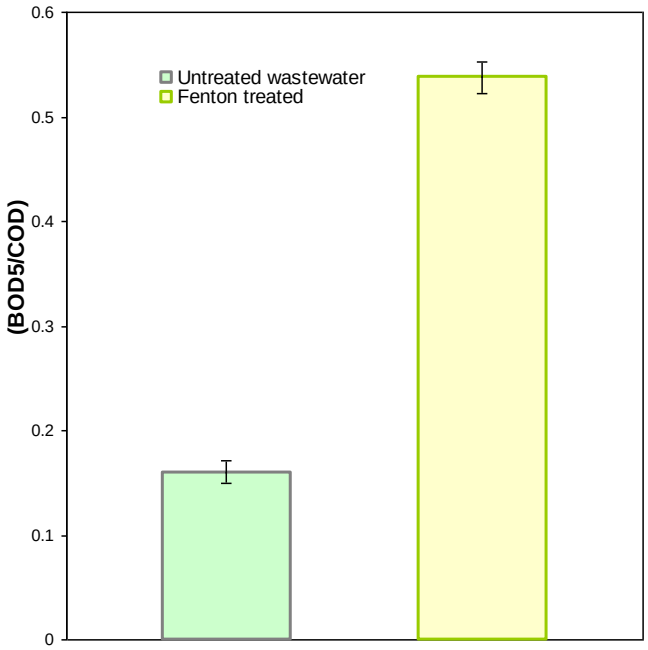



**Figure 3.11:** Biodegradability index ($BOD_5$/COD) of the untreated and Fenton treated effluent (Mean ± SD of triplicates shown).

After the MWF effluent was pre-treated with optimised doses of Fenton's reagents, it was subjected to a biological treatment step. This was achieved by introducing the Fenton treated effluent into a fixed film batch bioreactor. The rate of degradation and mineralisation of the effluent were monitored in terms of COD and TOC abatement by regular sampling (Figure 3.12). Fresh influent was introduced into the reactor when the COD concentration stabilised (Fill and draw cycles). It was observed that the percentage removal of the recalcitrant MWF constituents improved with time. Steady state conditions were achieved within 20 days, which was the time required to acclimatise the biomass to the substrate. At steady state conditions, COD removal of 92% and TOC removal of 86% were achieved after the biological treatment, as shown in Figure 3.12.

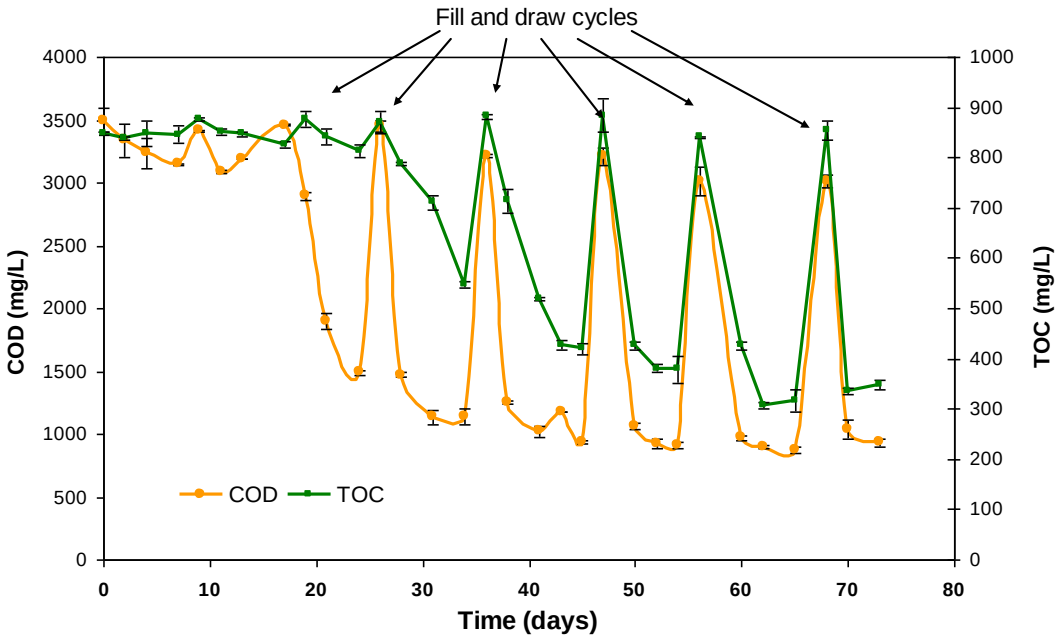



**Figure 3.12**: COD and TOC profile in the fixed bioreactor charged with Fenton treated effluent (Mean ± SD of duplicates shown).

In the light of observations and evaluations presented above, it can be concluded that Fenton's pre-treatment was successful at increasing both the extent and rate of biodegradation of the recalcitrant wastewater and was clearly capable of stimulating degradation of highly recalcitrant wastewater, generating an overall decrease of 92% and 86% in the COD and TOC, respectively (Table 3.10). The 92% reduction in recalcitrant COD levels achieved by employing Fenton's reagents with a subsequent biological step is a favourable alternative to energy demanding physical and chemical treatment options such as incineration, membrane separation or disposal into landfills for toxic wastewaters. This 2-stage treated non-toxic effluent could now be discharged into the local sewerage to be treated along with conventional municipal wastewater, where limits typically are set in the UK at 2000mg/L.

**Table 3.10:** Stepwise COD and TOC profiles in the hybrid treatment train

| | **Initial Wastewater** | **Fenton treated effluent** | | **Biologically treated final effluent** | |
|---|---|---|---|---|---|
| | Pollutant (mg/L) | Pollutant (mg/L) | %Reduction | Pollutant (mg/L) | %Reduction |
| **COD** | 11,500 | 4025 | 65 | 900 | 92 |
| **TOC** | 2100 | 945 | 55 | 300 | 86 |

## 3.6 Discussion

Fenton's reaction has proven to be effective in terms of pollutant removal rates, as well as operating expenses, for the treatment of toxic and/or refractory industrial wastewater. Fenton treatment has been shown to be an effective pre-treatment step for enhancing bioremediation by reducing overall toxicity with concurrent increase in bioavailability (Lu *et al.,* 2010, Goi *et al.,* 2010). In these integrated systems, Fenton oxidation is employed as a pre-treatment to enhance the biodegradability of wastewater containing recalcitrant or inhibitory pollutants (Guomin *et al.,* 2009, Wang *et al.,* 2008, Tekin *et al.,* 2006, Elmolla and Chaudhuri, 2009, Chen *et al.,* 2007). Fenton-biodegradation hybrid treatment strategy rests on the expectation that the parent molecule is usually more reactive chemically than biologically, while the opposite is true for the Fenton oxidation products due to introduction of functional groups such as -OH and -$CO_2H$ as depicted in Figure 3.13 (Pignatello *et al.,* 2006). The purpose of this treatment strategy is for the effluent to go from chemical oxidation to biological treatment, as soon as it becomes sufficiently biodegradable.

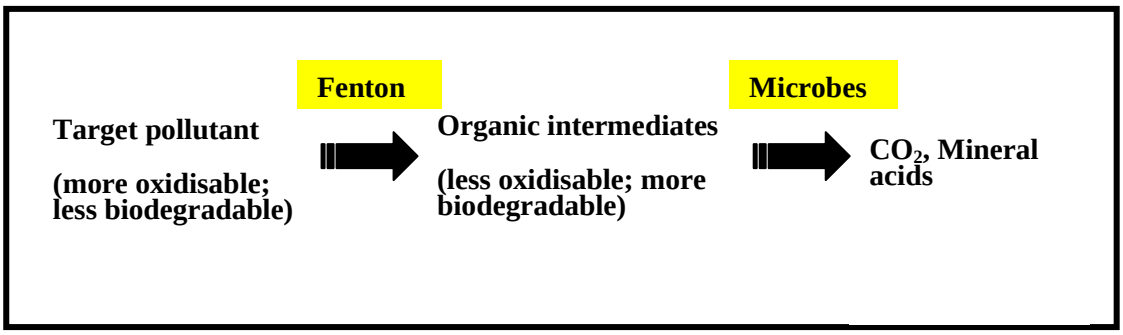



**Figure 3.13**: Rationale behind sequential Fenton-biological treatment plan

The feasibility of exploring the potential of Fenton-biological oxidation hybrid treatment has been investigated by several researchers for treatment of a vast array of industrial wastes; surfactant rich wastewater (Wang *et al.*, 2008), textile industry (Wei *et al.*, 2007, Rodrigues *et al.*, 2009, Tantak and Chaudhari 2006, Lodha and Chaudhari 2007), polycyclic aromatic hydrocarbons (PAHs) in aged soil samples (Valderrama *et al.*, 2009), petroleum-contaminated (Lu *et al.*, 2010), pharmaceutical wastewater (Badawy *et al.*, 2009, Tekin *et al.*, 2006, Elmolla and Chaudhuri, 2009), semiconductor wastewater (Lin and Jiang 2003), mixed industrial waste (Mandal *et al.*, 2010, Guomin *et al*, 2009, Lim *et al.*, 2003), olive processing wastewater (Kotsou *et al.*, 2004, Bressan *et al.*, 2004), pesticide wastewater (Chen *et al.*, 2007), mature landfill leachate (Wang *et al.*, 2009).

Hydrogen peroxide addition in excess, though it sometimes results in increased COD removal, should be avoided since this typically has toxic effects on the biological activity. There is always some residual concentration of hydrogen peroxide if an excess of $H_2O_2$ is used, and also it may act as a scavenger for the hydroxyl free radicals resulting in lower degradation rates. Hence, it is necessary to perform an optimisation exercise on the effluent in question for deciding the oxidant dose. Since $H_2O_2$ is typically toxic to microorganisms,

it is essential to be aware that excess $H_2O_2$ can deteriorate overall degradation efficiency for cases that Fenton process is followed by biological oxidation. Furthermore, excess amounts of ferrous would lead to greater production of sludge, which needs additional treatment and disposal. Consequently, the Fenton reagents were considered as independent variables in the factorial design in this study.

The effect of initial pH was investigated, with pH ranging from 2-7 and maximum degradation was observed at a pH around 3.0 (Figure 3.3). The lower degradation at pH's greater than 4 may be attributed to the shift in equilibrium of $Fe^{2+}$and $Fe^{3+}$ forms at higher pH. Majority of ferrous ions exist in the hexa-aquo $(Fe(H_2O)_6^{2+})$ state, in acidic solution. As the pH increases, this ion undergoes extensive hydrolysis leading to precipitation of ferric oxyhydroxides $(Fe_2O_3.nH_2O)_{(s)}$, which subsequently form $(Fe(OH)_4)$ at higher pH. The formation of this ferric hydroxide complex at pH's greater than 3.0 leads to a drop in the Fe(II) concentration and loss in their catalytic activity, thereby impeding the Fenton reaction (Pignatello *et al.,* 2006, Torrades *et al.,* 2011 Mandal *et al.,* 2010, Chen *et al.,* 2007, Tekin *et al.,* 2006).

In addition, hydrogen peroxide undergoes dissociation into $O_2$ and $H_2O$ (Eq. 3.9) at higher pH, hence lowering the amount of hydroxyl free radical generation (Elmolla and Chaudhuri, 2009). Hence, the decrease in dissolved iron and auto-decomposition of hydrogen peroxide at higher pH, leads to decrease in the generation and oxidation rate of hydroxyl radical, hence slowing down the rate and extent of COD reduction.

The work in this chapter shows that the reduction in treatment efficiency at doses greater than a critical $H_2O_2$ concentration (Figure 3.7 a, b) was probably due to the auto-scavenging effect of peroxide on hydroxyl radicals leading to formation of oxygen and water which can be expressed by Eq.3.18 and 3.19 (Wang *et al.*, 2008, Torrades *et al.*, 2011, Tantak and Chaudhari, 2006). In addition, excess $H_2O_2$ reacts with ferric ions to form hydroperoxyl radical, as shown in Eq. 3.20 (Elmolla and Chaudhuri, 2009). The oxidation potential of hydroperoxyl radical was much less than the hydroxyl radicals and hence the extent of COD reduction was compromised in the presence of greater concentrations of $H_2O_2$.

$$H_2O_2 + HO^{\bullet} \rightarrow H_2O + HO_2^{\bullet} \qquad (3.18)$$

$$HO_2^{\bullet} + HO^{\bullet} \rightarrow H_2O + O_2 \qquad (3.19)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^{+} \qquad (3.20)$$

The degree of pollutant degradation increased with increasing amounts of Fe(II) salts. This is because higher ferrous concentration caused greater generation of $OH^{\bullet}$ radicals (Eq. 3.1). In addition, $Fe^{2+}$ converted to $Fe^{3+}$, which acted as a coagulant, most likely of the hydrophobic oil residues present in the wastewater, resulting in secondary improvement in COD reduction. Concentration of ferrous iron greater than 8.0mM however, did not enhance COD and TOC reduction significantly rather slightly inhibiting COD reduction at higher $FeSO_4$ concentrations (Figure 3.6 a, b). This may be due to the scavenging of $OH^{\bullet}$, generated in the aqueous system, in presence of excess $FeSO_4$ concentration (Eq. 3.8). Furthermore, the $Fe^{3+}$ formed in the reaction mixture reacted with $H_2O_2$ (Eq. 3.5) to generate $Fe^{2+}$ and hydroperoxyl radicals $HO_2^{\bullet}$ in the reaction medium. The oxidation capacity of $HO_2^{\bullet}$ was less compared to $OH^{\bullet}$, which effected the overall COD and TOC reduction. The negative effects of excessive $Fe^{2+}$ concentration on treatment efficiency was

also reported by Mandal *et al.*, 2010, Lodha and Chaudhari, 2007, Neyens and Baeyens, 2003, Dogruel *et al.*, 2009, Bautista *et al*., 2008.

The work in this chapter showed an enhancement in the biodegradability of the recalcitrant MWF wastewater (evidenced by increase in $BOD_5$/COD from 0.160 to 0.538 (Figure 3.11). Several studies (Muszynski *et al*., 2007; Tekin *et al*., 2006; Lodha and Chaudhari, 2007; Rodrigues *et al*., 2009; Chen *et al.*, 2007, Lu *et al.*, 2010, Goi *et al.*, 2010) have reported a similar reduction in toxicity and enhancement in biodegradability for Fenton treated effluent. In another comparatively recent study (Seo *et al*., 2007), it was concluded that non-biodegradable pollutants such as ethylenediaminetetraacetic acid and triethanolamine were oxidised and degraded after ultrasonication-Fenton treatment of cutting fluid wastewater. In the present study, similar degradation of non-biodegradable recalcitrant pollutants might have occurred, thereby bringing about reduction in toxicity and an increase in biodegradability of the Fenton treated effluent. These complementary approaches confirm that the Fenton solution treatment brought about an improvement in the bioavailability and so biodegradability of the recalcitrant components of the waste MWF, which resulted in the decrease in the toxicity of the wastewater. This treated effluent was hence considered amenable for the next stage of biological treatment.

An interesting aspect of the biological processes employed in the combined treatment was the effect of acclimation on the degradation ability. An acclimation period is essential in order to gradually expose and adapt the microbial community to the intermediate oxidised compounds and maximise their removal efficiency. This was accomplished by increasing the feed concentration of the compound of interest over a period to allow for the growth of

microorganisms which could utilise the compound as a carbon or nutrient source. Typically, an acclimation period facilitates the development of appropriate enzyme mechanisms essential to induce biodegradation of the intermediary components. Specifically, stabilisation of the reactor was assessed by measurement of COD and TOC reduction. It took almost 20 days for start up of the reactors, i.e. to achieve steady state COD reduction. After 20 days, almost identical COD removal of >92% was observed in both the bioreactors. This suggests that acclimation of microbial culture to the reaction intermediates was more efficient than employing unacclimatised cultures in integrated treatment systems.

The overall costs of this hybrid treatment are represented by the sum of the capital costs, the operating costs and maintenance. Previous economic estimates showed that chemical costs would comprise more than 90% of the overall treatment costs for an integrated Fenton/activated sludge treatment system (Kitis *et al.*, 1999). Chemical costs assumed were £18/kg $FeSO_4.7H_2O$, £20/L (30% $H_2O_2$), £7/L $H_2SO_4$ (98%) and £20/kg NaOH (98% pellets). Major capital cost factors for the biological treatment process includes fabrication cost, air-supply system, air diffusion system and reactor maintenance which includes secondary clarification system. O&M costs for biological treatment would include labour, power, chemicals, material supplies and other costs. Results of detailed economic analysis conducted in previous studies showed that the chemical costs accounted for greater than 95% of the total costs in all cases (Kitis *et al.*, 1999). At the optimal dosage of Fenton's reagent (i.e., $[H_2O_2]$ =14.9mM, $[Fe^{2+}]$ =5.62mM), the total cost of chemicals including the Fenton's reagent (i.e., $H_2O_2$ and $FeSO_4$) and the acid/base for pH adjustment (i.e., $H_2SO_4$ and NaOH) was estimated to be £50/$m^3$ wastewater treated in addition to the biological treatment costs, which was mainly for the aeration system, total amounting to ~ £85-

105/$m^3$. By comparison, the operating cost for a typical Fenton system for treatment of phenol was estimated to be $2000/1000 gallons (£325/$m^3$) and $1,200/1000 gallons (£190/$m^3$) for activated sludge treatment method. (Mahamuni and Adewuyi, 2010, Mohajerani *et al*., 2009). These figures indicate that an integrated treatment system reduces the operational cost of a treatment scheme by several folds.

The findings reported in this chapter confirmed that the use of non-pathogenic microorganisms to remediate organic materials present in MWF wastewater is a favourable alternative to energy demanding physical and chemical treatment options. However, optimal performance of this biological process may require chemical enhancement. This component of the study provided strong evidence that optimisation of the Fenton dose regime reduced the quantities required for the overall remediation and made the process highly efficient. Lowering the concentration of hydrogen peroxide and ferrous iron reduced the quantity of toxic sludge generated by the process. However, the optimum reaction necessitated pH adjustments before and after Fenton treatment.

# CHAPTER 4

# REMEDIATION OF RECALCITRANT METALWORKING FLUID WASTEWATER EMPLOYING NANOSCALE IRON PARTICLES

## 4.1 Nanoscale Zero-Valent Iron Induced Oxidation

In Chapter 3, Fenton oxidation was demonstrated to successfully transform recalcitrant metal-working fluid (MWF) wastewater to biologically degradable intermediates, which facilitated a second-stage post biological treatment. This treatment necessitated pH adjustment before and after the treatment and addition of adequate amounts of ferrous iron and hydrogen peroxide, which was required for the production of hydroxyl free radicals. In this Chapter, the application of a novel treatment strategy with reaction very similar to the Fenton oxidation (alternate Fenton process), employing nanoscale zero-valent iron (elemental iron/ $Fe^0$) to remediate recalcitrant MWF wastewater, was investigated. With this treatment method, reactive hydroxyl free radicals are created *in-situ* in aqueous-phase by reaction between nanoscale zero-valent iron (nZVI) and oxygen (from air) in ambient conditions of temperature and pressure. The reactants in the process were nZVI and air, with the latter serving as the oxidant.

ZVI is an electron donor and a strong reducing agent, so can bring about oxidation of $Fe^0$ to $Fe^{2+}$ and the subsequent reduction of oxygen (by two-electrons gained from iron dissolution) to hydrogen peroxide, as shown by Equations 4.1 and 4.2 (Noradoun *et al*., 2003). The overall reaction depicting the oxidation of $Fe^0$ and reduction of oxygen is given in Eq. 4.3.

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \qquad (4.1)$$

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \qquad (4.2)$$

$$O_2 + Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2O_2 \qquad (4.3)$$

The ferrous iron and hydrogen peroxide (products of reaction 4.3) are the reagents employed in classic Fenton's oxidation. These products react to produce hydroxyl free radicals ($OH^{\bullet}$) through Fenton reaction, leading to oxidation and subsequent degradation of organic contaminants (Eq. 4.4)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \qquad (4.4)$$

Many reports have indicated that nano-iron has been accepted as a versatile tool for the remediation of groundwater, soil, and air on both the experimental and field scales. The combination of high reactivity and small size makes nano-scale particles highly flexible remediation tools for a variety of environmental applications. Nano-scale iron particles provide enormous flexibility for both *in situ* and *ex situ* applications for treatment of contaminated soils, sediments and solid wastes (Figure 4.17).

**Figure 4.17:** *Ex-situ* slurry reactor with nano-scale iron particles employed in remediation of wastewater. (Observatory NANO focus report 2010).

Since recalcitrant fractions of MWF components were successfully treated by Fenton's oxidation (chapter 3), the study presented in this Chapter focused on whether nZVI-induced oxidation could achieve transformation of refractory organics present in MWFs. The present study is the first to our knowledge to investigate a hybrid nZVI-induced oxidation, combined with biological oxidation for remediation of real industrial wastewaters. The experiments were conducted in two parts. The first consisted of determining optimum reaction conditions (pH, iron concentration, type of iron) to achieve maximum degradation (expressed in terms of percentage reduction in chemical oxygen demand (COD). The second part of the study deals with biological degradation of nZVI treated effluent.

## 4.2. Aims and Objectives

The present study reports the results of employing a novel hybrid approach (zero-valent iron oxidation (nZVI) followed by biological degradation) for treatment of a recalcitrant semi-synthetic MWF wastewater. The aims of the studies described within this chapter were:

- To determine the effect of pH on zero-valent iron induced oxidation of MWF wastewater.
- To determine the concentration of nano-scale particles required for the optimum oxidation of the organic components.
- To compare the reduction rates of commercially available granular Fe particles, nanoscale Fe particles and bimetallic (Fe/Ni) nano-scale particles for recalcitrant MWF wastewater.
- To investigate the feasibility of recycling the spent iron.

- To examine integrating the nano iron treated effluent with a post-biological treatment.

## 4.3 Materials and Methods

### 4.3.1 Wastewater characteristics

The recalcitrant semi-synthetic MWF wastewater samples (Hocut 795-B, Houghton International Inc.) were those obtained from Microbial Solutions Limited (as described in Chapter 3. The wastewater had an initial chemical oxygen demand (COD)=12,650 $mgL^{-1}$, five-day biochemical oxygen demand ($BOD_5$)=1945 $mgL^{-1}$, biodegradability index ($BOD_5$/COD)=0.154, total organic carbon (TOC)=4115 $mgL^{-1}$, pH=7.5 and suspended solids (SS)=2295 $mgL^{-1}$, respectively.

### 4.3.2 Chemicals

All reagents used in this work were of analytical grade and employed without any further purification. Ferric nitrate $(FeNO_3)_3.9H_2O$ and urea were obtained from Acros Organics, nickel nitrate from Sigma Aldrich, UK, iron powder (20 mesh size) from Alfa Aesar and tryptone soya broth (TSB) from Oxoid Ltd, England. All other chemicals were supplied by Fisher Scientific, UK. Reagents were prepared using 15 MΩ Milli-Q water.

### 4.3.3 Preparation and characterisation of nano Zerovalent iron

The ZVI nanoparticles were synthesised adapting a procedure described by Kingsley and Patil, 1988. Stoichiometric compositions (1:2.5) of iron nitrate (oxidiser) and urea (fuel)

were dissolved in a minimum quantity of water and the resulting solution was introduced into a muffle furnace at 673 K to make iron (II) and (III) oxides. Measured quantities of iron oxide were taken in test-tubes and crimp sealed. Hydrogen gas was then introduced into the test-tubes, via 12 cm syringe needles. A smaller needle (6 cm) was kept for the outgoing hydrogen gas to inhibit build-up of pressure within the system. Iron oxide taken in the test-tube was reduced to elemental iron, by flowing hydrogen at 623 K (in a furnace). Bimetallic Ni-Fe oxide was prepared similarly by mixing stoichiometric amounts of nickel nitrate and iron nitrate, and following the same procedure as above.

Transmission Electron Microscope (TEM) images and diffraction patterns of the synthesised iron oxide were obtained with a high resolution transmission electron microscope (HRTEM, JEOL 4000 EX). X-Ray Diffraction (XRD) is one of the primary techniques used by mineralogists and solid state chemists for the fingerprint characterisation of crystalline solids and determination of their structure. X-Ray Diffraction (XRD) was carried out using a Philips PW1830 diffractometer.

## 4.3.4 Analytical techniques

COD was analysed using dichromate solution as an oxidant, in a strongly acidic media, employing a Hach reactor HT200S and spectrophotometer (Hach DR 2800). Five-day biochemical oxygen demand ($BOD_5$) measurements were carried out in Oxitop (WTW). Hydrogen peroxide generated during the experiment was analysed using the N, N-diethyl-p-phenylenediamine (DPD) method, modified to minimise interference by Fe(II) and Fe(III) (Balmer and Sulzberger, 1999). Bipyridine was added to complex Fe(II) and EDTA

was added to complex Fe(III). The procedure consisted of the following steps: 0.4 mL of phosphate buffer solution (pH 6, 0.5 M phosphate) and 0.1 mL of bipyridine solution (0.01 M) were premixed in a 1 cm quartz cell; 2 mL of the sample, 0.02 mL of EDTA solution (0.01 M NaEDTA), and 0.03 mL of DPD (N,N-diethyl-phenylenediamine) reagent (1% in 0.1 M $H_2SO4$) were then added, followed by 0.03 mL of POD (horseradish peroxidase) reagent (about 0.8 g/mL). Absorbance was read at 551nm and the hydrogen peroxide concentrations of the samples were compared against a linear standard curve (0-500µM).

The toxicity of the effluent was assayed by *Acinetobacter baylyi* ADP1_recA_lux biosensor, as described by Song *et al.*, 2009 (detailed in Chapter 3). This chromosomally-based bioreporter expresses bioluminescence when exposed to any DNA damaging toxicants. The toxicity was quantified in terms of relative bioluminescence (absolute intensity of bioluminescence divided by $OD_{600}$).

### 4.3.5 nZVI oxidation experiments

All experiments were carried out at room temperature (293±1 K) in 25 ml test tubes, unless specified. Care was taken to ensure that the test tubes containing freshly prepared nZVI samples were not exposed to atmospheric air, as the elemental iron gets oxidised and catches fire instantaneously on contact with air. The test tubes were stoppered with rubber septa and crimp-sealed. To initiate the reaction, an aliquot of 10ml of MWF wastewater was added to freshly prepared nZVI. The particles were kept in suspension by magnetically stirring the solution. In order to avoid oxygen limitation in the system, air was continuously bubbled through the system at 150 mL $min^{-1}$. Liquid samples were collected at different time intervals and the reaction was quenched by filtering the nZVI precipitate from the

solution using a 0.22-µm syringe filter. Additional experiments were carried out to study the role of hydroxyl free radicals in the reaction, by addition of a large excess (100mM) of tertiary butanol (*tert*-butanol) as $OH^{\bullet}$ scavenger along with the wastewater. The amount of iron was decided based on ZVI-induced oxidation conducted at various concentrations of nano-iron (as detailed in section 4.4.5).

### 4.3.6 Biological treatment experiments

Aerobic biodegradation studies were carried out in 250 mL batch glass reactors, with a working volume of 100 mL, maintained at an rpm of 120 at $28^0$ C. The bioreactors were fed with nZVI oxidised effluent and a 5-membered bacterial consortia (grown in 1/10 w/v Tryptose Soya Broth, (TSB) and incubated at $28^0$ C for 12 hours, on an orbital shaker at 120rpm). The bacterial consortia consisted of *Agrobacterium radiobacter, Comamonas testosteroni, Methylobacterium mesophilicum, Microbacterium esteraromaticum* and *Microbacterium saperdae*, all of which was assembled specifically for the task and has previously been reported to be effective for bio-treatment of operationally exhausted MWF (van der Gast *et al.,* 2003).

## 4.4 Results

### 4.4.1 Characterisation of the synthesised nanoparticles

X-ray diffraction (XRD) pattern for the prepared mixed iron oxides is shown in Figure 4.1. XRD analysis of the nano-scale Fe particles demonstrated broad peaks relating to poor crystallinity. The dominant phase was iron (II) and (III) oxides, illustrated by broad diffraction peaks situated at 33.06, 35.54, 49.4 and 62.44 degrees 2-theta (Figure 4.1). The

poor crystallinity of these particles meant that Fe oxide peaks for various other smaller points could not be identified. The TEM micrograph indicated that the average particle size of the iron oxide is around 10-20 nm with spherical or pseudo-spherical form (Figure 4.2). The electron diffraction pattern given in Figure 4.3 revealed that a particle's core was polycrystalline. The diffraction patterns indicated the presence of a body centered cubic (bcc) Fe structure. Apart from the rings characteristic of bcc structure, the diffraction pattern revealed a diffuse pattern that could be attributed to the outer oxide shell layer.

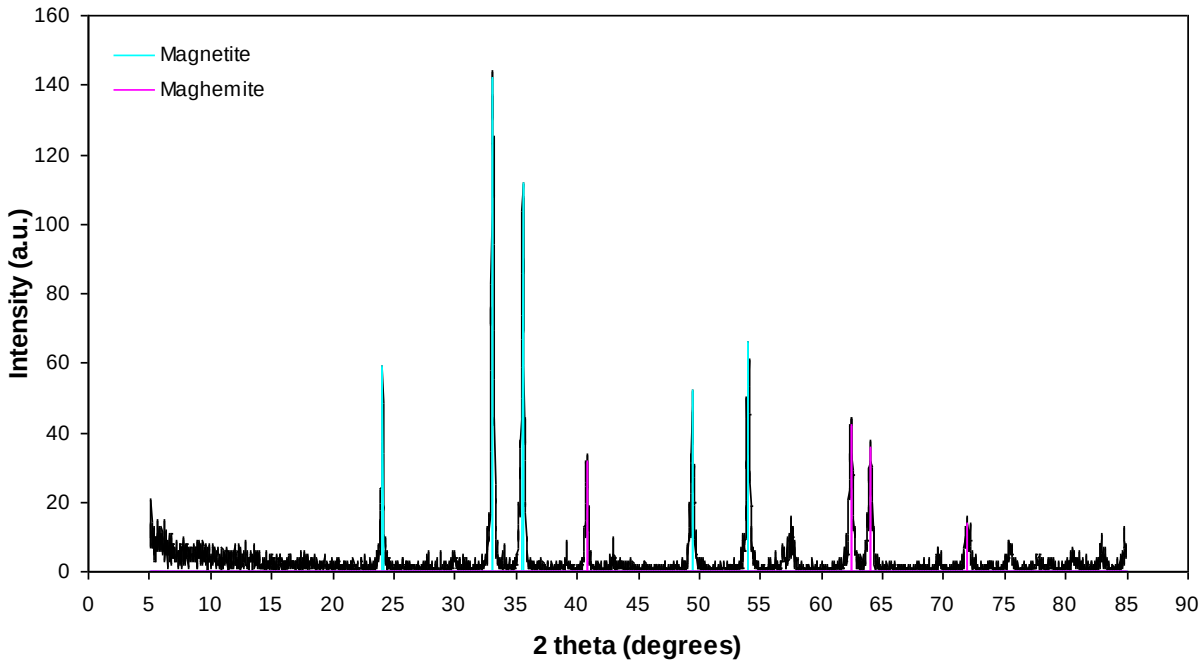



**Figure 4.1:** X-ray diffraction patterns of the synthesised iron oxide nano-particle.

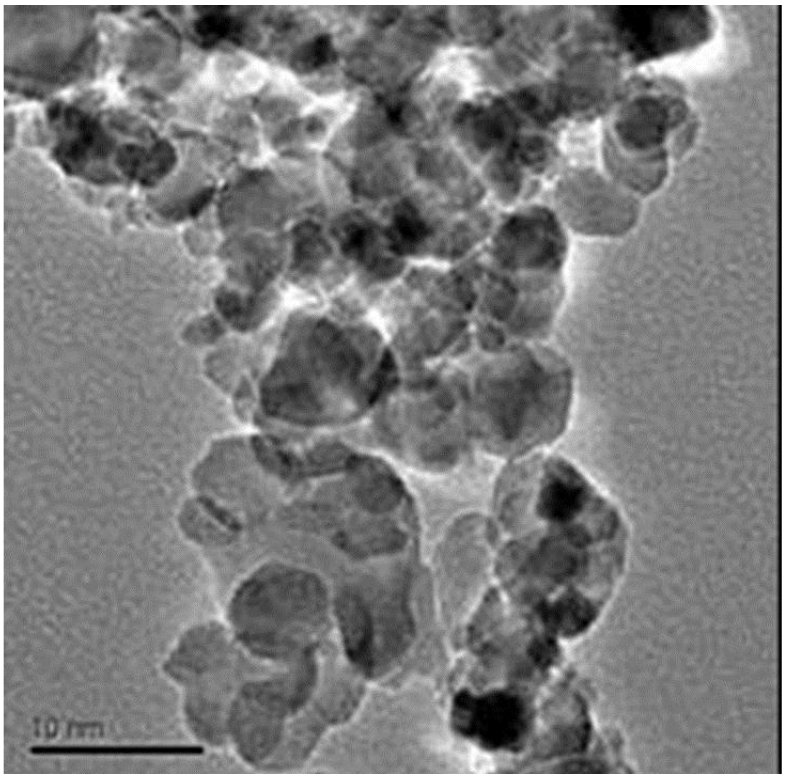



**Figure 4.2:** Transmission electron microscope picture of prepared iron oxide.

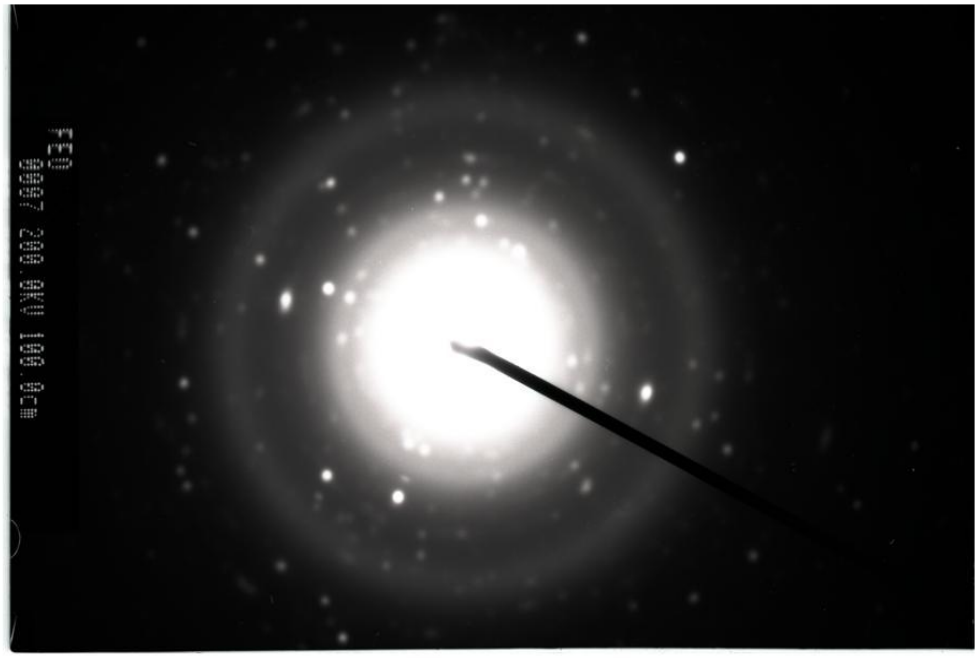


**Figure 4.3:** Electron diffraction pattern of the iron oxide nanoparticle.

## 4.4.2 COD Reduction

Degradation reactions start immediately after MWF comes in contact with nZVI bubbled with air. Figure 4.4 is a photograph of the wastewater before and after the ZVI induced oxidation. The nanoscale elemental iron before treatment appeared black in colour (Fig 4.4, left), which changed to rust colour after oxidation (Fig 4.4. right). This was due to the conversion of $Fe^0$ to ferrous and ferric oxides and hydroxides during the course of the treatment. The treated wastewater also appeared clearer.

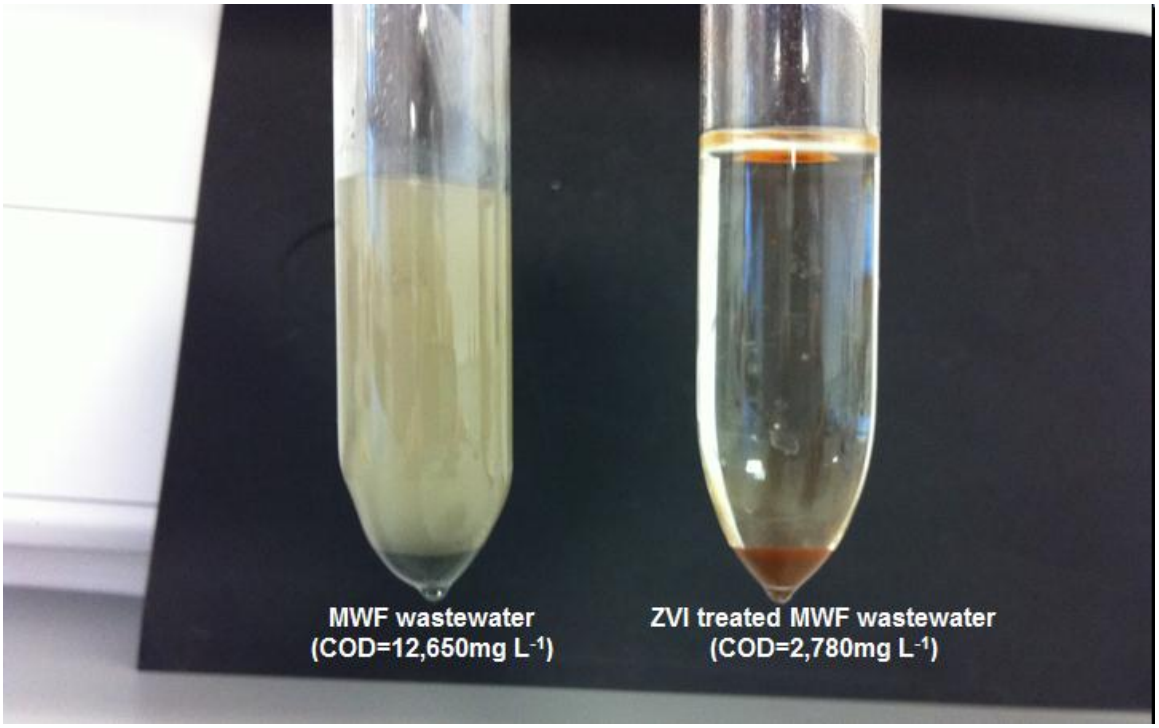



**Figure 4.4**: Photograph of ZVI treated wastewater ($COD_0$=12,650 mg $L^{-1}$, treatment time=60minutes, pH= 7.5, temperature=$20 \pm 2^0$C)

The COD reduction of the MWF was followed with time and estimated at different intervals. Figure 4.5 represents the ratio of wastewater COD to initial COD ($COD/COD_0$) with time. Two control experimental set-ups were run. The first set of control experiments

were carried out in absence of ZVI and the next set-up was conducted in presence of nZVI, but air was replaced with nitrogen.

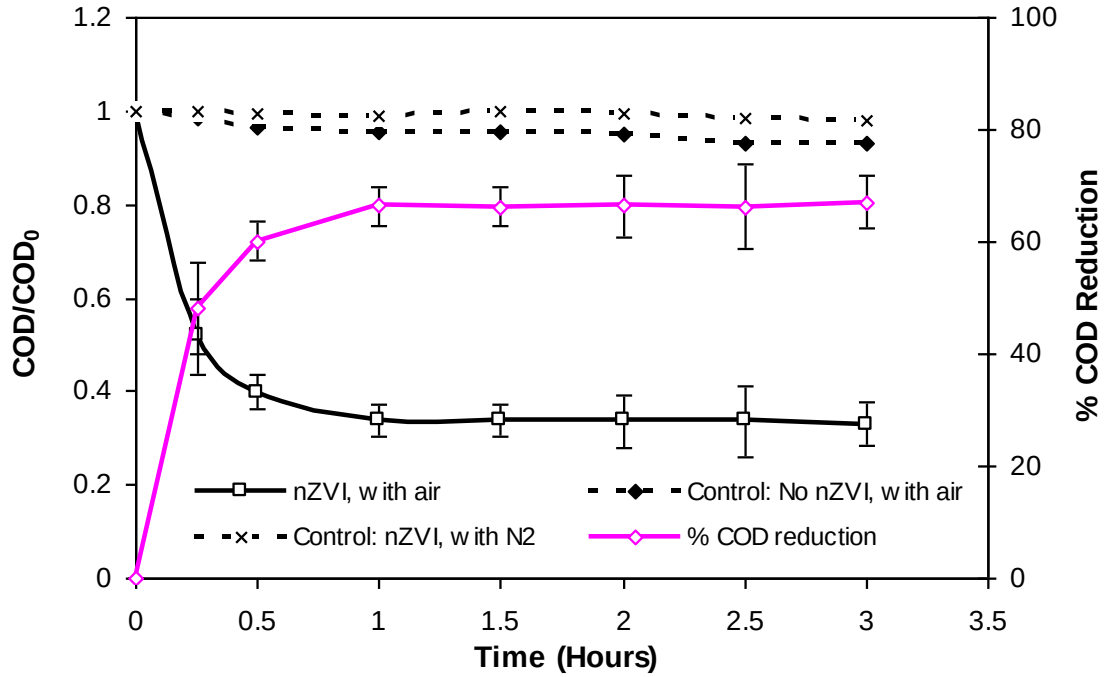



**Figure 4.5**: Reduction of COD by nZVI oxidation (nZVI concentration=5g/L, pH=3.0) of MWF wastewaters (Mean ± SD of triplicates shown).

A steady reduction in wastewater COD was detected during the initial 60 minutes of reaction (Figure 4.5). Thereafter, the decrease in COD was stabilised. Maximum rate was observed within the first 30 minutes, with 70% reduction in COD. Maximum decrease of 78% reduction in COD was achieved within the first hour of treatment time. The colour of the MWF wastewater began to change from grey-green (as shown in Figure 4.4) to deep rust colour within 15 minutes of reaction, indicating oxidation of the ZVI particles. The experiment was conducted at pH=3.0. Two sets of control experiments were conducted to determine the significance of oxygen and nZVI in the reaction. Figure 4.5 also summarises the results of these control experiments. The slight reduction in COD with air in absence of nZVI could be due to volatilization of organic compounds and adsorption of organics on iron surface during experimentation. There was no reduction in COD when air/$O_2$ was

replaced with nitrogen, even in the presence of nZVI. The results of these control experiments highlight the importance of oxygen and ZVI in degrading MWF components, and in eliminating the possibility of adsorbed MWF on nZVI surfaces. If any reduction in COD would have occurred in the presence of nZVI and absence of air, then the decrease in organic load could be ascertained to adsorption of organics on the surface of iron. The absence of such a phenomenon indicates complete oxidation of the organic substances due to the action of hydroxyl free radicals generated during the course of the reaction.

The concentration of *in-situ* hydrogen peroxide generated by the reduction of dioxygen (Eq. 4.2) was measured and Figure 4.6 represents the hydrogen peroxide detected in the reaction mixture throughout the reaction time, after the addition of nZVI. The concentration of hydrogen peroxide in the reaction mixture increased for nearly 45 minutes, reaching a peak $H_2O_2$ concentration of 92µmol/L and started to go down after this time. No residual hydrogen peroxide could be detected in the aqueous system after 75 minutes. The actual amount of hydrogen peroxide generated may, however, be more than the measured quantity. The $H_2O_2$ profile correlated with the degradation profile of the organics shown in Figure 4.5, confirming the role of hydrogen peroxide in the formation of ROS. This *in-situ* generation of hydrogen peroxide in the nZVI induced oxidation prevented the addition of an excess of extraneous $H_2O_2$, hence obliterating the requirement for transport and storage of this reagent.

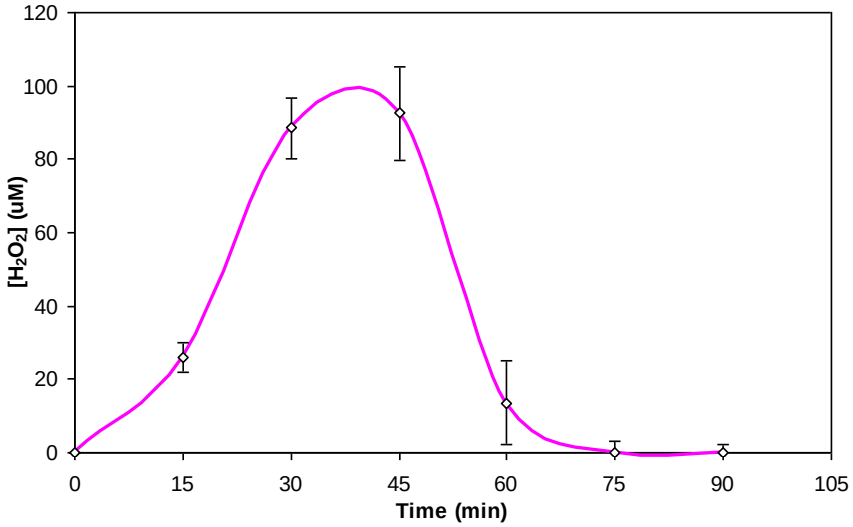



**Figure 4.6**: Hydrogen peroxide profile with time after the addition of nZVI (nZVI=5g/L, pH=7.5) (Mean ± SD of triplicates shown).

These reactions are initiated when $Fe^0$ is oxidised by oxygen (aq), to produce hydrogen peroxide (Eq. 4.5), through a two electron process.

$$Fe^0 + O_2 + 2H^+ \rightarrow Fe^{2+} + H_2O_2 \qquad (4.5)$$

Hydrogen peroxide generated in reaction (4.9) has two fates.

- Hydrogen peroxide could oxidise another $Fe^0$ (Eq. 4.6), in which case, MWF oxidation does not occur.

$$Fe^0 + H_2O_2 \rightarrow Fe^{2+} + 2OH^- \qquad (4.6)$$

- Alternatively, $H_2O_2$ could react with species such as $Fe^{2+}$, via a Fenton reaction (Eq. 3.7) and result in the production of hydroxyl radicals (Keenan and Sedlak, 2008b), which brings about the oxidation of MWF components.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^- \qquad (4.7)$$

### 4.4.3 Effect of particle size and Ni doping on ZVI

Two different sources of ZVI (commercial granular ZVI with mesh size 20 and nano sized Fe prepared in this study) were selected to investigate the size effect of ZVI on MWF treatment. Additional experiments were conducted employing nNi-ZVI to study the effect of a bimetallic system on oxidation capacity of recalcitrant waste MWF. Figure 4.7 illustrates COD reduction when the equivalent amount of granular iron, nano sized iron and nano sized Ni-Fe bimetal were used. It can be seen from the results that the percent COD reduction of MWF by n-ZVI addition was over two-fold greater than with the granular ZVI.

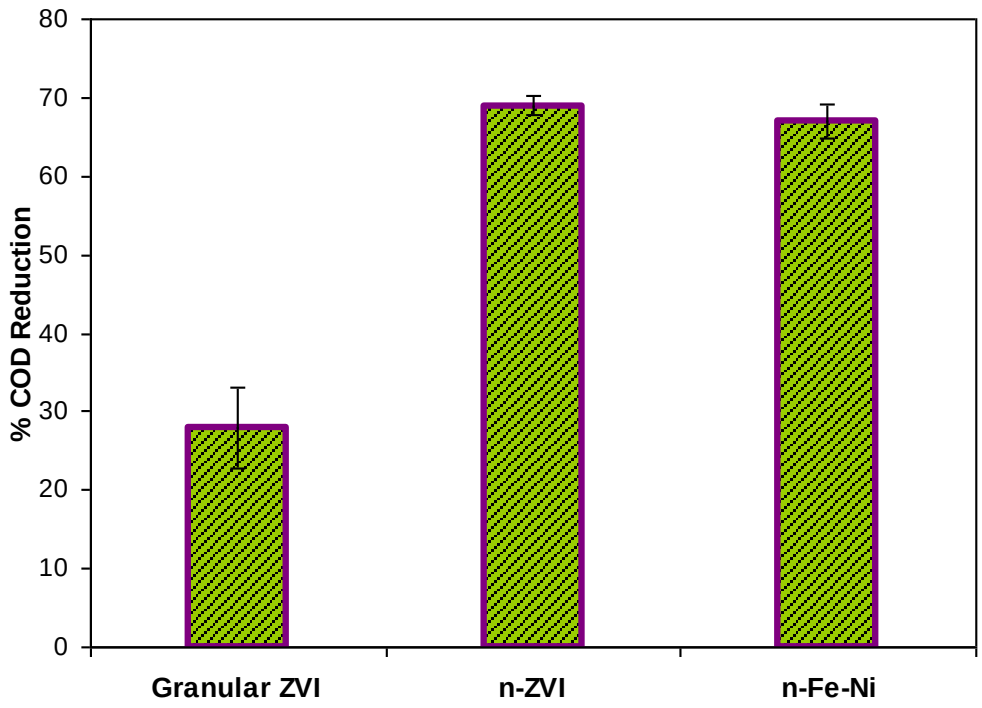



**Figure 4.7**: Effect of particle size and bimetallic iron particles on percentage COD abatement (reaction time=1 hour, pH 7.5, metal concentration=5 g/L) (Mean ± SD of triplicates shown).

In the present study, the effect of a nanoscale Ni-Fe bimetallic particle on COD reduction was also investigated. The results of the present study demonstrated that nNi-ZVI to have

comparable or lower reactivity as compared to nZVI. This was probably due to one of the MWF components (such as sodium salt of ethylenediaminetetraacetic acid (EDTA) which are typically added in MWF formulation as a chelating agent to complex with water hardness ions and stabilise the coolant) itself retarding peroxide ion consumption by nZVI (Keenan and Sedlak, 2008b). It is, however, worth noting that all the previous reported studies on bimetallic nanoparticles had been conducted on a single compound, taken as a model pollutant (Lien and Zhang 1999, He and Zhao, 2005, Wang and Zhang, 1997). In this study the effect of reactivity in a complex real wastewater system was investigated. A possible explanation for this variation from the general trend is that each of the various components present in the wastewater may have reacted very differently with the peroxide ion generated by reduction of the nZVI.

### 4.4.4 Effect of pH

The pH of the reaction medium plays a significant role in MWF treatment (van der Gast and Thompson, 2005) and in treatments involving various advanced oxidation reactions. Figure 4.8 illustrates temporal changes of nZVI treated wastewater, at pH 3.0.

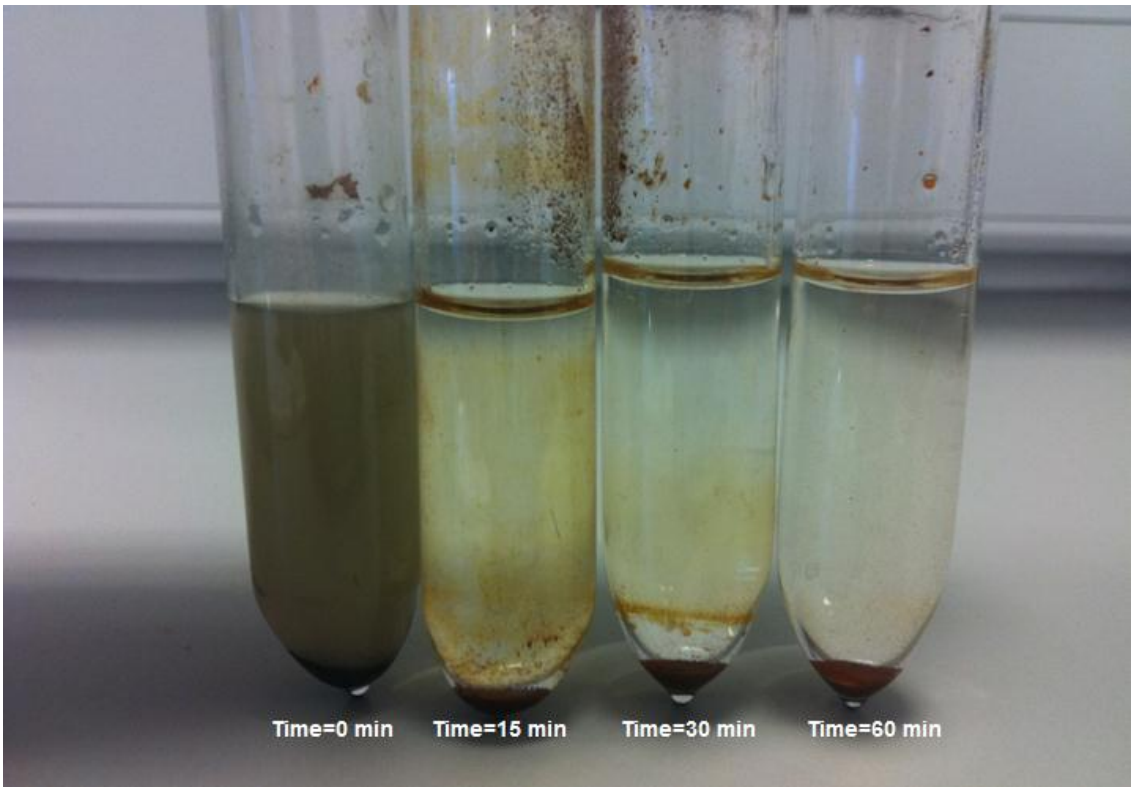



**Figure 4.8**: Photograph of the nZVI treated wastewater at pH 3.0 at varying time intervals (Reaction time= 15minutes, 30 minutes and 60 minutes).

nZVI oxidation was conducted under acidic pH (3.0) and in the neutral pH (7.5). The original pH of the wastewater was 7.5. pH amendment was done by adding $H_2SO_4$(1M). The aim of this part of the study was to check whether nZVI reaction would lead to reduction in COD under near-neutral conditions. Fenton's oxidation effectively occurs only under acidic conditions (pH 2.5-4). Figure 4.9 summarises nZVI induced COD reduction at pH 3 and at near neutral pH 7.5. The extent and rate of degradation of the MWF effluent was greater in acidic pH. The maximum observed decrease in COD occurred within 30 minutes of the start of the reaction, under both pH conditions examined. The rate of the reaction then increased very slowly to reach a maximum COD degradation at the end of 1 hour of the reaction. Further treatment failed to cause any detectable increase in the percentage COD reduction. Figure 4.9 shows the maximum reduction of 78% COD at pH 3.0, compared to 64% at a neutral pH, after 30 minutes of reaction. Control experiments at

both pH's were established in the absence of ZVI. Neither control experiment induced any detectable reduction from the initial COD. This indicates that the reduction in the COD of MWF at both pH's examined was attributed to the action of reactive oxygen species, most probably hydroxyl free radicals or reactive ferryl species. These were likely to have been formed from the combination of ZVI and oxygen. This suggests that decrease in COD concentrations detected could not be attributed solely to changes in pH of the medium.

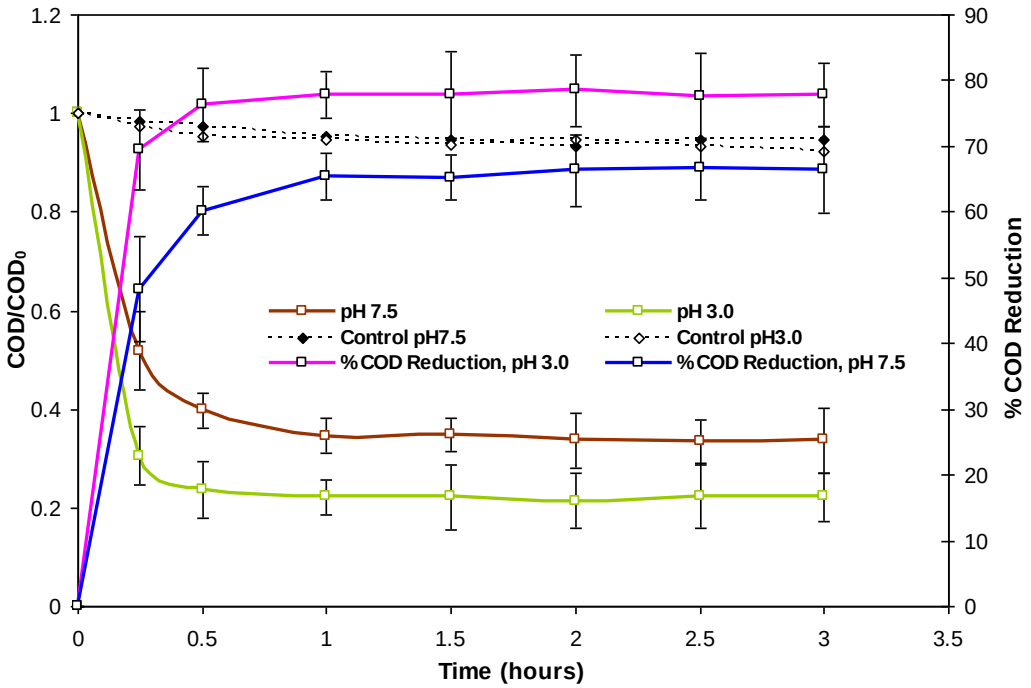



**Figure 4.9**: (a) COD degradation profile with time at pH 3.0 and 7.5. Control experimental results at the same pH are also shown in the figure (Mean ± SD of triplicates shown).

The rate of the reaction was three times faster when the starting pH was 3.0, when compared to the same at a pH of 7.5, which is illustrated in Figure 4.10, which depicts the pseudo-first order plots for COD reduction. The pseudo first order rate constant was -0.0139 under neutral conditions compared to -0.0391 under acidic pH.

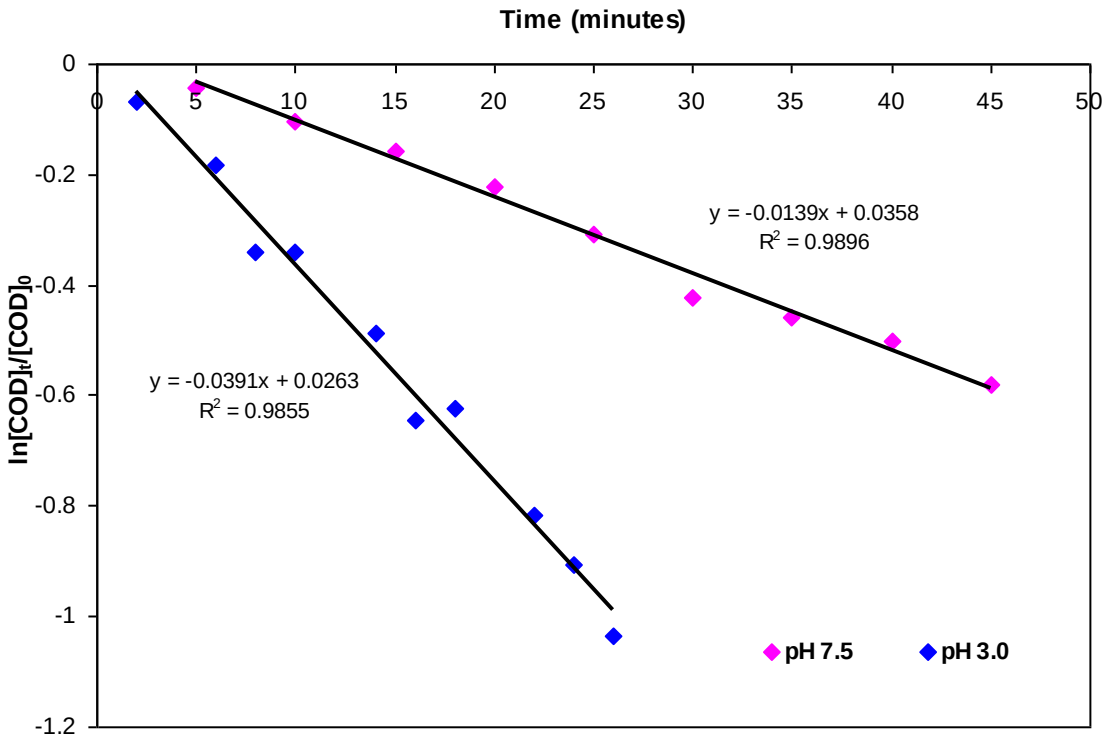



**Figure 4.10**: Pseudo first order plots for pH=7.0 and pH=3.0

Figure 4.11 illustrates the Arrhenius plot for reactions at pH 3.0 and 7.5. Experiments were conducted at temperatures of $20^0$ C, $30^0$ C, $35^0$ C, $40^0$ C, $45^0$ C and $50^0$ C run for 15, 30, 45 and 60 minutes for each temperatures. The activation energies were calculated to be 3.112 kJ $mol^{-1}$ at pH 3.0 and 5.810 kJ $mol^{-1}$ at neutral pH respectively, assuming the COD reduction followed a pseudo-first order reaction. The activation energies further confirmed greater reactivity at lower pH.

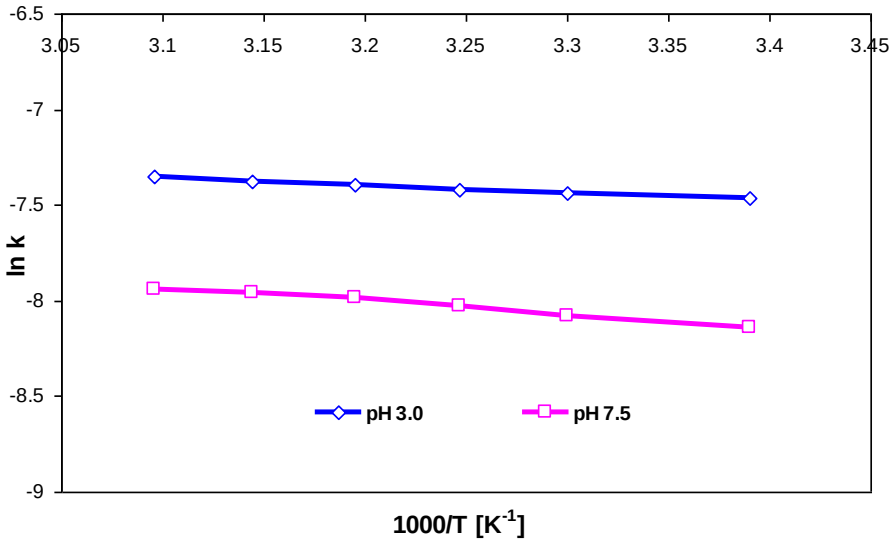



Figure **4.11**: Arrhenius plot of nZVI oxidation conducted at temperatures between 295 K and 323 K.

In order to elucidate the role of free radicals in the COD reduction reaction, *tert*-butanol, a known hydroxyl radical scavenger, was added to the reaction mixture to eliminate the presence of hydroxyl radicals in the solution, as *tert*-butanol acts as a highly effective scavenger of hydroxyl free radicals. Figure 4.12 illustrates that addition of *tert*-butanol, as an $OH^{\bullet}$ scavenger, quenched the oxidation of the contaminants at low pH (3.0). The reaction under pH of 3.0 brought down the effluent COD load by 80% while the addition of *tert*-butanol led to only 8% reduction in COD. This provided strong evidence that increased rates of COD reduction in acidic pH were attributable to hydroxyl radical ( $OH^{\bullet}$ ) production through a Fenton like reaction, via *in-situ* generation of hydrogen peroxide and Fe (II) during nZVI oxidation.

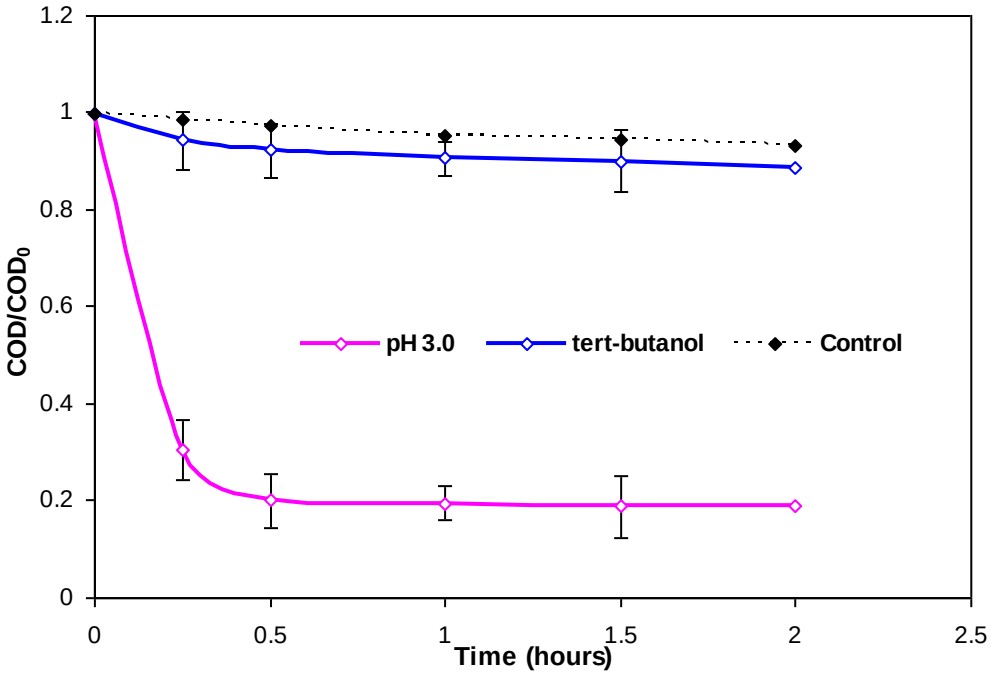



**Figure 4.12**: Effect of *tert*-butanol on COD abatement by nZVI oxidation (nZVI concentration 5g/L, pH 3) (Mean ± SD of triplicates shown).

Under neutral conditions, 68% reduction in the COD concentration was observed. An interesting observation was that under neutral experimental conditions, addition of *tert*-butanol did not seem to arrest the reaction completely. There was a 30% reduction in COD under this near-neutral condition. Reactions conducted under near-neutral conditions, depicted by Figure 4.13, suggests that an additional oxidant, possibly ferryl Fe (IV) species, that did not react competitively with *tert*-butanol, must have been generated in the neutral pH (Eq. 4.8) (Stieber *et al*., 2011).

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O \qquad (4.8)$$

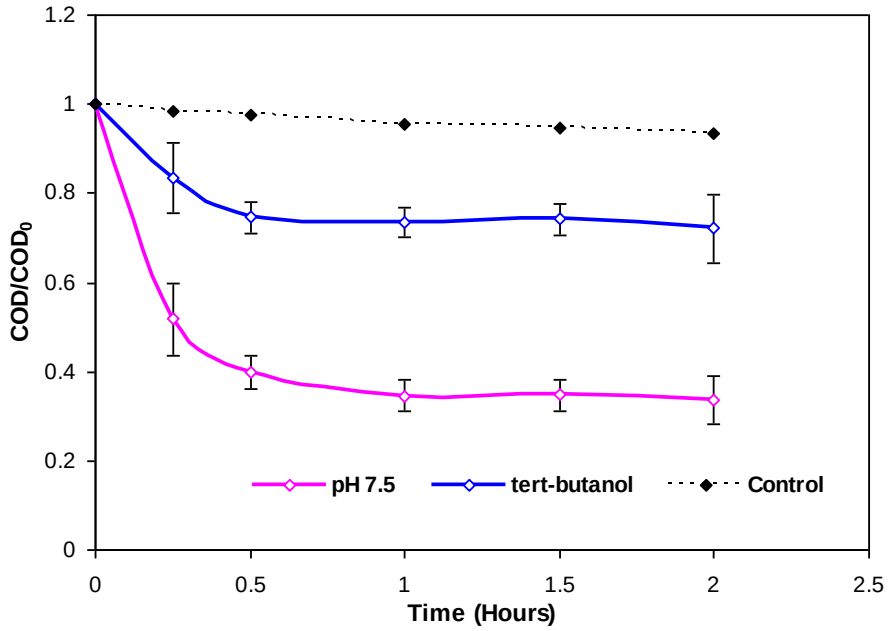



Figure 4.13: Effect of *tert*-butanol on COD abatement by nZVI oxidation (nZVI concentration 5g/L, pH 7.5) (Mean ± SD of triplicates shown).

Hydroxyl radicals react with organic compounds by the addition to the double bonds possessing sufficient electron density, or by hydrogen abstraction from alkyl groups hydroxyl groups, or by electron transfer. In contrast, Fe (IV) being a weaker oxidant than $OH^{\bullet}$, the reaction proceeds exclusively by an electron-transfer mechanism, since neither addition or hydrogen abstraction is possible (Keenan and Sedlak, 2008 a,b; Bossmann *et al.*, 1998; Hug and Leupin, 2003; Noradoun and Cheng, 2005). In addition, at higher pH there was greater affinity of the $Fe^0$ surface for $Fe^{2+}$ and the ability of $O_2$ to outcompete $H_2O_2$ for $Fe^{2+}$. Previous reported studies suggest that this would have resulted in reduction in the rate of generation of oxidising radicals and greater extent of generation of a passivating layer of iron oxide/hydroxide (Eq. 4.9, 4.10) (Joo *et al.*, 2005).

$$Fe(II) + O_2 \rightarrow O_2^{\bullet -} + Fe(III) \qquad (4.9)$$

$$Fe(II) + O_2^{\bullet -} \rightarrow Fe(III) + H_2O_2 \qquad (4.10)$$

### 4.4.5 Effect of nZVI concentration

The effect of initial nZVI concentration at pH 7.5 was studied over 60 minutes, by varying the amounts (1g/L – 20g/L) of nZVI added to the effluent. Figure 4.14 reflects the increase in the degree of degradation with increasing nZVI concentration, up to 5 g/L, after which there was little change in COD reduction. The reason for this trend may be related to the increase in concentration of ferrous iron that accompanied the rise in nZVI concentration. Elevation in the concentration of $Fe^{2+}$ in the aqueous solution consequently led to an increase in competitive scavenging of $OH^{\bullet}$, leading to formation of ferric oxides and hydroxides, which further passivated the nZVI surface (Eq. 4.11) (Joo *et al.*, 2005). The ferrous ion thereby replaced the organic compounds present in the wastewater as a dominant sink for $OH^{\bullet}$ and this brought about a reduction in rate of degradation at greater concentrations of nZVI.

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-} \qquad (4.11)$$

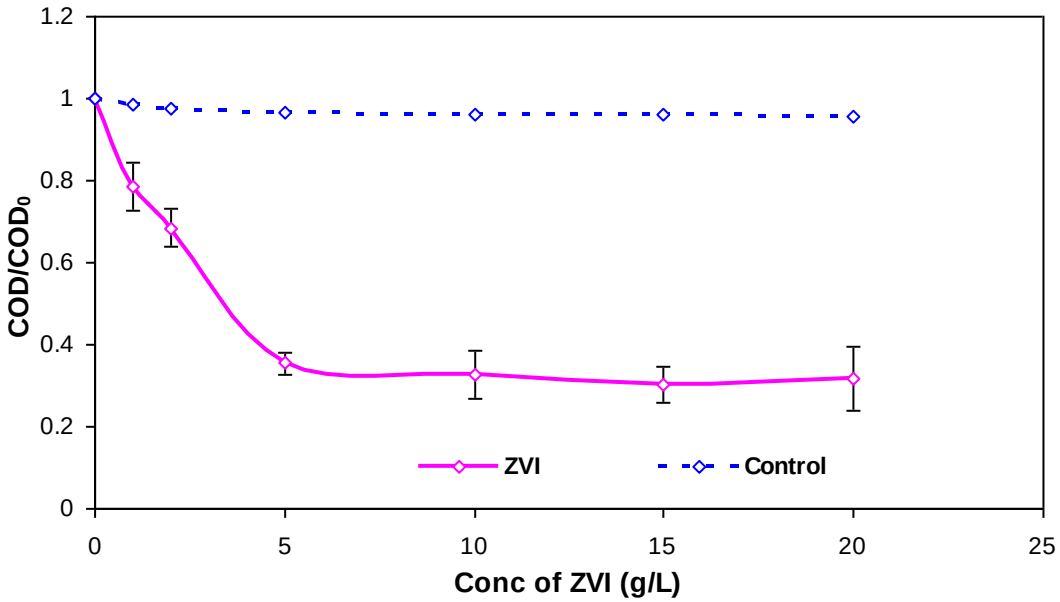



**Figure 4.14**: Effect of nZVI concentration on COD reduction (pH 7.5, reaction time=1 hour) (Mean ± SD of triplicates shown).

### 4.4.6 Recycling of spent nZVI

With increasing pressure on resources, together with costs, an important consideration in any treatment process is the amount of material employed. In this case, it may be possible to recycle most of the used / residual iron compounds by reprocessing. The prospect of reprocessing spent nZVI was investigated in this study by collecting residual iron oxide / iron hydroxide precipitate, after the nZVI oxidation experiments. The spent/oxidised iron nano particles agglomerated and settled down the reaction vessel owing to the precipitation of iron hydroxides and oxides. These settled particles were filtered out and the collected material was then dried and reduced to regenerate nZVI in hydrogen. The regenerated nZVI was used again to treat successive batches of MWF in the same way as the original nZVI experiments. It was found that the regenerated nZVI showed very similar COD reduction characteristics when compared to the freshly prepared nZVI COD reductions. The recycling of this catalyst without significant loss in reactivity makes this treatment method a greener and economic alternative for practical applications. The recycling of nZVI could have a significant impact on overall economy of the industrial scale plants, as only a small quantity of iron source was required to replenish the system, since most of the nZVI could be recycled within the system, and this eliminated any waste iron products.

### 4.4.7 Biodegradation studies

The conventionally employed parameters for assessing effluent such as COD are insufficient to describe the potential environmental impacts of treated industrial effluent, and hence an additional toxicity measure was employed. This was to confirm that the ZVI treated effluent was amenable for a second-stage biological degradation. Advanced oxidation based remediation methodologies, based on generation of hydroxyl free radicals,

may sometimes lead to the production of toxic reaction intermediates, whilst showing reduction in COD. The toxicity of the ZVI treated effluent was assayed by employing a chromosomally based bioreporter (*Acinetobacter baylyi* ADP1_recA_lux) bacterial biosensor. This biosensor expresses bioluminescence when exposed to any DNA damaging toxicants (Song *et al.*, 2009). With this sensor, increased luminescence was indicative of toxicity.

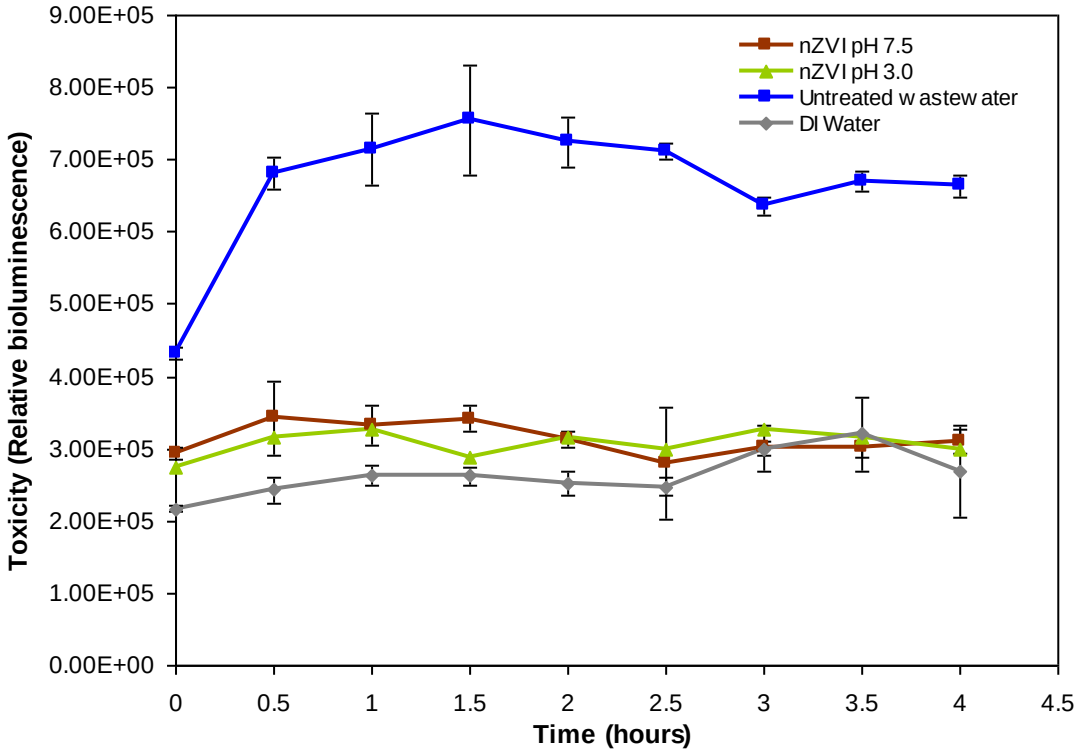



**Figure 4.15**: Effect of nZVI treatment on effluent toxicity (Mean ± SD of 8 replicates shown).

Figure 4.15 indicates reduced relative bioluminescence of the nZVI treated effluent demonstrating 85 % reduction in toxicity when compared to the untreated original MWF effluent. The toxicity of the treated effluent at both operating pH was comparable to that of deionised water. Furthermore, the biodegradability index denoted by $BOD_5$/COD (ratio gives information regarding the ratio of the readily biodegradable and recalcitrant

substances of the wastewater) increased from 0.154 to 0.512, after pre-treatment with nZVI. The reduction in the toxicity and the concurrent increment in biodegradability index may

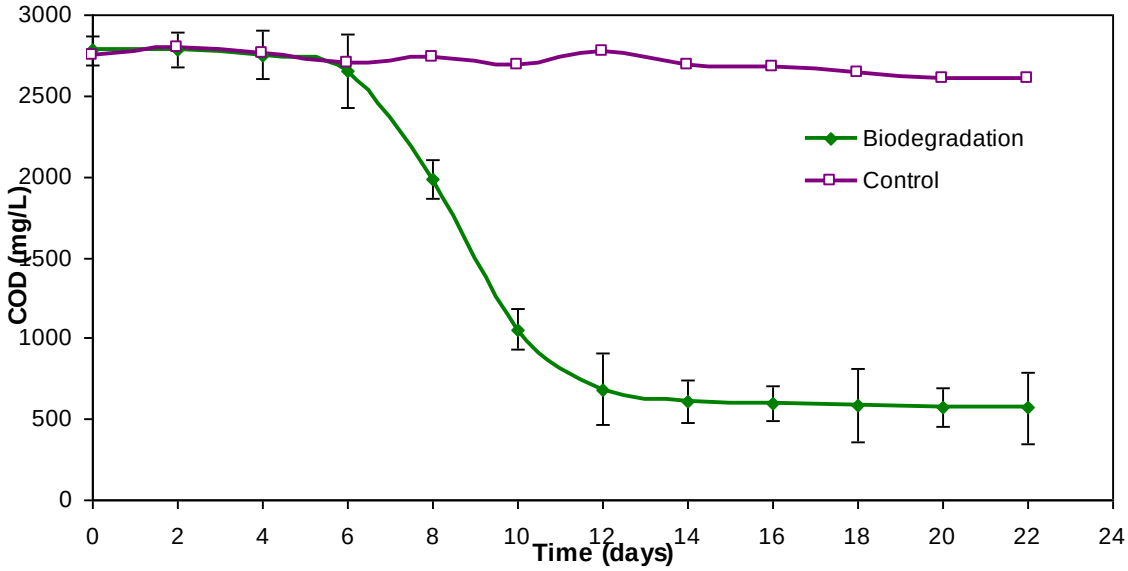



**Figure 4.16**: COD profile in the batch bioreactor charged with nZVI treated effluent (Mean ± SD of 3 replicates shown).

have been due to the fact that nZVI treatment was able to alter the chemical structure and properties of the non-biodegradable components of the effluents into compounds that were more bioavailable, hence making the nZVI treated effluent more susceptible to further processing by biodegradation. It was found that the microbial cultures employed required an acclimatisation period of 15 days, after which there was a steady decrease in the COD, as represented in Figure 4.16.

The ZVI pre-treated effluent when subjected to further biological degradation could achieve an overall 95.5% drop in COD. The above observation clearly demonstrates that pre-treatment with nZVI reduced toxicity of the MWF and was highly effective at stimulating the extent of biodegradation of the residual chemical constituents of the MWF effluents.

## 4.5 Discussion

ZVI-induced oxidation, in presence of oxygen generates ferrous ions (by oxidation of ZVI) and hydrogen peroxide (from reduction of oxygen). Although hydrogen peroxide was not added to the system, it was produced by the reaction of superoxide radicals and protons. This created the Fenton chemistry that was responsible for the degradation of organic pollutants, without addition of hydrogen peroxide. The main feature of this system was its ability to create *in-situ* reactive oxygen species in aqueous-phase, employing less expensive and nontoxic reagents, in ambient conditions of temperature and pressure. The major advantage of this system over that of conventional Fenton chemistry is that, while normally the reaction stops once all the added ferrous ions are used up in Fenton oxidation, the ZVI provided a constant source of the ions and hydrogen peroxide. In addition, relative to the homogeneous Fenton catalysts, ZVI treated organic constituents of the MWF over a wide range of pHs, without the need for extraneous addition of hydrogen peroxide. However, this reaction, and in particular the production of hydrogen peroxide, is limited by the solubility of oxygen in water.

Work in this chapter has demonstrated that degradation of recalcitrant MWF wastewater occurred in presence of nZVI and atmospheric oxygen. The decrease in the COD in ZVI-treated wastewater (Figure 4.5) was attributed to the reduction of dioxygen dissolved in the aqueous MWF wastewater. Production of *in-situ* hydrogen peroxide and kinetically facile oxygen species, including $H_2O_2$, $O_2^{\bullet-}$ and $HO^{\bullet}$, collectively referred to as reactive oxygen species (ROS) (Joo *et al.*, 2004), reacted with the MWF. Hydroxyl radicals are potent oxidising agents ($E^0$~2.8V) that can oxidise almost any available organics at diffusion-limited rates. Typically, they have rate constants of reactions with the majority of organic molecules, in the order of $10^6$-$10^9$ $mol^{-1}s^{-1}$ (Petrovic *et al.*, 2011). The lack of selectivity of

hydroxyl radicals is an advantage when dealing with highly contaminated wastewaters, such as MWF effluent.

The reaction efficiency could be hindered by two mechanisms: (i) the loss of $H_2O_2$, through the four-electron transfer process (Eq. 4.10) and (ii) precipitation of iron oxides, and hydroxides at neutral pH (Eq. 4.11) (Lee and Sedlak, 2008). Nano-iron particles have a core-shell structure with a $Fe^0$ core and an oxide or a noble metal shell. The ferric oxide or hydroxide layers are found to have properties similar to $\gamma$-$Fe_2O_3$ and $Fe_3O_4$ on un-reacted nZVI, which leads to reduction in the reactivity (or passivation of the surface) which is accompanied by a decrease in the rate of nZVI oxidation (Joo *et al.*, 2005; Keenan and Sedlak, 2008a,b; Feitz *et al.*, 2005). Furthermore, charge and mass transport through the oxide shell is necessary for sustained reaction, and the kinetics of these transport processes may be important determinants of contaminant reduction kinetics (Nurmi *et al.*, 2005).

A decrease in iron reactivity over time has been observed and reported for several studies investigating degradation of chlorinated organics such as trichloroethylene (TCE) (Lien and Zhang, 1999, Wang and Zhang, 1997). Although the exact mechanism of this reduced reactivity is unknown, some researchers offer plausible explanations. This includes the formation of surface oxide layers, a phenomenon confirmed through the oxygen electron energy-loss spectroscopy test (Liu *et al.*, 2005) which indicated that oxygen atoms concentrated in the shells of the particles which oxidises the iron core. Studies have suggested that coating or doping nanoscale zero-valent iron particles with noble metals such as nickel can greatly increase reactivity (Lien and Zhang 1999, He and Zhao, 2005, Wang and Zhang, 1997). This is because Ni or Ag, on the iron particle surface oxidises less rapidly than $Fe^0$, hence preserving the $Fe^0$core for chlorinate organics degradation. Nano-scale zerovalent attached to a noble metal forms galvanic cells in the coupled bimetallic

particles, wherein iron serves as the electron donor and preferably reacts with the contaminants, whereas the noble metal (cathode) is protected. Also, noble metals such as Pd may further promote dechlorination reactions by their catalytic function. Another possible explanation for the increased reactivity of bimetallic systems has been suggested (Lee and Sedlak, 2008 and Tee *et al.*, 2005). Reported studies suggest that bimetallic nickel-iron nanoparticles exhibit enhanced yields of oxidants compared to nZVI. Bimetallic Ni coated Fe particles produced 85% greater yields of formaldehyde, from the oxidation of methanol, relative to nZVI. Their study also concluded that Ni addition did not enhance hydroxyl radical formation and the enhancement in oxidant yield was observed over a pH range of 4-9. Their study put forward the view that nNi-Fe surface is less reactive towards hydrogen peroxide ($H_2O_2$) than the nZVI surface, which favours the reaction of $H_2O_2$ with dissolved Fe(II) (the Fenton reaction); hence an enhancement in reactivity.

In contrast to the aforementioned studies, results from the present study suggest that bimetallic nNi-ZVI had comparable or lower reactivity compared to nZVI (Figure 4.7). This study demonstrated that the size of the ZVI particles investigated had an enormous effect on the reaction reactivity. Exceptionally high reactivity was exhibited by nano-scale ZVI for degradation of recalcitrant MWFs. This was attributed to the characteristically large surface area, per unit of volume, as reflected in Figure 4.7. In terms of reactivity, many studies have concluded that nano-scale iron degrades contaminants more rapidly than conventional forms of granular Fe (Wang and Zhang, 1997, Lowry and Johnson, 2004, Choe *et al*., 2001, Ponder *et al*., 2000, Schrick *et al*., 2002). The high reactivity of the nano particles is mainly attributed to their small particle sizes, leading to very high specific surface area (~30 $m^2/g$), compared to that of conventional micro-scale iron (typically < 1 $m^2/g$), providing more sites on which reactions occur (Li 2006). For contaminants that are

degraded by Fe, such as TCE, there is a direct relationship between contaminant reduction rate and the surface area of Fe (Johnson *et al*., 1996). Furthermore, the observed rate constant was directly proportional to surface area of the particles and mass concentration. Due to the small size and large surface area of the nanoparticles, the surface-area normalized rate constant has been shown to offer the most practical, general descriptor of

contaminant degradation kinetics in the literature. Previous studies have shown that the surface-area-normalised rate constants for the nanoscale iron particles were approximately two to three times greater than those of the commercially available microscale iron particles (Wang and Zhang, 1997, Lien and Zhang, 2001).

Other possible reasons why nano-scale Fe might have exhibited enhanced reactivity include the greater density of reactive surface sites and enhanced intrinsic reactivity of surface sites (Nurmi *et al*., 2005). Particle shape, size and composition are known to be important properties that affect the chemical and physical properties of nano-scale particles (Nurmi *et al*., 2005). Thus, obtaining uniform nano-scale particles is important (Wang and Zhang, 1997). In addition to having an increased surface area at nanoscale, metal particles have high surface energies, thus are even more reactive than would be expected from just the increase in surface area (McDowall, *2005).*

This study also demonstrates that greater reduction in pollution load was achieved under acidic conditions (Figure 4.9, 4.10, 4.11). An advantage of conducting the nZVI reaction at lower pH is that the final pH of the effluent attained a pH of approximately 7, after a reaction time of 1 hour. There was no change in pH when the initial pH of the aqueous suspension was 7.5. Similar observations have been made in studies where attempts were

made to oxidise the herbicide molinate employing nano-scale zerovalent iron (Feitz *et al.*, 2005). Their study led to the conclusion that for every mole of oxygen initially consumed, assuming that $Fe^0$ was in excess, between 2 and 4 moles of $H^+$ ions were consumed, leading to a rise in pH. The rise in pH from acidic to near neutral was very beneficial for the second stage biological oxidation step. The nZVI treatment, at an acidic pH, ensured a greater reduction in COD and the final neutral pH made it beneficial for the subsequent biological oxidation treatment step. This eliminated the requirement to add alkaline reagents to adjust the pH to neutral range, which is essential in the case of Fenton treatments, where the pH of the final solution stayed at around 3 (Jagadevan *et al.*, 2011).

Work in this chapter has also shown that nZVI induced oxidation led to an increase in biodegradability index ($BOD_5$/COD) from 0.154 to 0.512 which furthermore led to a corresponding reduction in toxicity (Figure 4.15). The residual COD from the ZVI treated solution was amenable to a second stage degradation, in this case by bacteria activity. An acclimation period of 15 days was required for the bacterial consortia to acclimatise to the organic compounds present in ZVI treated solution. Microcosm studies showed an overall decrease of 95.5% COD after the ZVI-biological oxidation combinative treatment.

The results presented demonstrate that nanoscale ZVI particles may be technically feasible as a pre-treatment option for remediation of recalcitrant MWF wastewater. To help assess the economic viability of the process, an economic analysis was performed. Depending on the amount ordered and the modifications, commercially available nZVI costs around £100-125/kg of pure iron. The cost of preparing iron oxide in this study was around £75/kg of iron oxide (Assuming $Fe(NO_3)_{3.}9H_2O$ at the rate of £93/kg and urea at the rate of £25.5/kg).

Both prices sourced were from Fisher Scientific, UK. This iron oxide is further reduced under hydrogen atmosphere. So, the cost of hydrogen and the cost for running the electric furnace would further add on to the synthesis cost. This would bring the cost of synthesising nanoscale iron powder up to £85-90/kg. Thus with the treatment method developed, employing iron at a concentration of 5g $L^{-1}$, an estimated cost of £425/$m^3$ of recalcitrant MWF wastewater was calculated. It is worth noting at this point that the spent iron could be recycled and reused for further batches of wastewater. That would make great cost savings and suggest that even in today's market the technology would be economically feasible and its scale-up certainly worthy of further investigation.

Integrating ZVI-induced oxidation with aerobic biodegradation as investigated in this study is a novel treatment strategy and to our knowledge has never been investigated before, certainly not in the case of MWF effluent. *In-situ* generation of hydrogen peroxide, lack of a need for pH adjustment after treatment and the ability to recycle spent iron makes this treatment a sustainable option for remediation of operationally exhausted recalcitrant metalworking fluid. nZVI induced oxidation of the recalcitrant component of MWF thus appears to be complementary to aerobic biodegradation, particularly for treatment of highly recalcitrant MWF wastewater.

# CHAPTER 5

# OXIDATION OF RECALCITRANT METALWORKING FLUID EMPLOYING OZONE GAS

## 5.1 Overview of Gas-Induced Ozonation

Ozone is widely employed in water and wastewater treatment, due to its high oxidation potential. When applied in aqueous solution, substrate reactions occur partly by direct interaction with molecular ozone, and partly by indirect interactions with hydroxyl radicals, produced by the decomposition of ozone (Zouboulis *et al*., 2007, Haapea *et al*., 2002). The type of oxidant generated depends on reaction conditions such as pH. Under aqueous conditions, typical of natural waters and wastewaters, both direct and indirect reactions will proceed simultaneously, and the contribution made by indirect reactions (via radical attack) increases with increasing pH (Ning *et al.,* 2007).

At lower pH, molecular ozone reactions are predominant, where organic compounds are subjected to the electrophilic attack of ozone molecules which decompose into carboxylic acids as the final end products. The molecular ozone reactions are highly selective to the organic molecules, having nucleophilic/electrophilic moieties, such as carbon-carbon double bonds, -OH, $-CH_3$, $-OCH_3$, and other nitrogen, oxygen, phosphorus, and sulphur bearing functional groups. It is also known that compounds with aromatic structures and unsaturated bonds are selectively decomposed through ozonolysis (Iaconi *et al.,* 2003, Schepper *et al.,* 2009).

At high pH, ozone molecules are decomposed into free radicals, subsequently producing hydroxyl radical ($OH^{\bullet}$), which attack organic compounds. The radical reactions are non-selective, indiscriminate and highly reactive leading to very powerful chain reactions, which ultimately results in mineralisation of ozone-refractory organic compounds (Iaconi *et*

*al.,* 2003, Kwon and Lee, 2006). The hydroxyl radicals are able to oxidise ozone-resistant compounds, due to a higher redox potential (2.86 V for $OH^{\bullet}$ versus 2.07 V for $O_3$) and react fast with organic compounds and the water matrix itself.

## 5.1.1 Effect of ozone on degradation of two model pollutants

Alkaloamines and benzotriazole are common components employed in MWF formulations. They are added as additives to impart corrosion inhibition and as neutralisation agents to maintain an alkaline pH. The model pollutants focussed in the current research were monoethanolamine (MEA), triethanolamine (TEA) and benzotriazole (BZT).

### 5.1.1.1 Alkanolamines (Monoethanolamine and triethanolamine)

Alkanolamines like monoethanolamine (MEA) and triethanolamine (TEA) possess multiple functions in the MWF formulation. These amines are well known corrosion inhibitors and are neutralisation agents to stabilise the pH value in MWF. In addition, they possess antimicrobial properties. The antimicrobial effect of ethanolamines is suggested to be due to their surface-active properties which cause damage to the cell membrane (Bakalova *et al*., 2008). High concentration of these compounds in MWF formulations makes the MWF wastewater toxic to the microbial population employed in biodegradation. Alkanolamines include both amino and hydroxyl functional groups (Figure 5.1). Because of the amine functionality, they are basic compounds with acid dissociation constant (pKa) values in the higher pH range (pKa value are 9.68 and 7.7 for MEA and TEA respectively) (Bakalova *et al.,* 2008, Rabenstein *et al*., 2009).

$$H_2N-CH_2-CH_2-OH \text{ (Monoethanolamine)}$$

$$N-(CH_2-CH_2-OH)_3 \text{(Triethanolamine)}$$

**Figure 5.1**: Structure of MEA and TEA

The disposal of MEA and TEA containing wastewater is a problem, because they cannot be easily treated in wastewater treatment systems due to their toxicity effect and slow biodegradability. Despite the toxicity, several studies have reported biodegradation of these compounds at varying rates ((Rabenstein *et al*., 2009, Seo *et al*., 2007, West and Gonsior, 1996, Kim *et al*., 2010, Hawthorne *et al.,* 2005, Mrklas *et al*., 2004). MEA and TEA are biodegraded by a process that involves the hydrolysis of amines to ammonium and acetaldehyde. For the complete degradation of these amines, ammonium hydrolysed from MEA and TEA needs to be nitrified to nitrite and nitrate, by nitrification (Kim *et al*., 2010). Hence, nitrogen removal by nitrification/denitrification as well as biodegradation is imperative for successful treatment of these wastewaters.

#### 5.1.1.2 Benzotriazole

Benzotriazole (BZT) is a class of high production volume chemicals which are added as corrosion inhibitors and biocides in metalworking fluid formulations. It forms a thin complexing film on metallic surfaces, hence protecting the underlying metal from corrosion. It also provides a valuable source of reserve alkalinity in MWF formulations. (Weiss *et al*, 2006, Liu, 2011). In addition, BZT has been found to impart significant microbial toxicity and is quite resistant to microbial degradation (Molstad *et al.,* 2007, Jia *et al*., 2006). Efficient antibacterial performance is observed when added in concentrations between 1200-1500 parts per million (ppm) to in-use MWF (Karsa and Ashworth, 2002).

1 H-benzotriazole is weakly basic, of high polarity, is characterised by high water solubility (28 g $L^{-1}$), moderate tendency to partition into organic phase (log $K_{ow}$ 1.23) and hence may be very mobile in the aquatic environment (Weiss *et al*., 2006, Giger *et al*., 2006). Benzotriazoles contain a five-membered ring with three nitrogen atoms directly bonded to one another as substituents on a benzene ring (Figure 5.2).

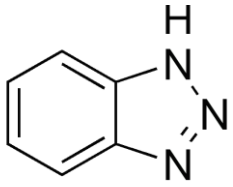



**Figure 5.2**: Structure of 1 H-Benzotriazole

Benzotriazoles have been classified as being toxic to aquatic organisms, causing long-term adverse effects. These compounds are very resistant to biodegradation and are toxic to microorganisms, plants and aquatic fauna. Several attempts to isolate organisms that could degrade benzotriazole as a C or N source have reported failure (Matamoros *et al*., 2010, Giger *et al*., 2006, Weiss *et al*., 2006, Rollinson and Callely, 1986). They also stated that there were no reports demonstrating evidence for the biodegradation of benzotriazoles. In addition to their recalcitrant nature, BZT is also an effective inhibitor of nitrification of nitrogenous compounds. It is a well-known nitrification inhibitor applied extensively in agricultural lands alongside nitrogen fertilizers (Callender and Davis, 2003, Puttana *et al*., 2001).

Although the aforementioned amines (MEA and TEA) are known to be biodegradable, the presence of BZT in MWF formulations is suspected to impart non biodegradability attributes to amines owing to the nitrification inhibition properties of BZT. This study focused on the fate of these target components (individually and as a mixture) by gas-induced ozonation. For benzotriazole, with its electron rich aromatic system, ozonation could be a viable treatment option (Leitner and Roshani, 2010, Hollender *et al.,* 2009, Weiss *et al*, 2006). In this study, the action of ozone on the degradation of a mixture of amines and BZT were examined, followed by investigating the feasibility of employing a post-biodegradation step.

Ozonation of organic compounds in water usually produces oxygenated organic products and low molecular weight acids that are more biodegradable. As a stand-alone technology, oxidation with ozone may not be economically favourable, mainly due to the applied high ozone doses. Therefore, a hybrid treatment approach integrating ozone oxidation with a robust and environment-friendly biological oxidation step for treatment of recalcitrant semi-synthetic waste MWF was investigated in this study. The experiments under this chapter were conducted in three parts. The first part consisted of performing gas-induced ozonation to a previously biologically treated recalcitrant MWF wastewater. The second part of this study examines the change in biodegradability index and toxicity of the pre-treated solution and checks the feasibility of combining the ozone treatment with a post-biological treatment. The last section in this chapter investigates the rate and extent of degradation of three toxic non-biodegradable MWF constituents, namely monoethanolamine, triethanolamine and benzotriazole.

## 5.2 Aims and Objectives

The overall aim of this part of the study was to investigate the role of ozone gas in improving biodegradability of recalcitrant MWF wastewater. The specific objectives were:

- To investigate the effect of ozone operating parameters such as ozone dose, contact time and pH on improving the biodegradability of MWF wastewater.
- To test the biodegradability of resultant intermediates.
- To test the feasibility of a coupled chemical-biological treatment of the recalcitrant component MWF wastewater.
- To test the hypothesis that reaction of $O_3$ and its concomitant $OH^{\bullet}$ radical with three recalcitrant compounds (monoethanolamine (MEA), triethanolamine (TEA) and benzotriazole (BZT)) would induce ring-cleavage and hydroxylated intermediates such as aldehydes and acids, which are more soluble and thus rendered more amenable to further biological degradation in the aqueous solution. Such an understanding is necessary for the design, application, and optimisation of an integrated treatment process of MWF wastewater.

## 5.3 Materials and Methods

### 5.3.1 Wastewater characteristics

Samples of a recalcitrant semi-synthetic MWF wastewater (Hocut 795-B, Houghton International Inc.) were obtained from Microbial Solutions Limited, Upper Heyford, Oxfordshire, a company which specialises in biological treatment of MWF effluent. Wastewaters were treated biologically in large-scale attached bio-filters, but the final effluent quality would not have conformed to the local discharge limit, without additional dilution with clean water. The pilot trial results demonstrated that about 80% COD in the wastewater could be removed through aerobic degradation in the biological pre-treatment step. The residual COD in the effluent was categorised as being refractory and resistant to removal by the normal biological process. In this study, the feasibility of employing gas-induced ozone gas for oxidation of recalcitrant chemicals to improve their biodegradability was investigated; in a subsequent step, the wastewater was treated by attached bio-filter process in the biological post-treatment section.

The content of the wastewater was very difficult to ascertain, due to the fact that the formulation details are proprietary and not available from the manufacturers and this obviously is due to commercial sensitivity of the information. The poorly defined composition of the wastewaters made detailed evaluation of the extent and rate of degradation of specific compounds and determination of intermediary components generated due to ozonation very difficult. The only information available regarding the chemical constituents of the fluids used in this study was obtained from the health and safety data sheet provided with the fluid concentrate. According to these data, the majority of the semi-synthetic MWF formulations consisted of 7-9% w/w monoethanolamine, 5-8%

w/w triethanolamine and 0.2-0.5% w/w benzotriazole as additives (Appendix 1: Seppic formulations). The physico-chemical parameters of this wastewater are summarised in Table 5.1.

**Table 5.1: MWF wastewater characteristics**

| Wastewater characteristics | pH | SS (mg/L) | COD (mg/L) | $BOD_5$ (mg/L) | $BOD_5$/COD | TOC (mg/L) | T-N (mg/L) |
|---|---|---|---|---|---|---|---|
| | **7.32** | **905** | **3100** | **395** | **0.127** | **675** | **278** |

## 5.3.2 Experimental set-up and ozone generation

Ozone was produced from feed gas air using a TRAILIGAZ ozone generator (Ozotech Burgess Hill, UK), which is capable of producing up to 9g $hr^{-1}$ of ozone. Compressed air was supplied as feed gas for the production of ozone at a rate of 0.550 L $min^{-1}$ (STP) and the ozone production rates were 11.84 mg/min and the inlet ozone concentration was 22 mg $L^{-1}$. Typically applied doses were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg $O_3$/mg COD of effluent corresponding to contact times of 10, 20, 30, 40, 50, 60, 70 and 80 minutes, respectively. Ozonation experiments were carried out in batch mode. The experiments were performed in a laboratory batch column reactor, consisting of a 18.5 cm long cylindrical wash bottle with 4 cm internal diameter. The gas containing ozone was bubbled into the liquid by means of a ceramic porous diffuser. The exhaust gas stream that was not retained in liquid mass exited from the top of the column through a wash bottle containing potassium iodide (KI) solution (2%) that traps dissolved ozone. The off-gas was vented to an ozone destruction unit. Figure 5.3 illustrates the experimental set up. Once the ozone generator was stabilised, ozone was fed to the reactor containing MWF recalcitrant wastewater with a COD of 3,100 mg $L^{-1}$. All experiments were performed at room

temperature using 100 mL sample, for various reaction contact times between 10 and 80 minutes.

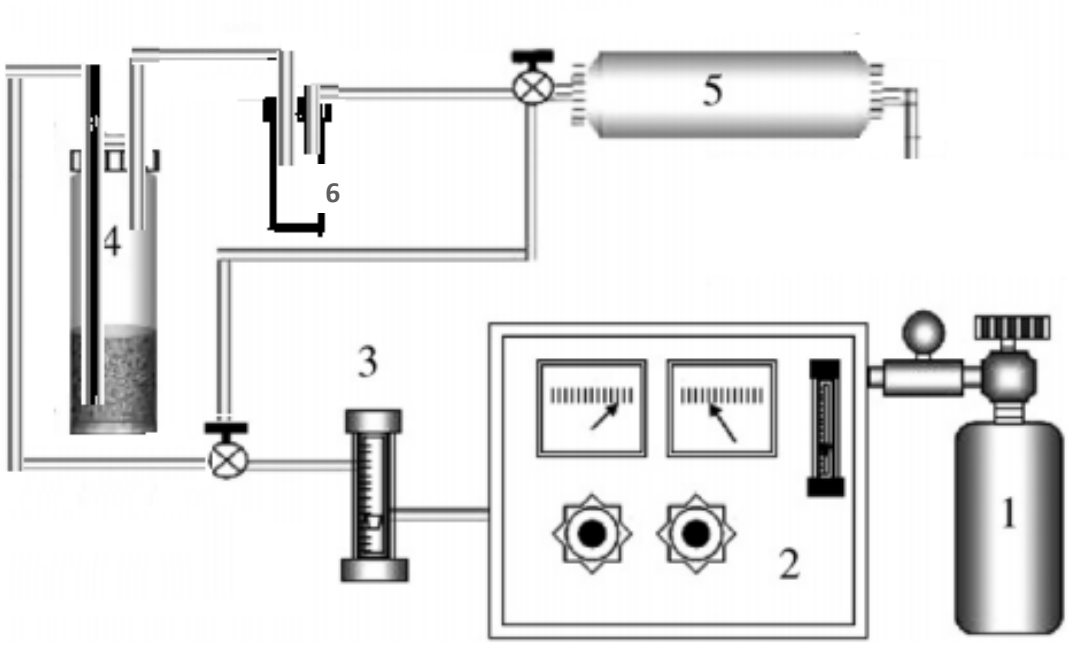



**Figure 5.3** : Experimental setup: 1. Air cylinder; 2. Ozone generator; 3. Flow-meter; 4. Reactor; 5. Ozone destructor, 6. 2% KI solution.

## 5.3.3 Determination of aqueous ozone

After ozonation, the residual concentration of ozone was measured by the standard indigo method; this has been widely used for pure ozone solutions or inorganic-buffered ozone solutions (Bader and Hoigni, 1981). In solution, ozone rapidly decolorizes indigo ($k > 10^7$ $M^{-1}s^{-1}$), and the decrease in UV absorbance of the indigo solution is linear with increasing concentration of ozone (Ning and Graham, 2008, Bader and Hoigni, 1981). A stock indigo solution of $2.5X10^{-3}M$ was prepared from potassium indigo trisulphonate and phosphoric acid. A working indigo solution of $1.25X10^{-4}M$ and pH 2 was prepared from the stock solution, sodium dihydrogen phosphate and phosphoric acid. The indigo method requires the indigo solutions to be fresh, and previously prepared solutions which were discarded

when the absorbance decreased to less than 80% of its initial value, typically within two weeks. The concentration of aqueous ozone was determined by UV spectrometry at 258 nm in a 5cm UV cell (molar extinction coefficient is 2,950 L $mol^{-1}cm^{-1}$). All experiments were performed under ambient conditions in an environmentally controlled laboratory where the room temperature was $21 \pm 1^0 C$ .

The term applied ozone dose is described by the following equations:

- Applied ozone dose (mg $L^{-1}$) =

$$\frac{\text{gas flow (L min}^{-1}\text{) x ozone concentration in inlet gas (mg L}^{-1}\text{) x Time(min)}}{\text{Volume of sample (L)}}$$

## 5.3.4 Chemicals and reagents

All reagents used in this study were of analytical grade and employed without any further purification. 1H-Benzotriazole 99% was obtained from Acros Organics. Alkanolamines (triethanolamine and monoethanolamine) were procured from Sigma Aldrich. All other chemicals and solvents were supplied by Fisher Scientific, UK. Reagents were prepared using 15 MΩ Milli-Q water.

## 5.3.5 Analytical techniques

In order to assess the treatability of the MWF effluent and the changes in its quality, the following parameters were analysed: total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand ($BOD_5$), absorbance at 254 nm (level of unsaturated

bonds), absorbance at 400 nm (colour), pH, and aqueous ozone concentration and toxicity tests.

TOC measurements provided a measure of mineralisation and were determined by direct injection of the sample into a Shimadzu TOC-$V_{CPH}$ analyser, provided with a non dispersive infrared (NDIR) detector and calibrated with standard solutions of potassium phthalate. COD measurements provided a measure of the relative oxidation of the organic mixture. This was analysed using dichromate solution as an oxidant, in a strongly acidic media, employing a Hach reactor HT200S and spectrophotometer (Hach DR 2800). $BOD_5$ measurements were carried out in Oxitop (WTW), according to the supplier's operating instructions. All $BOD_5$ measurements were conducted after adjusting pH to neutral range. pH measurements were made using a pH meter (Jenway 3345) and probe (model 924005, Keison Products, England). The level of unsaturated carbon bonds and colour were measured as the absorbance values at 254nm and 400 nm respectively in a Shimadzu UV-1800 spectrophotometer.

The toxicity of the ozone treated effluent was assayed by *Acinetobacter baylyi* ADP1_recA_lux biosensor, as described by Song *et al.*, 2009 in order to determine the toxic nature of the solution. This chromosomally based bacterial reporter expresses bioluminescence when exposed to any DNA damaging toxicants, and acts as a proxy of the response to toxic stress. The toxicity was quantified in terms of relative bioluminescence (absolute intensity of bioluminescence divided by $OD_{600}$).

### 5.3.5.1 Analysis of amines

The two amines selected for focus in this study (monoethanolamine and triethanolamine) were analysed by gas chromatography (GC) using a modified version of the method presented elsewhere (Marzo *et al*., 1990). The amines were measured using a gas chromatograph (Perkin Elmer, Clarus 600), equipped with a flame ionisation detector (FID). Impurities were removed from the MWF by centrifugation at 4000 rpm for 5 minutes followed by 0.45 µm filtration. Samples (1µL) were injected (split mode, 1:10) on to 15m X 0.25mm internal diameter Restek Rtx-5 amine (crossbond 5% diphenyl/95% dimethyl polysiloxane), fused silica column. Isothermal operation at $50^0$ C for 5 minutes was followed by a temperature increase of $20^0$ $min^{-1}$ up to $300^0$ C. Detector and injector were maintained at $300^0$ C and $250^0$ C respectively. Helium carrier gas flow rate was maintained at 1.5 mL $min^{-1}$.

### 5.3.5.2 Analysis of benzotriazole

The analysis of benzotriazole was performed using high performance liquid chromatography (HPLC), using a previously described method (Schmitt and Muzher, 1981). The liquid chromatographic system consisted of a Waters Associates model 2695, equipped with a Cl8 column, and photodiode array detector at a wavelength of 254nm. An aqueous buffer solution (0.08M) was prepared by dissolving sodium acetate trihydrate, followed by drop-wise addition of glacial acetic acid until a pH value of 6.0 was reached. The mobile phase was a 40:60 v/v mixture of methanol and aqueous buffer, used at a flow-rate of 1.5 ml/min under isocratic conditions. All solvents, standards and sample solutions were filtered (0.45 µm membrane filters) before injection. The volume injected was 10 µL.

## 5.3.6 Ozonation of model pollutants

The pure model/test pollutants in isolation were first subjected to ozonation to test whether these compounds are amenable to degradation by ozone gas. The initial concentrations of the pollutants were fixed on the basis of the usual concentration found in semi-synthetic MWF formulations (Appendix: Seppic formulations). According to these data, most of the semi-synthetic MWF formulations comprise of 7-9% w/w monoethanolamine, 5-8% w/w triethanolamine and 0.2-0.5% w/w benzotriazole as additives, which works out to be 3500 $mgL^{-1}$, 4250 $mgL^{-1}$ and 100 $mgL^{-1}$ of MEA, TEA and BZT, respectively. pH of 2 stock buffer solution was prepared by dissolving 10g of analytical grade sodium dihydrogen phosphate and 7 ml analytical grade phosphoric acid (d=1.7g $ml^{-1}$, 85%) in 1L Milli-Q high purity water (Ning and Graham, 2008). Higher pH buffer solutions were obtained by adding different quantities of 2M sodium hydroxide solution to the stock pH 2 buffer.

## 5.3.7 Biological treatment experiments

Aerobic biodegradation studies were carried out in 250 mL batch glass reactors, with a working volume of 100 mL, maintained at an rpm of 120 at $28^0$ C as detailed in chapter 3.

## 5.4 Results

### 5.4.1 Effect of contact time and ozone dose

Increasing ozone dose increases the mass transfer rate of ozone to the effluent and this, in turn, increases the reaction rate. Figure 5.4 investigates the effect of contact time and ozone dose (which is calculated based on the ozonation time) on COD removal for recalcitrant MWF wastewater. The ozone consumption determined for waste MWF in this study was in the range of 0.5–3.0 g $O_3$/g of COD removed. Contact time of 15, 30, 45 and 60 minutes for initial COD concentrations ($COD_0$) of 3100 mg $L^{-1}$ were investigated. Nearly 30% removal in COD was observed within 60 minutes of the reaction time. It has previously been reported (Walker and Armstrong 1995) that high ozonation rates result in a greater degree of removal for certain organic compounds. High concentration MWF wastewaters would therefore require linear enhancement in ozone dose and contact time, depending on the organic content of the wastewater (COD/TOC) as evidenced by Figure 5.5.

There is an optimal ozone dosage which enables the maximum increase in subsequent biodegradability and a high efficiency of the ozonisation processing. Further ozonisation did not help in enhancing biodegradability and was in fact disadvantageous. Although more exhaustive chemical oxidation of MWF wastewater by ozone gas may have resulted in more complete mineralisation; the dose of ozone in such cases would have far exceeded the level that is economically feasible for water treatment.

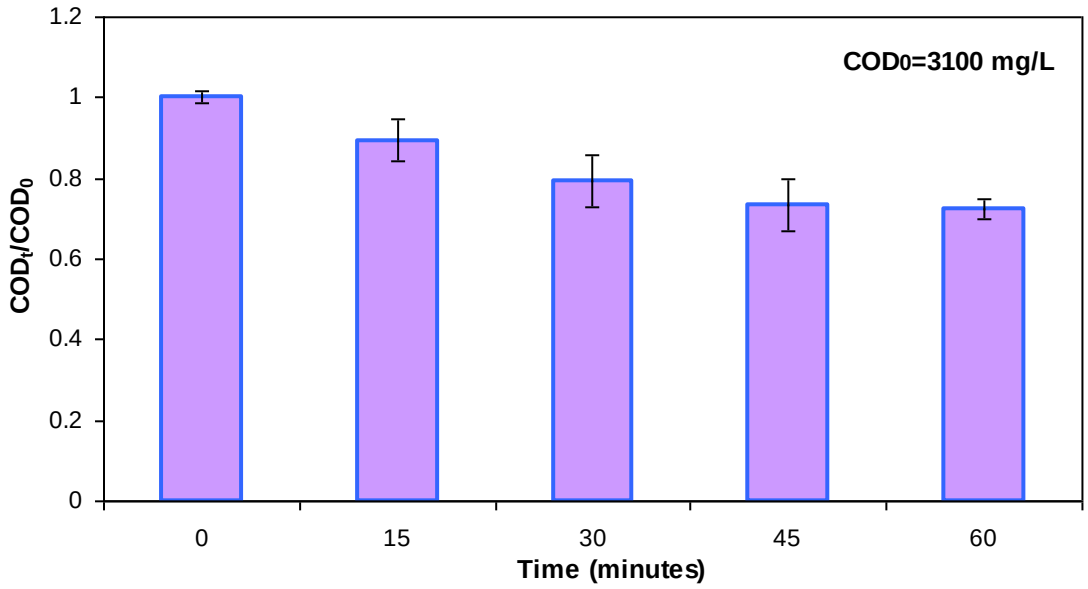



**Figure 5.4**: Effect of contact time and ozone dose on COD removal (unaltered pH (7.4), Ozone dose= 0.5–3.0 g $O_3$/g of COD) (Mean ± SD of triplicates shown).

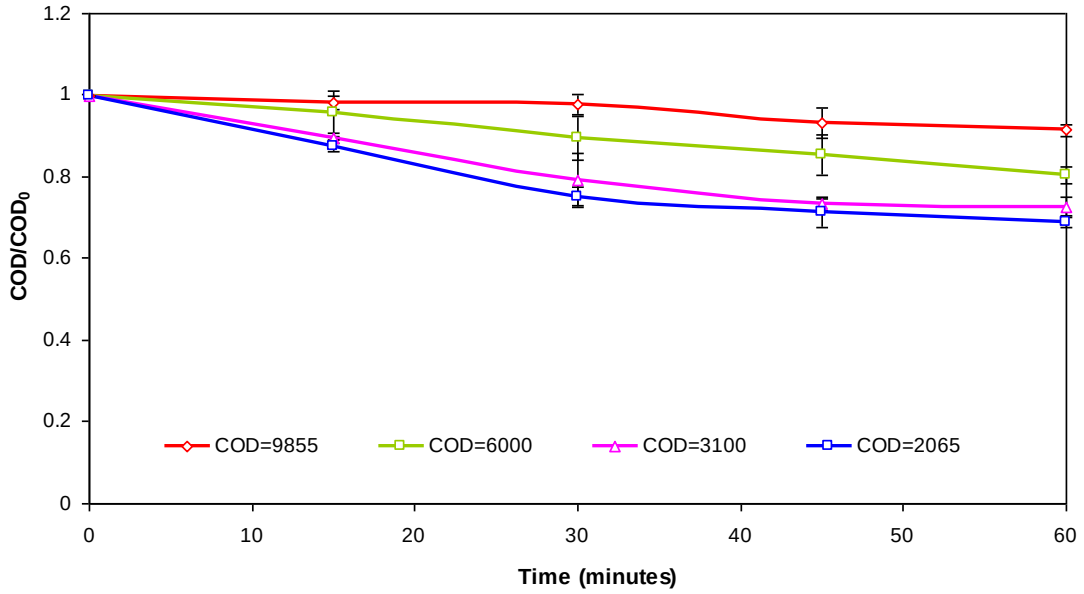



**Figure 5.5**: Effect of contact time and ozone dose on COD removal of wastewater with varying strength (unaltered pH (7.4), Ozone dose= 0.5–3.0 g $O_3$/g of COD) (Mean ± SD of triplicates shown).

## 5.4.2 Absorbance at 254nm (Aromaticity)

The reduction of UV absorbance at 254 nm has been identified as an appropriate surrogate to assess oxidation of organic compounds (Wert *et al*., 2009). The value of the absorbance at 254 nm ($A_{254}$) is indicative of the level of unsaturated carbon bonds, such as aromatic compounds, contained in a water sample. After the ozone treatment, a decrease in absorbance at 254nm was observed over a wide range of pH. Figure 5.6 shows more than 50% reduction in absorbance was observed even at the lowest ozone dosage of 1.0 mg $O_3$/mg COD. Further increase in the ozone dosage and prolonging the contact time failed to lower absorbance below 1.5 achieved at a dosage of 1.0 mg $O_3$/mg COD. The reduction varied from 12.5% to 53% for the highest ozone doses with a typical value being in the region of 50%.

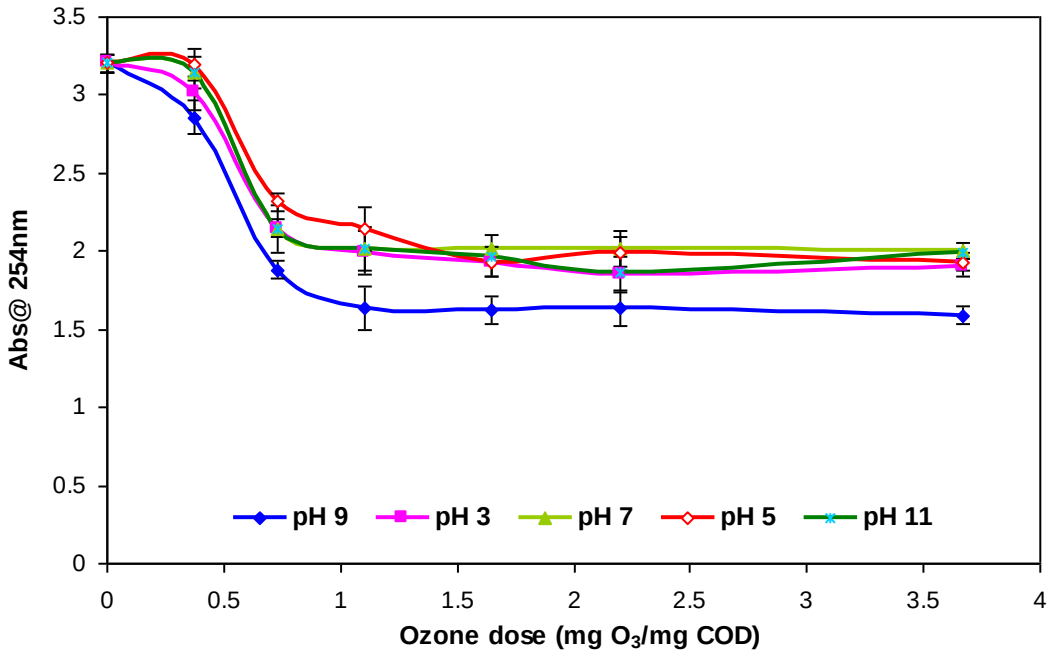



**Figure 5.6**: Effect of gas-induced ozonation on ultraviolet absorbance at 254nm ($COD_0$=3100 $mgL^{-1}$) (Mean ± SD of triplicates shown).

Reduction in $A_{254}$ absorbance is attributed to the reaction of ozone with double bonds and splitting of the aromatic rings of the organics in the MWF wastewater leading to formation of simpler organic matters. These compounds are generally recalcitrant to biodegradation, and a decrease of absorbance usually results in an increase in the biodegradability potential of the sample.

### 5.4.3 Absorbance at 400nm (Colour)

Ozone gas is a bleaching agent and it is known to decolourise effluents from paper and pulp, olive mill and textile industry. The reduction in colour could be attributed to the direct attack of ozone on carbon double bonds in the chromophoric groups, resulting in the formation of bleached products, such as aliphatic acids, ketones and aldehydes. The reduction of absorbance at 400 nm ($A_{400}$**)** has been identified as an appropriate surrogate to assess decolourisation of waste effluents (Wert *et al.,* 2009). Figure 5.7 shows the effect of ozonation in $A_{400}$. The initial dark colour of the wastewater sample was decolourised by the oxidation via gas-induced ozonation under all pH investigated even at a short contact time of 15 minutes (Figure 5.8). The final $A_{400}$ values of the treated samples under all pH investigated were comparable.

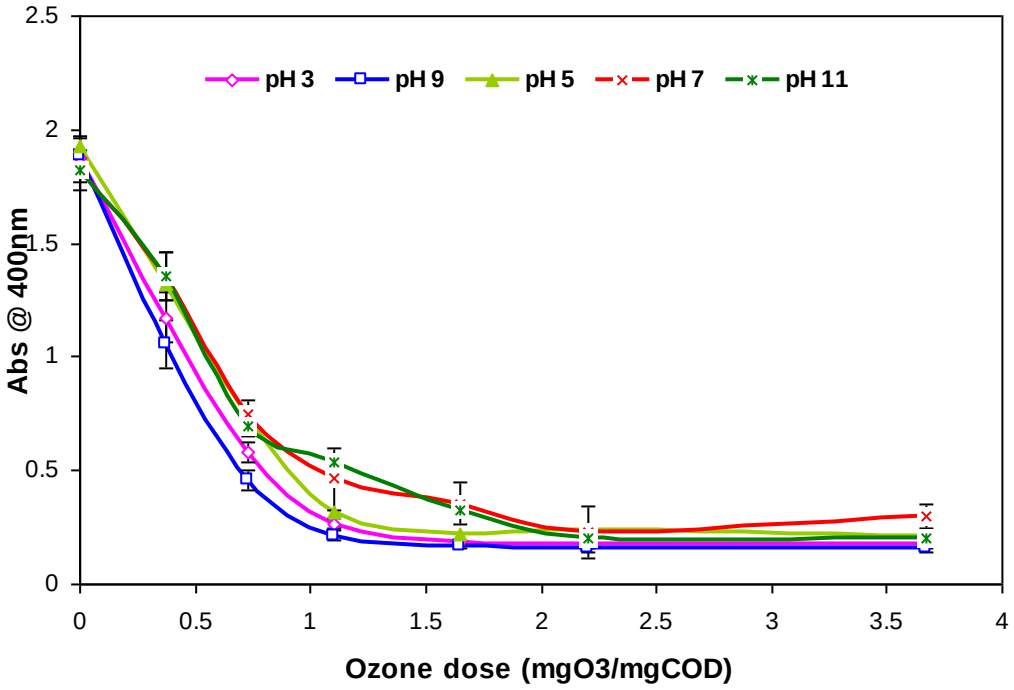



**Figure 5.7**: Effect of gas-induced ozonation on ultraviolet absorbance at 400nm. (Mean ± SD of triplicates shown).

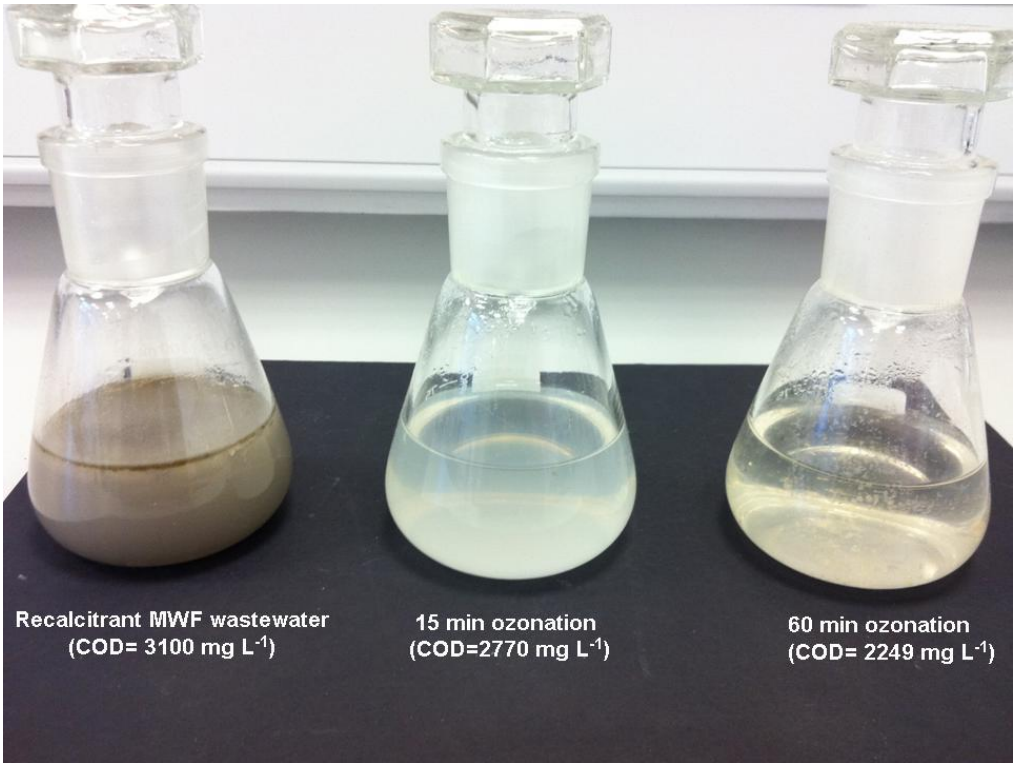



**Figure 5.8**: Pictorial representation of decolourisation of MWF wastewater by ozonation.

## 5.4.4 Effect of pH

### 5.4.4.1 Variation in pH with ozonation time

The wastewater pH has been reported to play an important role in the aqueous phase ozone oxidation of organic pollutants. The effect of initial pH values of the aqueous solution on the ozonolysis of recalcitrant MWF wastewater was investigated at different pH in the range of 3.0-11.0 with an initial COD concentration of 3,100 mg $L^{-1}$ and at room temperature. In Figure 5.9, it can be seen that the initial pH values of 5, 7, 9 and 11 decreased with ozonation, until a steady state was reached. This is due to the generation of by-products of an acidic nature (inorganic acids and organic anions) led to a decrease in the solution pH (Souza *et al.*, 2010, Benitez *et al.*, 2001). The decrease in pH was very pronounced in the initial phase of the process (particularly for alkaline solutions) because there was a greater ozone consumption and degree of pollutant degradation /mineralisation at elevated pH. It was also observed that with an initial pH of 3 there was a slight pH increase until relatively stable values were reached, close to those observed for the other initial pH values.

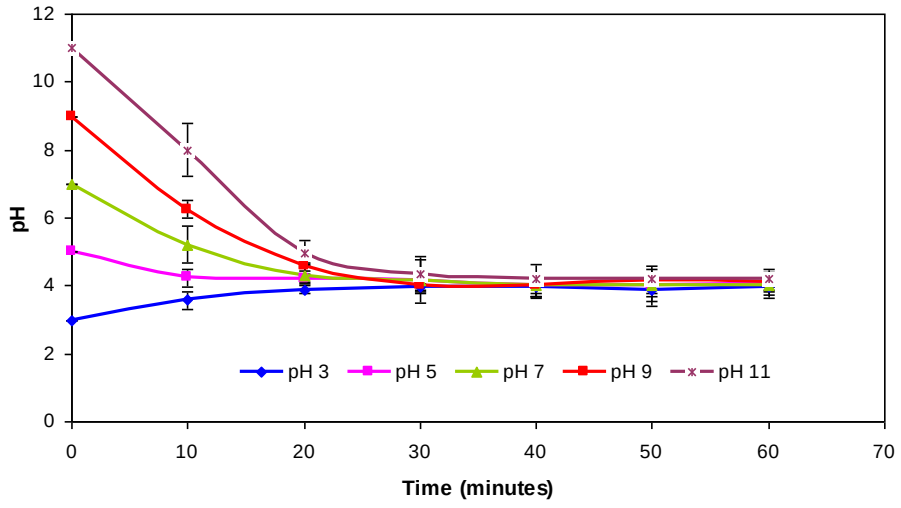



**Figure 5.9**: Variation in pH with ozonation time for recalcitrant MWF (COD = 3100mg $L^{-1}$, room temperature) (Mean ± SD of triplicates shown).

### 5.4.4.2 Effect of pH on COD removal

The effect of ozone on the COD of an effluent depends on the effluent matrix, the initial COD value, on the treatability of the water sample (whether the compounds are easily oxidisable or recalcitrant) and on other factors that can affect the production of free radicals, such as pH and alkalinity. The effect of initial pH on COD removal of recalcitrant MWF during a gas-induced ozonation process was studied under basic and acidic conditions. The results from a number of runs are presented in Figure 5.10. In all experiments, the trend was that the total COD was systematically reduced with increasing ozone dose and pH (until 9.0).

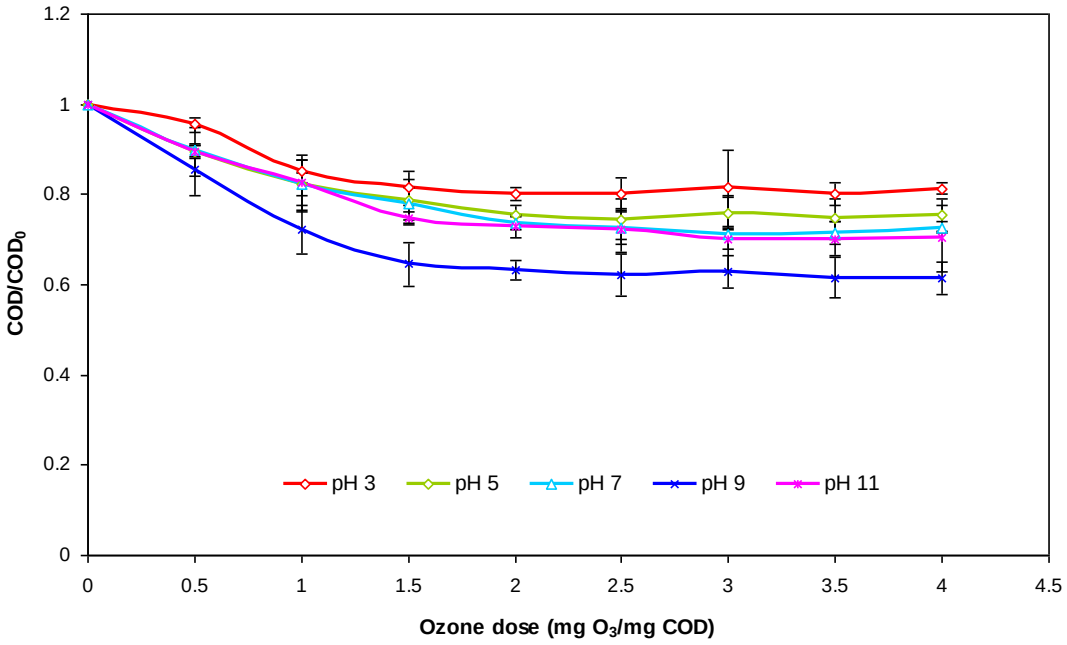



**Figure 5.10**: Effect of initial pH on COD removal ($COD_0$=3,100 mg $L^{-1}$, temp=293K). (Mean ± SD of triplicates shown).

This study demonstrated increased elimination rates of COD under alkaline pH, reaching its maximum at pH 9 (COD reduction=39%). This is because hydroxyl radicals initiate and

propagate oxidation processes at elevated pH. Reduction rates of 20%, 24.7% and 28.5% COD were achieved at pH 3.0, 5.0 and 7.0, respectively. For pH 3.0 and 5.0, an increase in COD with increasing ozone dose was detected. The increase in COD may have been due to organic molecules being oxidised by ozonation, resulting in small organic molecular fragments, such as acetic acid, aldehydes, ketones, which are not completely mineralised under the oxidative conditions described, contributing to an increase in COD with ozonation time (Souza *et al.*, 2010, Bijan and Mohseni 2005). Due to the complex nature of wastewaters, organic substances were not completely degraded by ozone, resulting in the formation of several aforementioned by-products. At pH> 9 (11.0), a drop in COD degradation was noticed when compared to pH 9.0. This may be due to decreased stability of ozone which hinders the formation of $OH^{\bullet}$ radical. Figure 5.11 depicts the pseudo-first order plots for COD reduction. The calculated degradation rate constants were $3.2x10^{-3}$, $4.8x10^{-3}$, $5.2x10^{-3}$, $7.9x10^{-3}$, and $6.1x10^{-3}$ at pH 3.0, 5.0, 7.0, 9.0, 11.0 respectively. These results also indicate that the degradation rate constants increase with increase in pH, with a maximum degradation rate at pH 9.0.

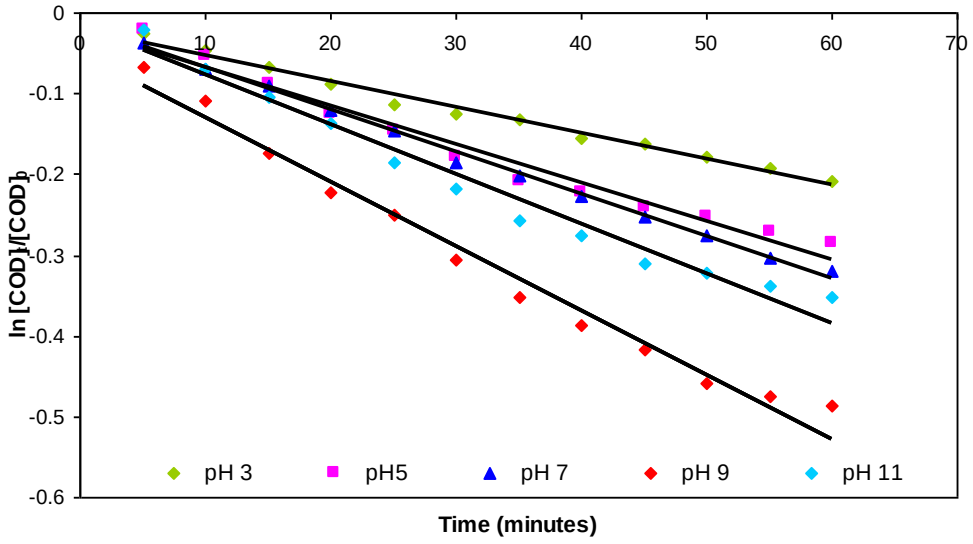



**Figure 5.11**: The effect of pH on the COD degradation rate (initial COD=3,100 mg $L^{-1}$, Temperature 293 K) (Mean ± SD of triplicates shown).

Changes in the COD of wastewater observed in this study were the results of the oxidation state of carbon in the organic matter and variations in the concentration of organics in the wastewater. The average oxidation state (AOS) of carbon is an indicator of the degree of oxidation of carbon atom in a chemical compound. It is defined by $AOS = 4 - (1.5 * \frac{COD}{TOC})$, where AOS takes values between -4 (i.e. for methane) and +4 (i.e. for carbon dioxide) and TOC and COD are expressed in mass concentrations. Higher AOS values consequently indicate a higher oxidation state of the organics in the mixture.

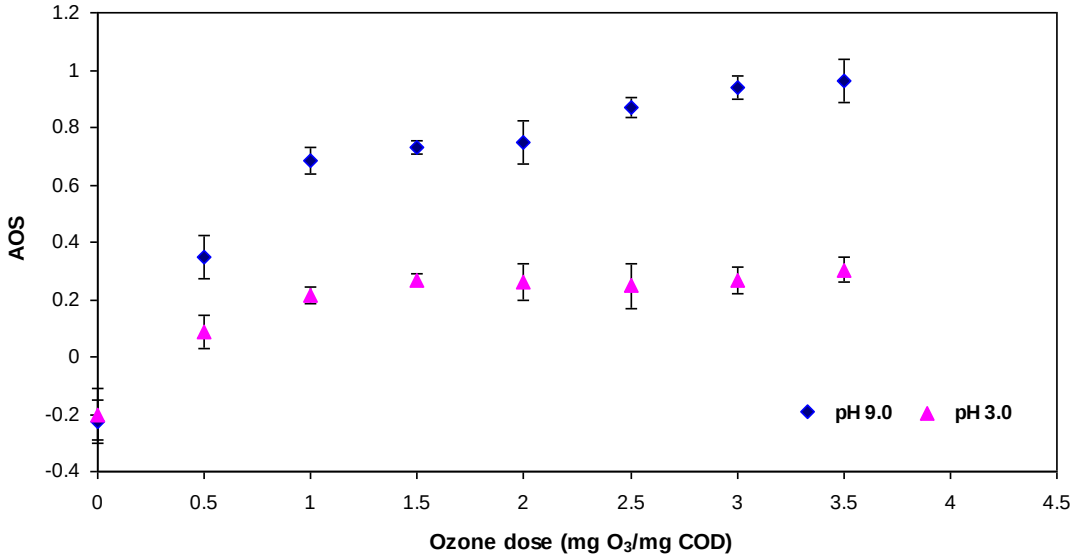



**Figure 5.12**: Effect of ozonation on average oxidation state of carbon in MWF wastewater under acidic (pH=3.0) and alkaline (pH=9.0) conditions (Mean ± SD of triplicates shown).

Figure 5.12 shows a comparison of the AOS values obtained during direct and advanced ozonation at pH 3.0 and 9.0, respectively. It can be seen that the AOS increased to nearly 1.0 from -0.2 under alkaline conditions, when compared to AOS value of 0.2 in acidic pH. This proves that the organics in the reaction mixture had become more oxidised after ozone treatment. Ozonation therefore facilitated the decomposition of exposed chemical structures

and the addition of oxygen (or ozone) to the molecules, thereby leading to their greater oxidation state and lower COD values.

### 5.4.4.3 Effect of pH on TOC removal

The effect of pH on TOC removal was studied and Figure 5.13 illustrates TOC degradation curves under varying pH (3-11). An increase in TOC removal was attained with an increase in pH, dissolved ozone dosages and at longer ozone contact times. TOC removal observed under varying ozone dosages and contact times employed in the batch experiment, ranged from 1.6 to 12.5%. The results showed that the change in TOC was lesser compared to the reduction in COD. The maximum decrease in TOC was 12.5% at pH=9.0. The TOC reduction for the other pH investigated (3, 5, 7 and 11) were 1.86%, 4.13%, 7.81% and 8.53% respectively, with greater TOC degradation occurring with increasing pH. This could possibly be due to larger concentrations of hydroxyl free radicals generated at greater pH. The TOC removal at 60 minute contact time, with a dissolved ozone dosage of 4.0 mg $O_3$/mg COD, was only marginally greater (less than 0.5%) than at a dissolved ozone dosage of 2.0 mg/L at 30 minute contact time, for all pH investigated.

This negligible reduction in TOC provides further evidence to suggest that mineralisation was difficult to achieve under the applied ozone doses and that the reaction lead to the formation of intermediate products. In order to achieve effective TOC reduction, a significant part of the organic carbon must be completely oxidised to $CO_2$ and the doses examined in this study were unlikely to have led to a large degree of complete oxidation.

The supplied ozone was therefore assumed to be primarily consumed in converting the parent organic contaminants to intermediates, thereby reducing the system COD.

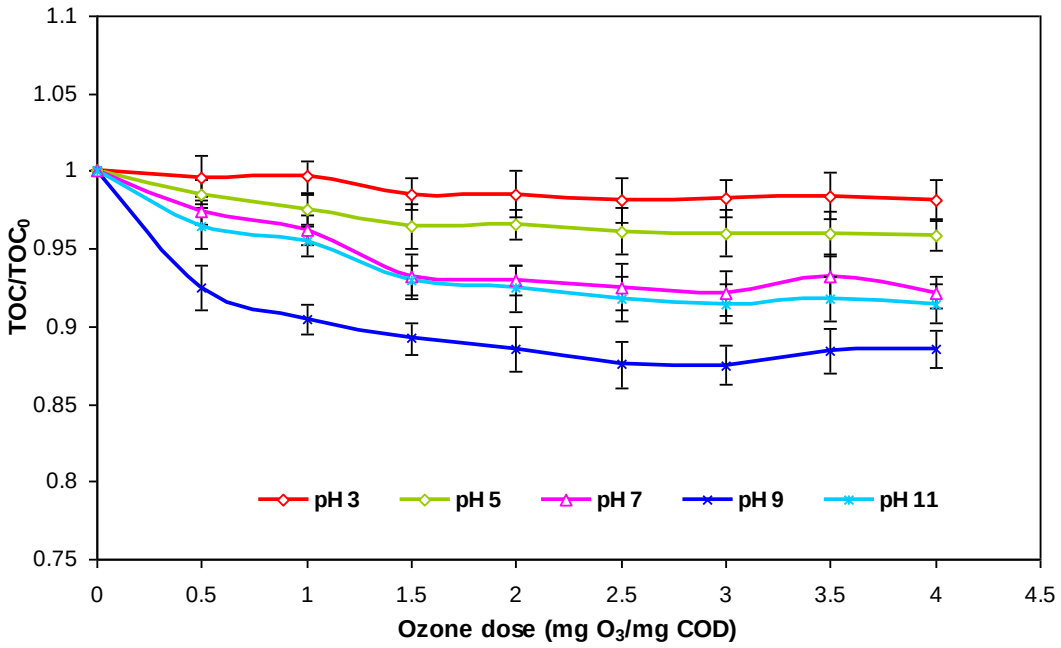



**Figure 5.13**: Effect of initial pH on TOC removal ($TOC_0$=675 mg $L^{-1}$, temp=293 K) (Mean ± SD of triplicates shown).

The results of COD and TOC degradation assessments, which highlighted accelerated degradation and higher elimination rates at increased pH, are in agreement with previously reported observations where ozonation of various organics have been investigated (Ning and Nigel 2007, Popiel 2009, Iaconi *et al.*, 2003, Schepper *et al.*, 2009, Bijan and Mohseni, 2005, Lin and Kiang, 2005, Munter 2001, Bijan and Mohseni, 2004, Souza *et al.*, 2010).

### 5.4.4.4 Effect of pH and ozonation on biodegradability

The effect of initial pH on COD and $BOD_5$/COD of the recalcitrant MWF effluent during the ozonation process was studied under basic and acidic conditions (Figure 5.14). Given

the sensitivity of the ozone oxidation to pH (Bijan and Mohseni, 2005, Lee *et al.*, 2009, Oeller *et al.*, 1997), this study helped determine the condition that enabled the greatest improvement in biodegradability during the chemical treatment. Figure 5.14 shows that under basic operating conditions (i.e. pH=9) biodegradability improved (monitored as $BOD_5$/COD enhancement) as did COD removal. Recorded COD reductions were 18.75% and 38.5%, resulting in biodegradability ($BOD_5$/COD) of 0.243 and 0.301 under pH of 3.0 and 9.0 respectively at maximum ozone dose of 4.0 g $O_3$/L from an initial value of 0.127.

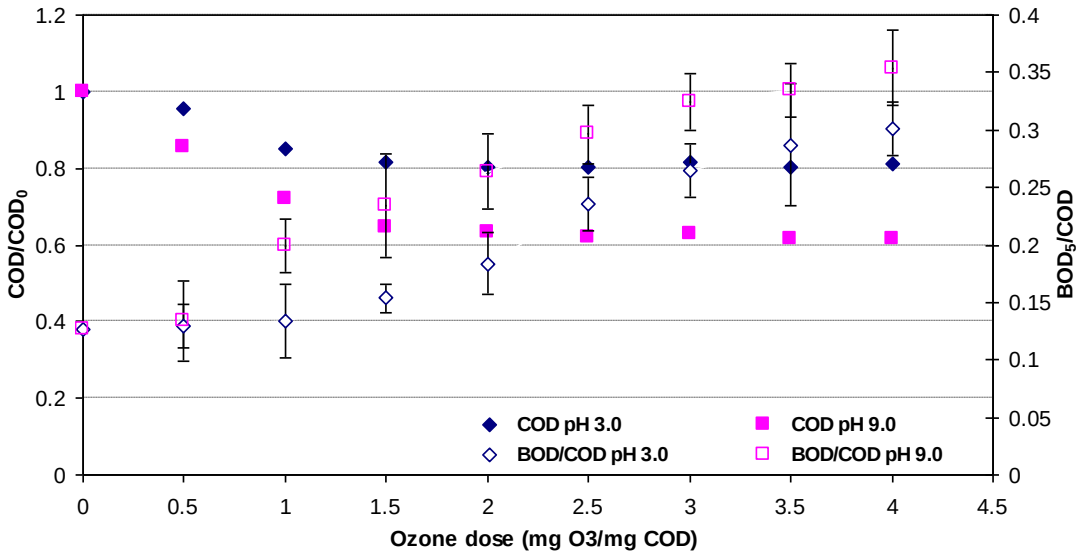



**Figure 5.14**: Effect of change in pH on COD removal and biodegradability (Mean ± SD of triplicates shown).

The superior performance of ozonation under basic pH conditions was largely due to the reaction of organics with molecular ozone and the generation of oxidising hydroxyl radicals (Glaze *et al.*, 1987). Given the significantly higher kinetic rate constants for the reaction between $OH^{\bullet}$ and typical model organic pollutants in the wastewater (Glaze *et al.*, 1987), the oxidation potential of ozone is enhanced under basic conditions when oxidising radicals are produced which participated in the reactions. Ozonation under acidic conditions is more

limited since the reaction of ozone is selective towards unsaturated organics of the wastewater. Furthermore, the oxidation ability of ozone is far lower than the oxidizing radicals (e.g. hydroxyl radical) formed under basic pH (Munter 2001).

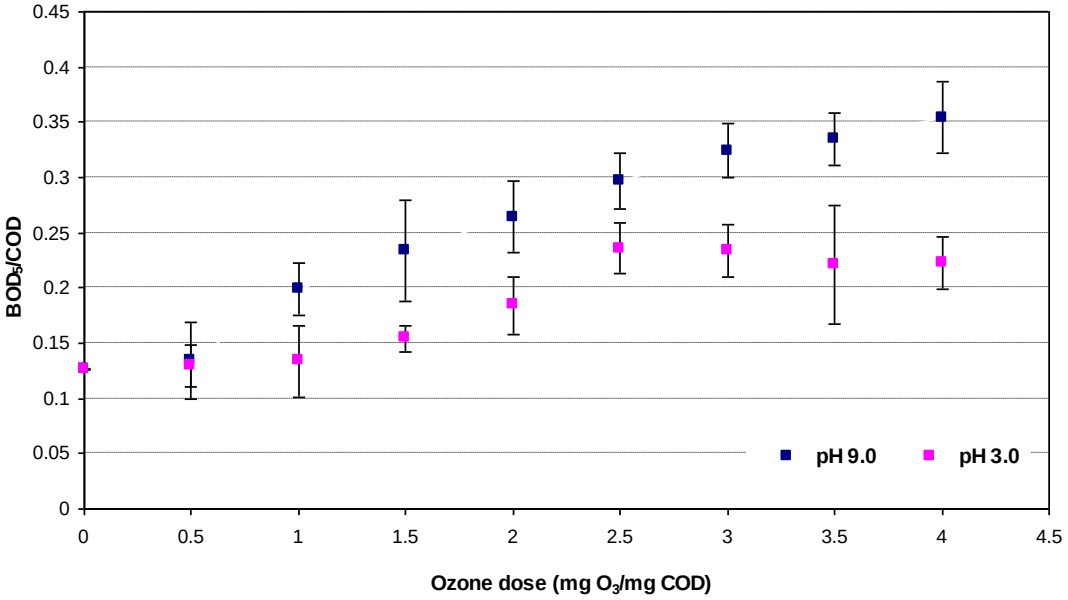



**Figure 5.15**: Effect of pH on biodegradability index ($BOD_5$/COD) (Mean ± SD of triplicates shown).

Ozonation under alkaline conditions enhanced the total amount of biodegradable compounds, measured as $BOD_5$/COD, from 0.127 to 0.354 for the overall ozone dosage of 4.0 g $O_3$/mL wastewater. Partial oxidation of dissolved organic matter by ozone was the desired effect of the chemical pre-treatment and indicates that the oxidising power of ozone was not wasted on mineralisation of organic compounds (COD removal), which can be done at lower cost, in a subsequent biological treatment. Similar enhancement in biodegradability following ozonation pre-treatment has been observed by several researchers (Morais *et* al., 2008, Coelho *et al.*, 2009, Bijan and Mohseni, 2004, Bijan and Mohseni, 2005). It is, however, worth noting from Figure 5.15 that the $BOD_5$/COD value under acidic conditions declined after 45-50 minutes of ozonation time, corresponding to a

dose of 3 mg $O_3$/mg COD. This may have been due to toxic by-products generated under ozonation.

### 5.4.4.5 Effect of direct (pH=3.0) and advanced (pH=9.0) ozonation on toxicity

The toxic effects of the raw and treated effluents were estimated by employing chromosomally based luminescence sensor *(Acinetobacter baylyi* ADP1_recA_lux), (Song *et al.,* 2009) which displays luminescence only in presence of any DNA damaging toxicants. With this sensor, increased luminescence was indicative of toxicity. Toxicity was quantified in terms of relative bioluminescence (absolute intensity of bioluminescence divided by $OD_{600}$). Figure 5.16 illustrates the effect of ozonation by-product toxicity under acidic (pH 3.0) (direct ozonation) and basic pH (9.0) (advanced ozonation) conditions. It can be clearly seen that direct ozonation generated more toxic effluent. Toxicity was found to reach a pronounced minimum for advanced ozonation at ozone doses varying from 2.0-4.0 mg $O_3$ $mg^{-1}$ COD.

Advanced ozonation led to a sharp decline in toxicity, thereafter it remained stable. However, toxicity of the effluent under direct ozonation increased considerably after 50 minutes of ozonation (3 mg $O_3$/mg COD). Increased toxicity during direct ozonation under acidic conditions could have been due to the formation of new toxic by-products and bio-sorbable matter, adding to the overall toxicity. This view is supported by the evidence of the lower biodegradability observed under acidic conditions in the previous section (5.4.4.4.). Advanced ozonation thus resulted in a significant lowering of final effluent toxicity and generates more bio-removable COD resulting in a more effective interaction

between chemical and biological oxidation stages. This study thus provides evidence that COD and toxicity removal efficiencies performed best at elevated (pH 9.0), when compared to an acidic pH of 3.0.

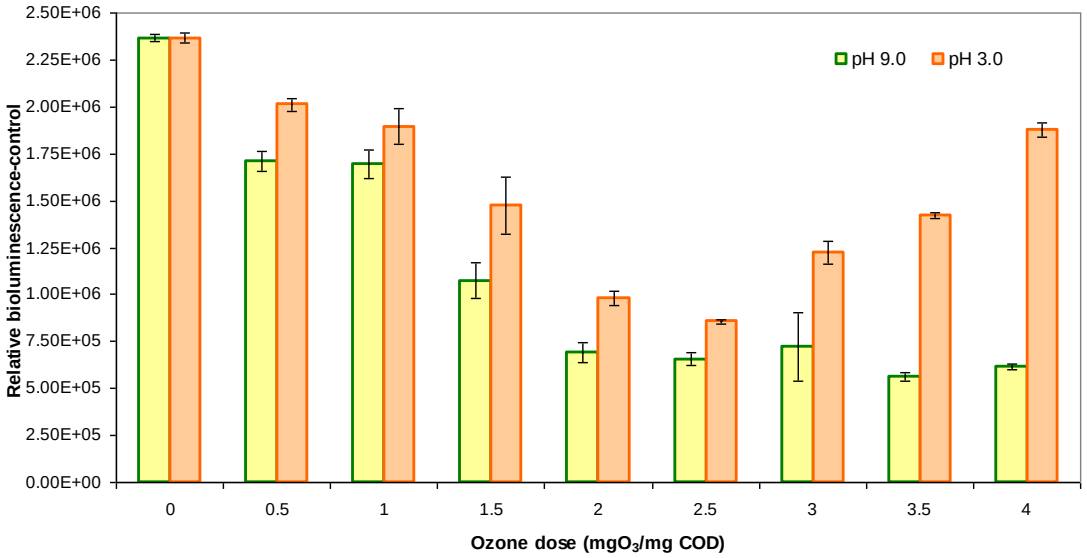



**Figure 5.16**: Effect of direct and advanced ozonation on toxicity (Mean ± SD of triplicates shown).

## 5.4.5 Biodegradation studies

The ozone pre-treated samples demonstrated an enhancement in biodegradability (monitored by $BOD_5$/COD) and lowering of toxicity. The reduction in the toxicity and the concurrent incremental change in the biodegradability index may have been due to the fact that ozone treatment was able to change the structure and properties of non-biodegradable compounds into those which were more bio-available, hence making the ozone treated effluent more susceptible for a further biodegradation process. Residual ozone is very toxic to aquatic organisms and hence steps were taken to ensure complete removal of residual ozone before subjecting the ozone treated effluent to a post-biological oxidation step. During the start-up, all reactors were fed with dilute operationally exhausted MWF

(COD=8,000-10,000 $mgL^{-1}$) diluted with tap water as a carbon source. An acclimation period was necessary in order to gradually expose the microbial community to the potential inhibitory nature of the toxic organic compound; this allows for the development of appropriate enzyme producing agents that are essential to induce biodegradation of MWF components. Stabilisation of reactor was assessed by measurement of COD reduction. In this study, almost 45 days were required for start up of the reactors, i.e. to achieve steady state COD reduction.

After acclimation of the cultures to MWF components, the amount of ozonated wastewater was then increased step by step. This percentage was increased progressively until achieving 100% of the ozonated solution as sole carbon source. The bacterial consortia consisted of *Agrobacterium radiobacter, Comamonas testosteroni, Methylobacterium mesophilicum, Microbacterium esteraromaticum and Microbacterium saperdae*, all of which were enriched from operationally exhausted MWF and have previously been reported to be effective for bio-treatment of waste MWF (van der Gast *et al*., 2003).

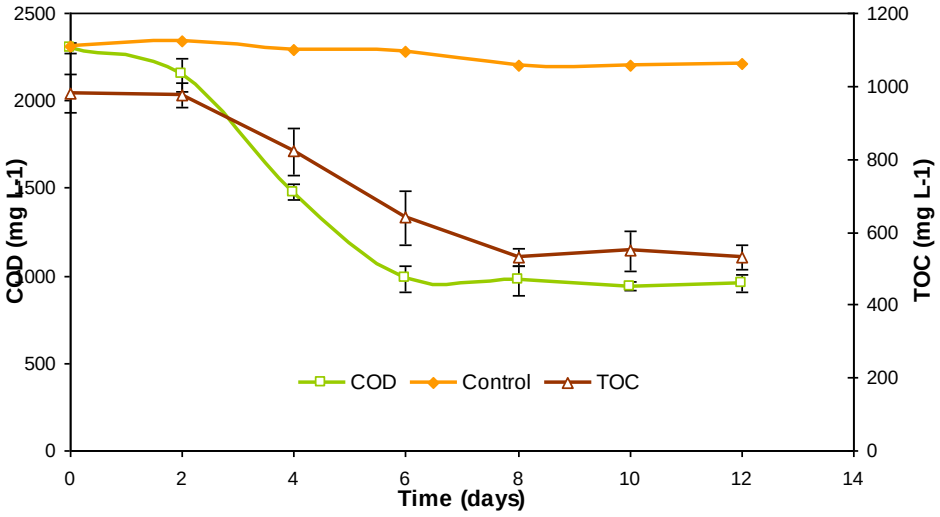



**Figure 5.17**: COD and TOC profile in the fixed bioreactor charged with ozone treated effluent (Mean ± SD of triplicates shown).

Aerobic biological experiments conducted employing acclimated microorganisms confirmed the suitability of the biodegradation after the chemical oxidation pre-treatment. Aerobic biodegradation experiments carried out using acclimated cultures were demonstrated to further reduce the remaining COD content to a value of around 850mg $L^{-1}$ COD in 8 days (Figure 5.17). The ozone treated effluent when further subjected to biological degradation achieved an overall 72.38% and 54.7% drop in COD and TOC respectively. The above observation clearly demonstrates that pre-treatment with ozone reduces toxicity of the MWF and is capable of enhancing the extent of residual pollutants biodegradation. The integrated chemical-biological system seems a feasible approach for treating recalcitrant compounds in MWF wastewater. Evidence provided here suggests that pre-treatment by chemical oxidation is effective at transforming otherwise recalcitrant MWF components in to biologically degradable intermediates.

## 5.4.6 Effect of ozonation on degradation of pure model pollutants

This experiment was conducted without adjusting the pH and was run for 15 minutes (~4000 $mgL^{-1}$ ozone). Figure 5.18 (a) and (b) are the GC and HPLC chromatographs illustrating the effect of ozone on degradation of amines and BZT respectively. It can be clearly seen from the chromatographs that all three compounds were amenable to degradation under gas-induced ozone gas. The oxidation condition examined was set at a ozone delivery of 8.9 mg/L. Initial concentration of BZT concentration was 100 mg $L^{-1}$ and that of MEA and TEA were fixed at 3500 mg $L^{-1}$ and 4250 mg $L^{-1}$.

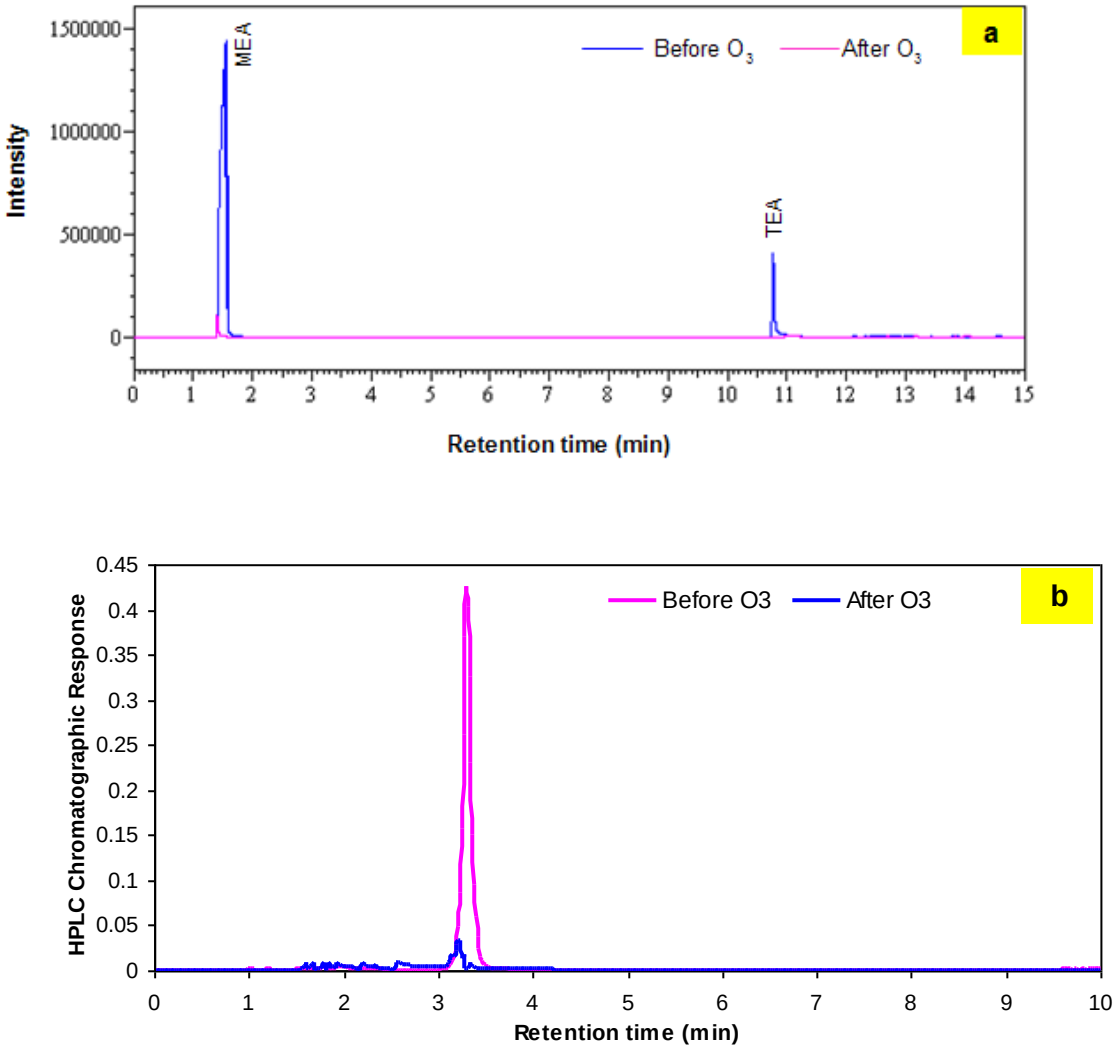



**Figure 5.18**: Effect of ozonation on degradation of (a) GC chromatograph of MEA and TEA (b) HPLC chromatograph showing BZT ([MEA]=3500mgL$^{-1}$,[TEA]=4250mgL$^{-1}$,[BZT]=100mgL$^{-1}$, Time=15 minutes, ozone dose=4000mgL$^{-1}$)

### 5.4.6.1 Effect of reaction time and ozone dose on MEA, TEA and BZT degradation

After confirming that all compounds are oxidised by ozone, mixtures of the amines and BZT were prepared. The rationale behind this step being that since BZT was being oxidised under the influence of ozone, its reduction would not arrest complete oxidation of the amines, via inhibiting nitrification, and hence we would be able to achieve effective oxidation of both these compounds simultaneously.

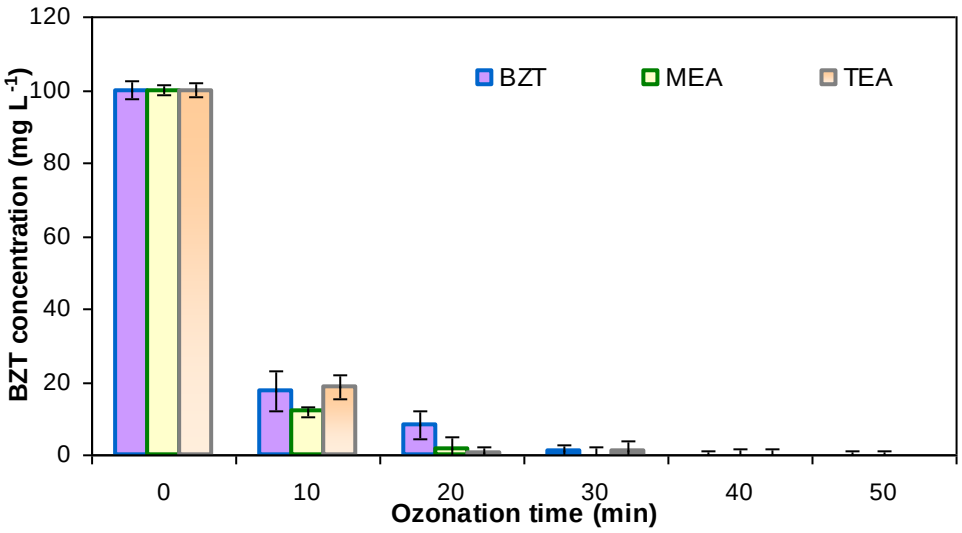



**Figure 5.19**: Effect of contact time on degradation of a mixture of amines (MEA, TEA) and BZT ([MEA] =3500mgL$^{-1}$, [TEA] =4250mgL$^{-1}$, [BZT] =100mgL$^{-1}$) (Mean ± SD of triplicates shown).

Figure 5.19 summarises the effect of contact time and ozone dose on removal of MEA, TEA and BZT in the mixture. This was investigated in the ozone dose range of 0.5–2.5 mg $O_3$/mg of MEA, 0.33-2.20 mg $O_3$/mg of TEA. Contact time of 10, 20, 30, 40 and 50 minutes were investigated. Nearly 89% MEA removal was observed within 10 minutes of ozonation, which reached 99% on a further 10 minutes of ozonation. MEA was undetectable after 30 minutes of ozonation. For TEA, 81% removal was seen in the first 10 minutes, which reached greater than 99% in 20 minutes reaction time. At the end of 30 minutes, the concentration of TEA was below detectable limits (BDL). The degradation profile of BZT was much more gradual. This demonstrated 82%, 92% and 98% BZT removed at the end of 10, 20 and 30 minutes, respectively. BZT was below detectable limits at 40 and 50 minutes of ozonation. The above figure clearly demonstrates that almost complete removal of the pollutants was achieved after 30 minutes of ozonation. All further experiments were therefore performed for 30 minutes.

### 5.4.6.2 Effect of ozonation on formation of by-products

It is a very common observation that the decline in the main pollutant by ozonation is accompanied by generation of toxic intermediary products which may inhibit a subsequent post-biodegradation step. Therefore, it was imperative to assess temporal breakdown compound formation. The formation of by products, both of amines and BZT, are shown in the chromatographs for amines (Figure 5.20 and 5.21). It was not possible to identify precisely the intermediary products since GC-MS or LC-MS were unavailable to undertake the analysis.

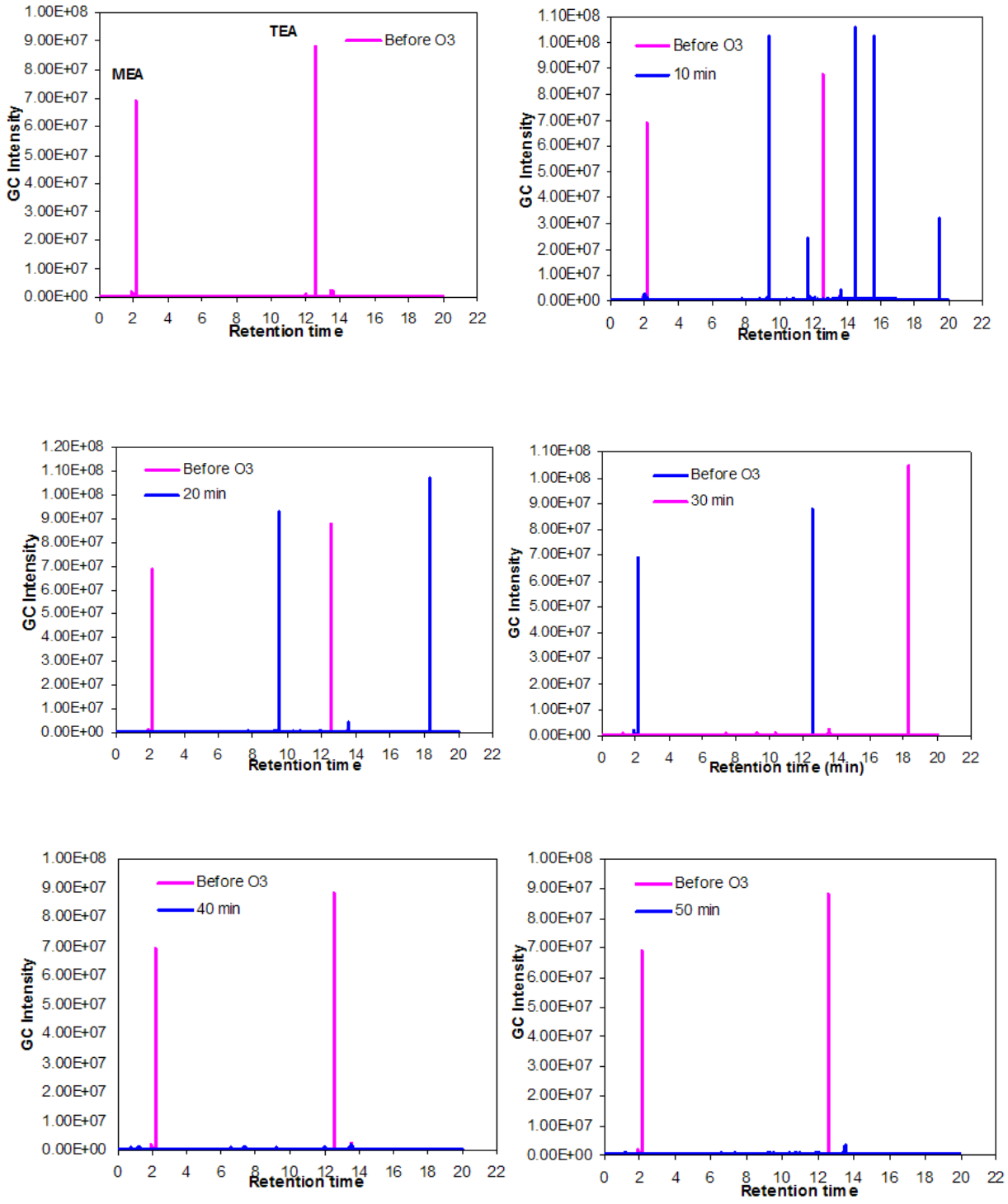



**Figure 5.20**: GC Chromatograph of MEA and TEA and the intermediary products generated at different time intervals.

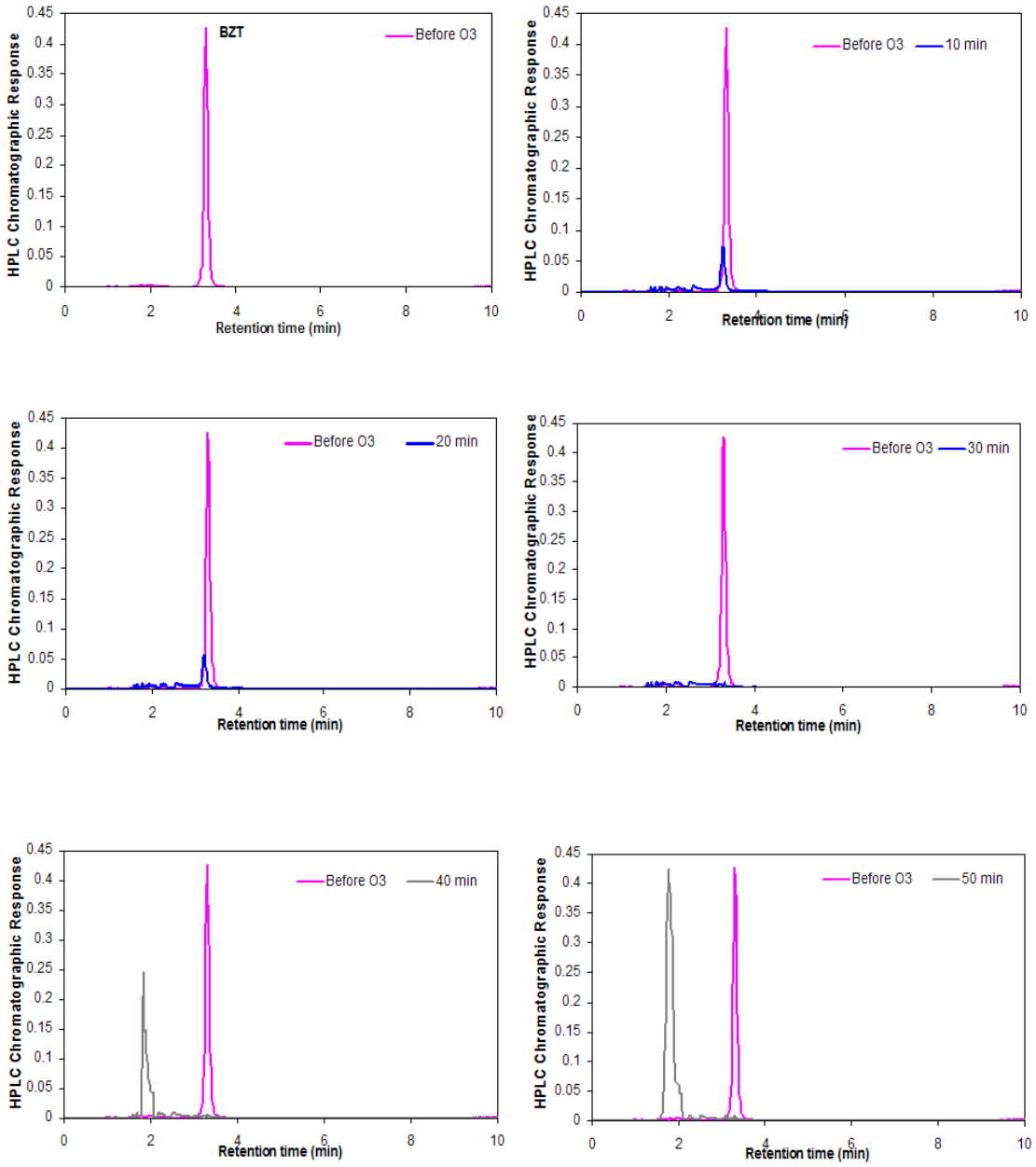



**Figure 5.21** HPLC chromatograph of BZT and the intermediary products generated at different time intervals.

### 5.4.6.3 Effect of ozonation on toxicity

Toxicity analyses were performed on the ozone treated effluent to evaluate the potential stress impact of the by-products generated. Figure 5.22 demonstrates that the initial mixture of amines and BZT (depicted in red line) was highly toxic to the biosensor. About 90% reduction in toxicity was observed after a treatment time of 10 minutes and 20 minutes; although the 20 minutes treatment was less toxic. This phase corresponds to generation of several by-products from amine degradation and reduction in concentration of BZT and both the amines shown in GC and HPLC chromatographs (figures 5.20 and 5.21). The presence of intermediary products from amines during 10 and 20 minutes did not appear to be toxic in nature.

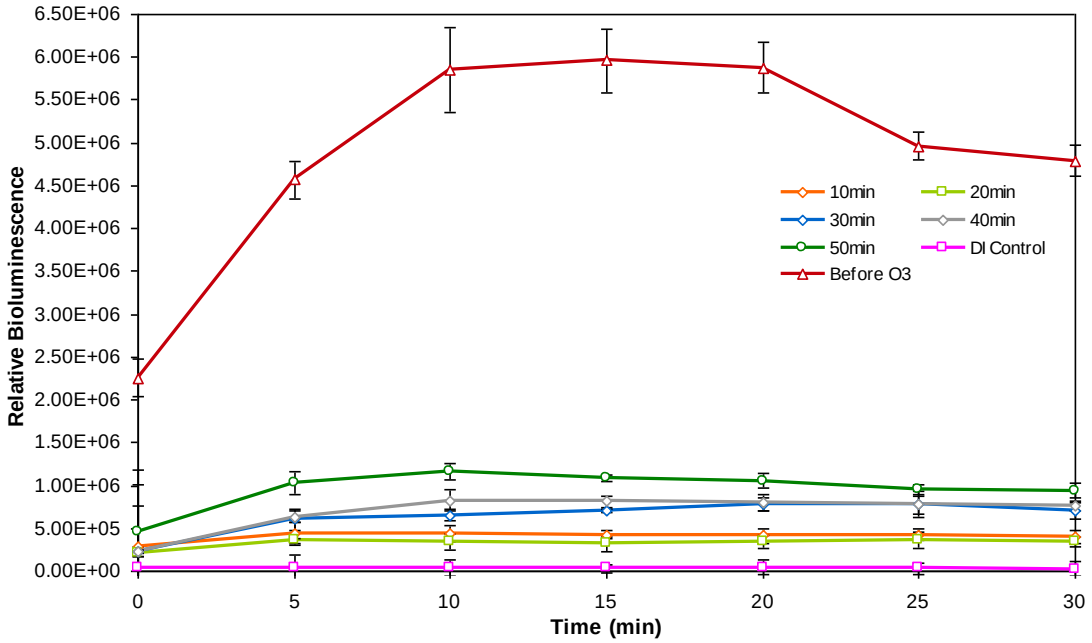



**Figure 5.22**: Effect of contact time and ozone dose on toxicity (Mean ± SD of 8 replicates shown).

The toxicity however increased with treatment time greater than 20 minutes. GC and HPLC chromatographs confirmed that toxicity at 30 minutes was due to two new by-products

generated from the amines. The enhancement in toxicity after 30 minutes of ozonation appeared to be due to these intermediary products from amine decomposition, as no by-products were observed in BZT degradation during this time scale. Reaction times of 40 and 50 minutes also generated toxic intermediates. No peaks of any kind were visible in the GC chromatographs and hence toxicity during 40 and 50 minutes was attributed to the by-products of BZT degradation (as evidenced from the HPLC chromatographs which increased in concentration from 40 to 50 minutes). Hence it could be concluded from these results that there was formation of highly biodegradable intermediate products in the first stages of the degradation; further oxidation of these intermediates lead to the generation of toxic intermediates.

#### 5.4.6.4 Effect of pH on degradation of MEA, TEA and BZT

In order to investigate the role of molecular ozone and hydroxyl radicals in the degradation of MEA, TEA and BZT, ozonation was performed under varying pH (3.0, 5.0, 7.0 and 9.0). As seen from Figure 5.23, the percentage degradation of BZT increased with increasing pH. There was 82.22, 80.21, 78.13 and 76.9 % degradation at pH 3.0, 5.0. 7.0 and 9.0, respectively. This result suggests that percentage degradation of benzotriazole decreased with increasing pH, but this was marginal. . Specifically, the rate of the reaction was 3.5 times faster when the starting pH was 3.0, when compared to that at pH of 9.0, which can be clearly seen from Figure 5.24 depicting the pseudo-first order plots for BZT reduction.

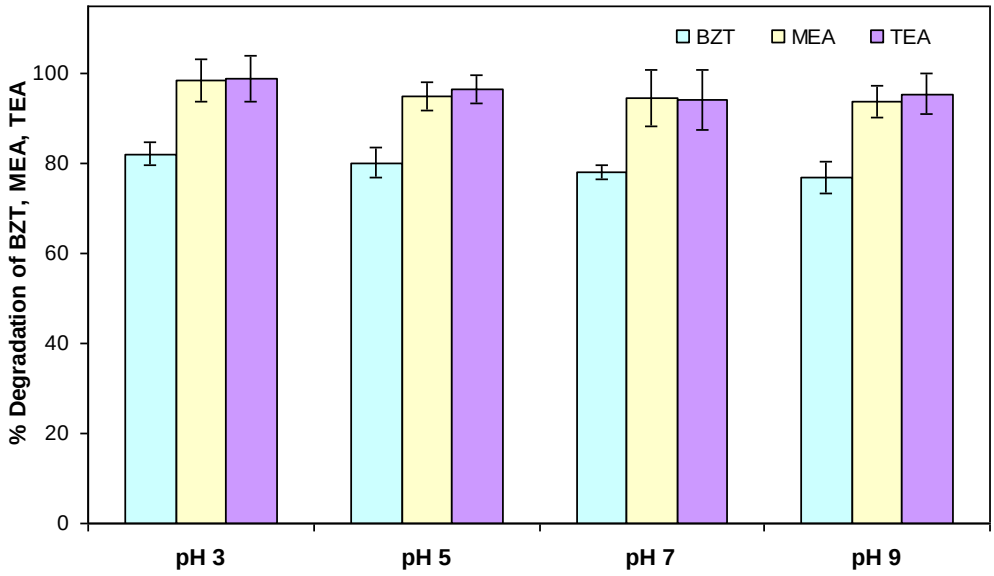



**Figure 5.23**: Effect of pH on % degradation of MEA, TEA and BZT ([MEA] =3500mgL$^{-1}$, [TEA] =4250mgL$^{-1}$, [BZT] =100mgL$^{-1}$, Reaction time=15 min) (Mean ± SD of triplicates shown).

The kinetic studies indicate that compound decomposition rates were observed to be much higher at acidic than those in near-neutral and alkaline pH, which suggests that the decomposition of BZT was mainly driven by molecular ozone rather than the radical mechanism. The kinetic study enabled evaluation of the kinetic constants for the substrate reduction at varying pH. The pseudo-first order rate constants for degradation of benzotriazole was -0.1463, -0.0883, -0.0586 and -0.0409 at pH of 3.0, 5.0, 7.0 and 9.0, respectively. Similar to the present study, the rate constants in other studies were found to vary from 6.2x10$^{9}$ M$^{-1}$s$^{-1}$ at pH 10.2 to 1.7x10$^{10}$ M$^{-1}$s$^{-1}$ at pH 2.0 (Leitner and Roshani, 2010), indicating a higher degradation under acidic pH. For the amines, almost complete degradation of MEA and TEA occurred in all the pH investigated (Figure 5.23). Almost similar compound degradation was achieved under all pH examined. The GC and HPLC chromatograms analysis revealed a decrease in the concentrations of MEA, TEA and BZT

with change in pH as depicted in Figure 5.25 and Figure 5.26. Figure 5.27 is an alternative representation of the change in concentration of BZT under varying operating pH.

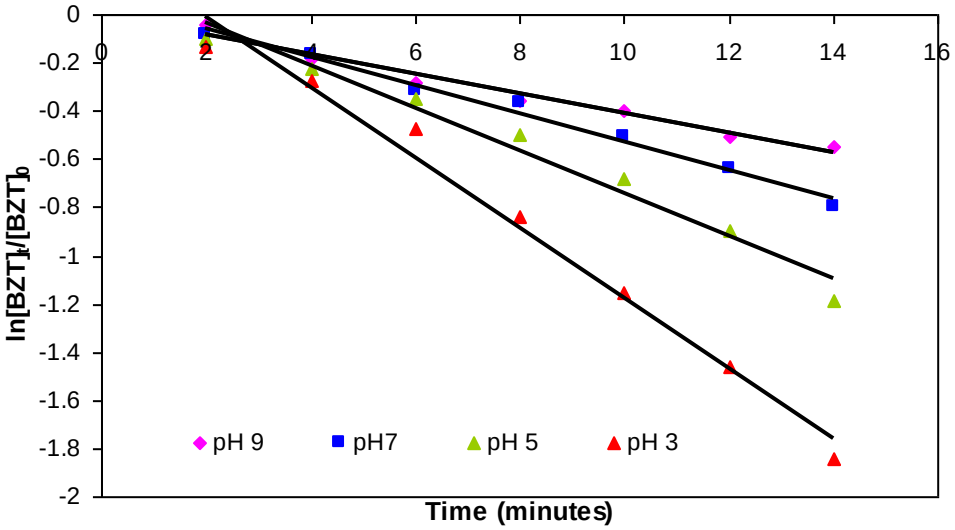



**Figure 5.24**: Kinetic analysis of benzotriazole degradation by Ozone.

Given the significantly higher kinetic rate constants for the reaction between molecular ozone and the pollutants, the oxidation potential of hydroxyl radical was enhanced under acidic condition, when molecular ozone was produced and participates in the reactions.

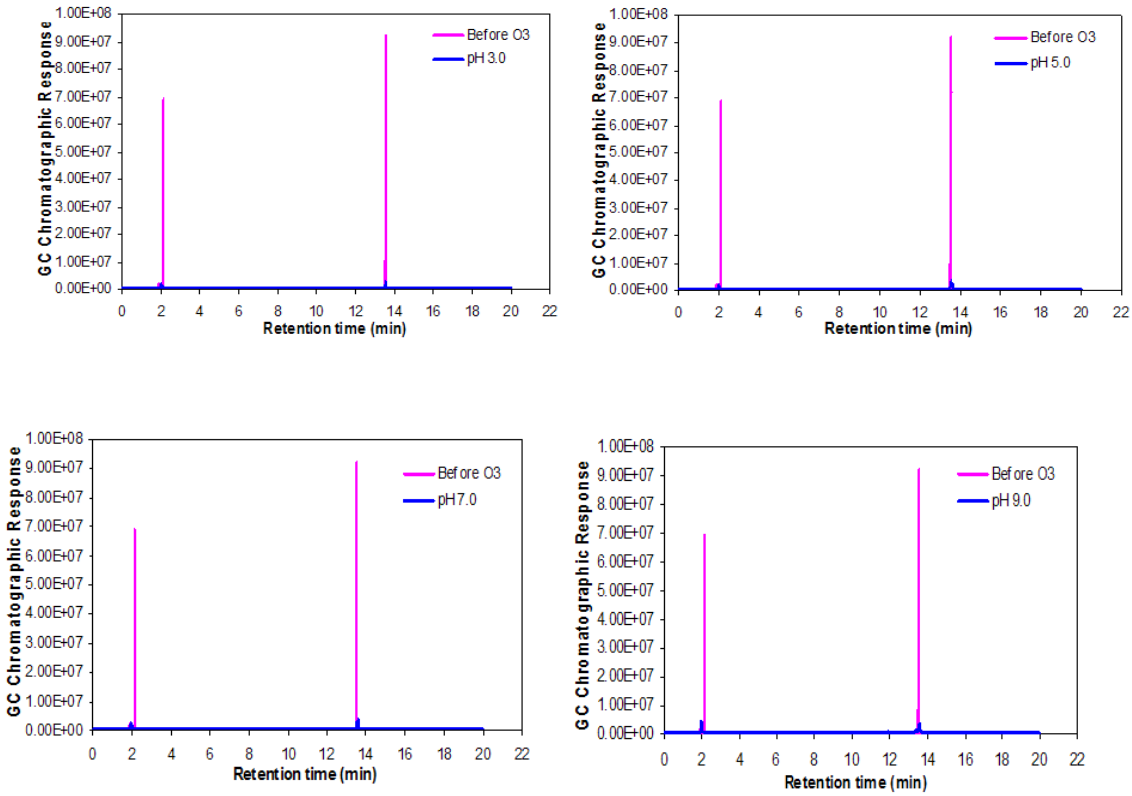



**Figure 5.25:** GC Chromatographs of change in concentrations of MEA and TEA under varying pH (Reaction time=15 min).

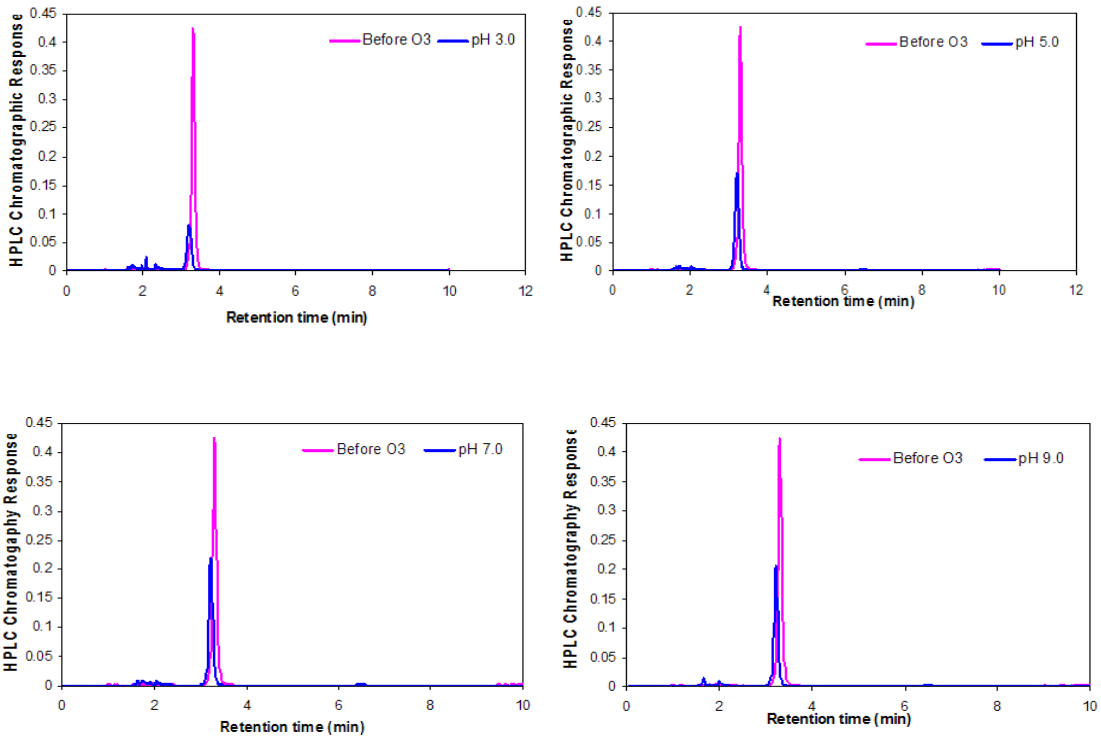



**Figure 5.26**: HPLC Chromatographs of change in concentrations of BZT under varying pH (Reaction time=15min).

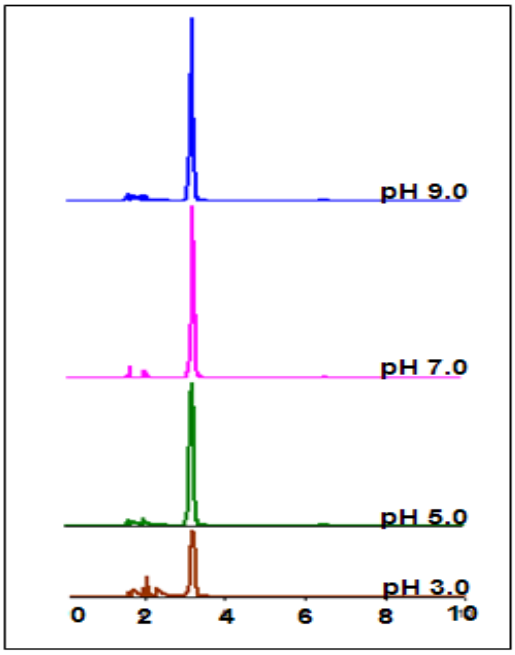



**Figure 5.27:** HPLC Chromatographs of change in concentrations of BZT under varying pH (Reaction time=15min).

These reaction mechanisms were examined by undertaking tests in the presence of tert-BuOH (tertiary butanol), which is a powerful hydroxyl radical scavenger and relatively un-reactive with molecular ozone. The reaction rate constant of tert-BuOH with molecular ozone is extremely small (0.003 $M^{-1}s^{-1}$) compared to that of hydroxyl radicals ($7.6x10^{8}$ $M^{-1}s^{-1}$) (Ning and Graham, 2008). Thus, if the role of hydroxyl radicals was a significant one, it would be expected that the degradation efficiency of BZT would be greatly reduced in the presence of tert-BuOH. An excess of tert-BuOH (250 mg $L^{-1}$) was used in the experiments in order to maximise the radical scavenging. Figure 5.28 summarises the results and shows the difference in compound removal efficiency in the presence and absence of tert-butanol. It is clear that the decomposition of BZT and amines (MEA and TEA) were caused by reactions with molecular ozone. The degradation of all these compounds were unaltered in the presence of a hydroxyl radical scavenger as these

compounds were transformed by direct reaction of molecular ozone as opposed to hydroxyl free radicals. Similar action of molecular ozone on BZT was also observed by Leitner and Roshani, (2010), Weiss *et al*., (2006).

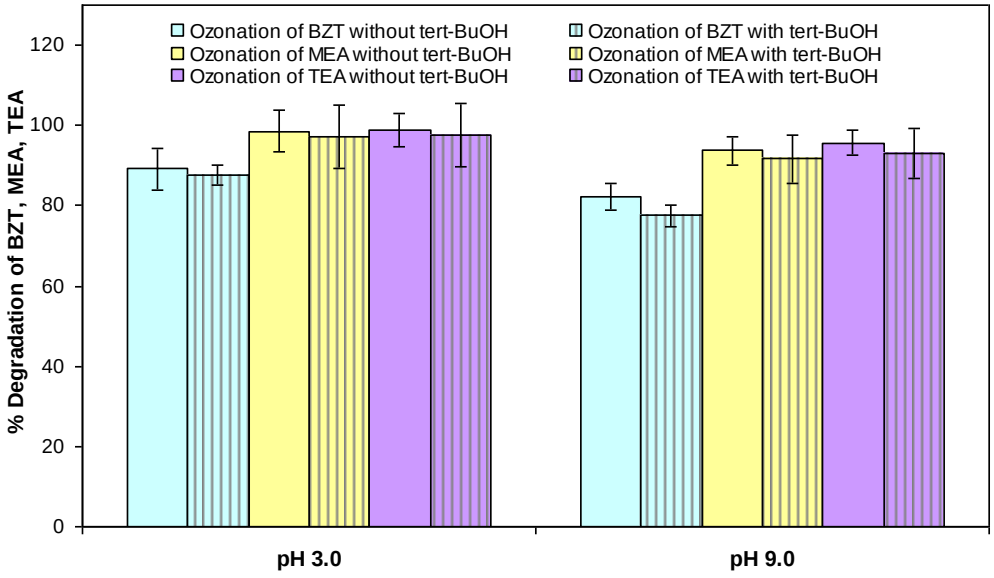



**Figure 5.28**: Effect of *tert*-butanol on percentage degradation of MEA, TEA and BZT by ozone-induced oxidation (Reaction time=15 min).

Almost complete removal of three recalcitrant additives found in MWF formulations were therefore achieved by gas-induced ozonation. Ozonation proved to be very effective in removing benzotriazole, monoethanolamine and triethanolamine under varying pH investigated in this study. The ozone dosage required to remove benzotriazole was adequate to remove amines from the same wastewater.

## 5.5 Discussion

An integrated ozone pre-treatment and biological oxidation has been investigated for the remediation of recalcitrant organic matter from pulp and paper industry (Morais, *et al.,* 2008, Oeller *et al.*, 1997, Bijan and Mohseni 2004), phenolic compounds (Contreras *et al.,* 2003, Amat *et al.,* 2003), tannery wastewater (Iaconi *et al.,* 2009), olive related industries (Rivas *et al.,* 2000), polycyclic aromatic hydrocarbon contaminated soils and wastewater (Derudi *et al.,* 2007, Rivas *et al.,* 2009, Zeng *et al*., 2000). These studies showed that the pre-treatment step enhanced and stimulated the secondary bio-oxidation, achieving a total reduction of 65-96% organics in remediation of these industrial wastewaters. Ozonation pre-treatment is not a new concept, but the results shown in this study provides evidence that ozonation is an effective tool for remediation of metalworking fluid wastewaters.

Ozone dose plays a vital role in improving the biodegradability of recalcitrant wastewaters. By ozonation, part of the refractory organic portion undergoes oxidation and generates numerous oxidation products which are usually in a more biodegradable form than the reactant compounds. The aim of ozonisation in this study was not complete mineralisation of recalcitrant products, but rather formation of a variety of oxidised bioavailable intermediates, whose concentrations depend on the reaction time. The time and ozone dosage therefore needs to be carefully optimised for an integrated ozone-biological treatment strategy. This is because excessive oxidation of these refractory compounds may have given rise to compounds which may be toxic or may not provide sufficient metabolic value to the micro-organisms adopted in the post-biological treatment step (Amat *et al.,* 2003, Lee *et al.,* 2009).

This study demonstrated that ozonation provided effective breakdown of the unsaturated hydrocarbons and aromatic compounds even at a short contact time of 20 min (Figure 5.6). Significant reduction of this parameter is consistent with established reaction mechanisms whereby molecular ozone readily reacts with both unsaturated and aromatic compounds. The MWF wastewater employed in this study was recalcitrant and hence reduction in $A_{254}$ absorbance indicated an increase in biodegradability. This reduction in the $A_{254}$ value also corresponded with an increase in biodegradability of the wastewater by 1.8–3.5 times based on the increase in $BOD_5$/COD ratio before and after ozonation. Similar observations have been reported with filtered biological treated wastewater and other effluents (Wang and Pai (2001), Imai *et al*., 2009, Assalin *et al*., 2007).

Higher rate and the greater extent of COD reduction of MWF wasteeater at elevated pH was attributed to the reaction of organics with molecular ozone and oxidising radicals, including hydroxyl radical, that are effectively formed at high pH (Figure 5.10, 5.11) (Glaze *et al*., 1987). A different trend was observed when benzotriazole was subjected to ozonation. This is because benzotriazoles (with an aromatic ring) is subjected to direct electrophilic attack by molecular ozone under low pH. The process starts with the rapid reaction of ozone and hydroperoxyl radical (Eq. 5.1). Propagation reactions involve the formation of other radical species such as $HO_3^{\bullet}$ (Eq. 5.2) that eventually lead to the formation of hydroxl radicals (Eq. 5.3) (Rosal *et al*., 2009). By the reaction between ozone and the super-oxide anion radical the ozonide anion radical $O_3^-$ is formed, which decomposes immediately giving $OH^{\bullet}$ (Munter 2001), thereby resulting in production of two $OH^{\bullet}$ radicals from three ozone molecules (Eq. 5.4) (Munter 2001). The rate of the attack by $OH^{\cdot}$ radicals is typically 106 to 109 times faster than the corresponding reaction

rate for molecular ozone. The oxidation ability of ozone is by far lower than hydroxyl radical formed under basic pH, as seen for several compounds found in various plant effluents (Bijan and Mohseni 2005, Lin and Kiang, 2005, Munter 2001, Bijan and Mohseni, 2004, Souza *et al.,* 2011. In a study on the oxidation of ozone on two alkylphenol compounds, the contribution of indirect reaction with hydroxyl radicals, was found to represent over 50% of the total degradation at pH 7, and this increased substantially (by a factor of 23 and 33) with pH increasing from 6 to 9 (Ning *et al.,* 2007). The ratio of exposure to the molecular ozone exposure ($R_{ct}$) parameter calculated in their study indicated that the rate constants for indirect radical attack were approximately five orders of magnitude greater than the corresponding rate constants for the direct compound degradation by molecular ozone.

$$O_3 + HO_2^- \rightarrow HO_2^\bullet + O_3^{\bullet -} \quad (5.1)$$

$$O_3^{\bullet -} + H^+ \rightarrow HO_3^\bullet \quad (5.2)$$

$$HO_3^\bullet \rightarrow HO^\bullet + O_2 \quad (5.3)$$

$$3O_3 + OH^- + H^+ \rightarrow 2OH^\bullet + 4O_2 \quad (5.4)$$

The extent of radical reaction, whether through the use of ozone at elevated pH or by applying an AOP, depends largely on the nature of the water quality, and specifically on the presence of scavenging or inhibiting species, such as natural organic matter and alkalinity anions (carbonate and bicarbonate) (Rice, 1997, Ning *et al.*, 2007).

Ozonation under acidic conditions predominantly involves the ionic process (via molecular ozone) whereby the organics within the wastewater are subjected to the electrophilic attack

by ozone molecules that decompose into carboxylic acids as final end products (Popiel *et al.,* 2009). The molecular ozone reactions are highly selective towards the organic molecules containing nucleophilic moieties such as carbon-carbon double bonds, -OH, -$CH_3$, -$OCH_3$, nitrogen, oxygen, phosphorus, sulphur bearing functional groups together with aromatic compounds and unsaturated bonds (Iaconi *et al.,* 2003, Schepper *et al.,* 2009).

Molecules which are more sensitive to oxidation react first with small ozone doses, and produce more biodegradable molecules with alcoholic and carboxylic functional groups. At higher ozone doses, biodegradable compounds (both initially present and others formed as a result of ozonation) compete for the available ozone. In addition, very high doses of ozone may produce compounds which are of low metabolic value to microbes employed in post-biological treatment. Therefore it is not always worthwhile to increase the ozone dose too high, and it is imperative to determine the optimum ozone dose corresponding to maximum COD removal and $BOD_5$/COD value. Partial ozonation is often preferred over total mineralization for its relative lower cost resulting from lower ozone dosage. The main goal of partial ozonation is the formation of biodegradable COD which is subsequently removed in a less costly biological treatment stage. The quotient of $BOD_5$/COD, which compares the amount of oxygen consumed biologically in 5 days with the total oxygen required for chemical oxidation of the compounds in the sample is an established measure of how easily the wastewaters can be degraded biologically. It is regarded as the biodegradability indicator of the portion of biodegradable organics in the wastewater. An increase in the $BOD_5$/COD ratio of a sample means that the intermediate products formed were biologically more accessible and palatable and is indicative of improved biodegradability. As a reference, a $BOD_5$/COD ratio of 0.4 is generally considered the cut-off point between biodegradable and difficult to biodegrade waste (Metcalf and Eddy,

2002). For instance, domestic wastewater typically has a $BOD_5$/COD ratio of between 0.4 and 0.8. The waste MWF, as received, showed a low initial biodegradability ratio of 0.127, indicating non-biodegradable characteristics of the effluent. Overall, from the results of this research it can be concluded that ozonation of wastewater under basic condition provided more effective use of ozone for converting non-biodegradable compounds into more biodegradable components that could be removed in the subsequent biological treatment (0.127 to 0.354) (Figure 5.15) . This is, in particular, important because operationally-exhausted MWF effluent possesses a high pH, which would eliminate any further need for manipulation of the wastewater prior to ozonation. Such increases in $BOD_5$/COD ratio are due to the production of more biodegradable compounds and improvement in the oxidation state of the organics (monitored as reduction in the oxygen consumed chemically per unit of carbon).

It is widely recognised that the ecotoxicologic effects of MWF components (biocides, corrosion inhibitors, extreme pressure and anti wear agents, emulsifiers, and surfactants) cause major problems regarding the disposal of MWFs and their adverse environmental impact (Muszynski *et al*., 2009). Established pollution parameters such as BOD, COD and TOC, although important for effluent monitoring, are not sufficient to describe the impact of wastewater constituents on the environment and post-biological treatment. Despite the fact that chemical parameters of treated wastewaters do not exceed any discharge limits, toxicity towards aquatic organisms might still be an issue in MWF effluents. Toxicity bioassays are particularly relevant when the precise composition of effluents is not known, as in the case of spent MWFs, where not all significant constituents can be identified. There have been some studies that have shown that oxidation products, generated from gas-induced ozonation, caused an increase in wastewater toxicity while chemical parameters

indicated a decline in contaminant load (Schepper *et al*., 2009). The toxic effects of high ozone doses could be attributed to by-products that might be formed during the reaction of ozone with the residual pollutants. The efficiency of the partial ozonation process therefore depends on both toxicity and COD removal. Advanced ozonation thus resulted in significant lowering of final effluent toxicity and generates more bio-removable COD resulting in a more effective interaction between chemical and biological oxidation stages. This study thus provides evidence that COD and toxicity removal efficiencies performed best at elevated (pH 9.0), when compared to an acidic pH of 3.0 (Figure 5.16).

Alkanolamines exhibited greater than 95% antimicrobial activity at greater ozone dose and elevated pH (Figure 5.22). One possible reason for this could be that amines exist primarily as protonated cations at pH values lower than 9. Uncharged species (found at higher pH) are more liable to diffuse through microbial membranes than a charged one which makes the former more toxic.

The major operating cost for the ozone oxidation process is the cost of electricity for ozone generation. The energy requirement for ozone synthesis, using air as a feed gas ranges from 22 to 33 kWh/kg $O_3$, including air handling and contacting ozone with water (Munter 2003).The operational costs for the production of 1 kg of ozone are therefore calculated to be typically in the range of 3.30 to 4.95 GBP (assuming cost of 15p per kWh). With these figures, it is possible to give an estimated cost for the above discussed dosage ranges. A dosage of 4g ozone/g COD would lead to GBP £160/$m^3$ of wastewater with COD of 10,000mg $L^{-1}$. The amount would vary linearly according to the COD of the wastewater.

In this part of the study, the effectiveness of ozonation on the breakdown of the unsaturated hydrocarbons and aromatic compounds with simultaneous reduction of pollution load for recalcitrant MWF was investigated. Enhancement in biodegradability indicates that the ozone treated wastewater is amenable to a second stage biological oxidation. Furthermore, ozonation has proved to be effective in degradation of toxic non-biodegradable MWF components such as monoethanolamine, triethanolamine and benzotriazole, which suggests that pre-treatment with ozone would facilitate biological oxidation of MWF wastewater.

# CHAPTER 6

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

Metalworking fluids (MWFs) are employed in metal processing operations for regulation of heat generation and for abatement of friction caused by contact of the tool on the work piece. They are composed of a complex mix of various well-defined organic compounds and operate in an extreme environment. This together with extended periods of use, bio-deterioration and thermal degradation inevitably results in the MWF becoming operationally exhausted. Typically the waste MWF fluids have a very high pollution load (COD > 120 g $L^{-1}$), which adds to the challenge in terms of developing sustainable ways of disposal. Until recently, most of the waste MWF has been disposed either by incineration or via landfills (Cheng *et al*., 2005). Incineration of such wastes is associated with evolution of nitrogen oxides, sulphur dioxide and hydrogen chloride, and disposal into landfill results in contamination of the soil and potential leaching of toxic compounds to the underground water table. The 2000/76/EC and Environmental Agency 2004 Directives issued by the European Union and Environmental Agency in UK (EA) now imposes a strict framework for the disposal of these wastes into landfills (Cheng *et al*., 2005). These regulations have created a growing interest in the development of environmentally benign and sustainable disposal methods of MWF wastewater. Also the driver is for on site treatment, which cuts out transport and associated carbon foot-print.

Physical treatment of MWF, employing membrane filtration, has been widely employed to demulsify semi-synthetic formulations, leading to separation of oil from the aqueous phase (Lin and Gomez, 2005, Benito *et al.,* 2001, Benito *et al.,* 2002, Chang *et al.,* 2001, Hilal *et al.,* 2004). These membranes have several operational drawbacks, such as frequent membrane fouling and are very expensive. Other methods such as incineration, reverse

osmosis and vacuum evaporation are very energy demanding, have high capital costs and requires specialist equipment/technicians to run them; hence not a preferred option. Biological treatment of waste MWF has been investigated by several researchers as it is the most sustainable, environmentally benign and economic option (Muszynski *et al.*¸2009, van der Gast *et al.*, 2003, Rabenstein *et al.*, 2009, Kim *et al*., 1994).

In this study the remediation of residual recalcitrant MWF obtained from a previously biologically treated facility (Microbial Solutions Ltd) was investigated. The recalcitrant MWF wastewater samples (Hocut 795-B, Houghton International Inc.) were obtained from Microbial Solutions Limited, a company which employs a repository of non-pathogenic bacteria to treat contaminants in metal working fluid wastewater (http://www.microbialsolutions.co.uk/). Using the approach developed by Microbial Solutions demonstrated that biological treatment in most instances was successful in degrading the majority of the simpler and more bioavailable constituents of the MWF wastewater, leaving behind complex recalcitrant and potentially toxic organic compounds.

The residual COD of bio-treated waste received for this work were typically in the range of 10,000-15,000 $mgL^{-1}$. The present investigation specifically focused on the remediation of the recalcitrant components of metalworking fluids. Since it seemed unlikely that no degree of manipulation would lead to biological degradation of the recalcitrant component, the approach was to embrace other technologies with greater oxidative potential and harmonise these with the established biological approach. Thus it was anticipated that the biological step would deal with the degradable component and the more robust oxidative step was exploited for the recalcitrant component.

The overall aim of the study was to compare three different chemical pre-treatment methodologies to determine the most effective way of harmonising these chemical oxidation processes with biological degradation. The experimental programme was designed to test three treatment methodologies; Fenton treatment, nanoscale zerovalent iron (nZVI) treatment and gas-induced ozonation, and assessed a wide range of experimental variables to determine the optimal conditions for each process.

## 6.2 Hybrid Fenton Oxidation-Microbial Treatment for Recalcitrant MWFs

Advanced oxidation processes, such as Fenton's oxidation, have been employed for treatment of a wide variety of toxic and recalcitrant industrial wastewaters. This has been successfully employed for treatment of non-biodegradable MWF wastewater (Seo *et al*., 2007) wherein a combination of ultrasonication and Fenton process was used to treat wastewater with COD of 12,000 mg $L^{-1}$. High concentrations of Fenton reagents ($H_2O_2$=10% v/v; $FeSO_4$=3 $gL^{-1}$) were employed by Seo *et* al., achieving a 98% removal of COD. Such high quantities of reagents would inadvertently lead to greater treatment costs and is hence not a viable practical option.

The feasibility of employing the Fenton reaction as a pre-treatment step to a biological oxidation was investigated. Thus in this way Fenton treatment was harmonised with a post-biological step for treating the recalcitrant component of MWF wastewater. The role of Fenton's oxidation in this case was not to achieve complete degradation of the organic load,

but for partial degradation of the recalcitrant compounds facilitating a post-biological treatment step.

Fenton oxidation usually requires consumption of one mole of $Fe^{2+}$ for each hydroxyl free radical produced, demanding a high concentration of Fe(II) and hydrogen peroxide. High concentrations of ferrous iron inevitably lead to greater amounts of sludge being generated, which would need to be treated separately. Addition of excess hydrogen peroxide has a scavenging effect on the free hydroxyl radicals, hence compromising the reaction efficiency. In addition, un-reacted residual $H_2O_2$ would be toxic, or certainly stressful to exposed microbial populations employed in the post-biological step. It was therefore imperative to conduct in-depth optimisation studies of the Fenton reagents to ensure that the chemically treated effluent was compatible with the inevitable exposure to the microbial cells employed in the subsequent biological step.

A statistical tool, namely central composite design, was employed for dose optimisation of ferrous sulphate and hydrogen peroxide. The independent variables were concentrations of hydrogen peroxide and ferrous sulphate. Percent removal of COD, TOC and toxicity were selected as the main response parameters. The optimal conditions for removal of COD, TOC and toxicity were obtained as summarised here: $[H_2O_2]$ = 14.91mM and $[Fe^{2+}]$ = 5.62mM. The observed decrease in pollution load was in agreement with the model predicted values, which validated the adequacy of the model. Hence these values were selected as optimal concentrations for pre-treatment of the recalcitrant MWF components. Under these reagent doses, COD and TOC reduction values were 64.73% and 55.93% respectively, with a concurrent 91% decrease in the toxicity. Maximum rate and extent of

COD removal occurred in acidic pH (3.0) conditions which facilitated maintenance of the iron in solution. The biodegradability index ($BOD_5$/COD) of the Fenton treated wastewater increased from 0.160 to 0.538 after the chemical treatment step. The toxicity and the biodegradability index provided evidence that Fenton treated effluent elevated biodegradability in the post-biological treatment step.

The Fenton treated effluent required (a) pH adjustment and (b) an iron extraction step prior to the second stage biological step. The Fenton treated effluent was introduced into fixed film batch bioreactors to determine the efficiency of the integrated treatment. An acclimation period was found to be necessary in order to gradually expose the microbial community to the intermediate oxidised compounds and maximise their removal efficiency. It took almost 20 days for start up of the reactors, i.e. to achieve steady state in terms of COD reduction. At steady state conditions, an overall COD removal of 92% and TOC removal of 86% were achieved after hybrid chemical-bacterial treatment.

Hybrid Fenton oxidation-microbial treatment proved to be an effective and efficient way to treat the residual refractory MWF waste. The pH of the Fenton treated effluent remained at 3.0. Therefore, pH adjustment, before and after treatment, was essential. This neutralisation step will inevitably add to the operational costs of the treatment step. Bacterial cultures isolated from operationally exhausted MWF have been extensively studied and characterised (van der Gast *et al*., 2002, 2003). It will be worth investigating if any of these cultures are acidophilic and specifically their response to each component of the Fenton reagents. Such a characterised acidophilic culture/consortia, with metabolic capability,

would prove immensely beneficial in reducing the overall costs levied on such a hybrid treatment system.

## 6.3 Novel Hybrid Nanoscale Iron Induced Oxidation of Recalcitrant MWFs

In this age of depleting resources, it is very important to find and develop techniques and approaches for wastewater treatment using sustainable methods, and processes that minimise the use of toxic reagents. nZVI induced oxidation is a recent introduction with reaction processes that are very similar to the Fenton oxidation system. The main feature of this system is its ability to create *in-situ* reactive oxygen species (hydroxyl free radicals), in aqueous-phase, employing nZVI at ambient temperature and pressure conditions. Relative to Fenton's oxidation, the heterogeneous nZVI reaction with atmospheric oxygen (from air) effectively treated the organic pollutants, whilst avoiding the addition of hydrogen peroxide, over a wide range of pH. nZVI has been employed for degradation of several test/model pollutants such as methanol, ethanol, 2-propanol, benzoic acid, molinate transformation, chlorophenol and arsenic (III) removal (Kennan and Sedlak, 2008, Joo *et al*., 2005, Noradoun *et al*., 2003, Kanel *et al.,* 2005). To our knowledge, this study is the first of its kind to investigate a combination treatment integrating nZVI pre-treatment with a post biological degradation, particularly of an effluent which is so recalcitrant.

The *in-situ* production of hydrogen peroxide, generated by the reaction between nZVI and dioxygen from air, eliminates the need for extraneous addition of $H_2O_2$. All the *in-situ* hydrogen peroxide that had been generated was demonstrated to be completely utilised for

the oxidation of the MWF compounds. The absence of residual peroxide at the end of treatment makes this approach compatible with subsequent biological steps. The rate and extent of COD reduction of chemical components was greater in acidic pH (78%), when compared to neutral pH (66%) conditions. This was confirmed by the activation energy in acidic pH which was lower than those in neutral conditions. An interesting point worth noting is that the final pH of the ZVI treated solution, at pH 3, reached neutrality even though the starting pH was highly acidic. This useful feature makes the treated effluent compatible for further biological treatment, whilst avoiding the need for neutralisation, hence saving in reagent costs and issues of logistics.

Thus the results of this study established that pre-treatment encompassing ZVI oxidation, at pH 3.0, was capable of reducing the toxicity and chemical oxygen demand by 85% and 78%, respectively. This pre-treated effluent was clearly capable of stimulating biodegradation of the residual pollutants, leading to an overall decrease of 95.5% in COD at the end of the combined treatment. The results of this study confirm that the reduction in COD in nZVI-induced oxidation was due to complete reaction of the MWF components, with hydroxyl free radicals or ferryl species generated from the reaction between *in-situ* hydrogen peroxide and ferrous iron. Any adsorption of the organic compounds on the surface of iron was ruled out. The residual iron oxide/hydroxide, after treatment, was successfully recycled, recovering the nZVI for further use. This recycling without significant loss in reactivity makes this treatment method a “greener” alternative for practical applications. The recycling of nZVI could have a significant impact in overall economy of the industrial scale plants, as only a small quantity of iron is required to

replenish the system. This is because most of the nZVI could be recycled within the system and this eliminates any waste iron products.

## 6.4 Combined (Ozonation-Biodegradation) Treatment of Recalcitrant MWFs

Gas-induced ozonation is well known to break open the structure of recalcitrant molecules, which is attributed to the combined action of molecular ozone and the hydroxyl free radicals, generated from ozone. Electrophilic attack of ozone molecules bring about decomposition of recalcitrant organic compounds into biocompatible final end products such as carboxylic acids (Iaconi *et al.,* 2003, Schepper *et al.,* 2009).The feasibility of employing ozone to oxidise toxic and bio-refractory MWF components combining with a post-biological degradation was thus investigated.

The results of this research indicate, over the range of variables studied, that ozonation of recalcitrant MWF achieved an organic substrate removal of 27.45% and 8.5%, after 60 minutes of the reaction, for wastewater of COD=3100 $mgL^{-1}$ and 9855 $mgL^{-1}$ respectively. Higher strength wastewater needs greater amounts of ozone to oxidise the components. There was a corresponding increase in biodegradability, decolourisation and removal of aromatic compounds with increasing inlet ozone dose. An increase in $BOD_5$/COD with increasing ozone dose was observed for all experiments, conducted under elevated pH, with MWF wastewater. The reduction in biodegradability and enhancement in toxicity for experiments conducted in acidic conditions may have been due to formation of toxic by-products during ozonation. This study demonstrates that there is an optimum ozone dosage

which enables maximum increase in biodegradability and high efficiency of the ozonisation process. Further ozonisation did not assist in increasing the biodegradability and was clearly disadvantageous. The combined treatment led to 70% reduction in recalcitrant COD.

To gain insight into the nature of the reactions and intermediates generated during ozonation, degradation kinetics of three highly toxic and non-biodegradable MWF components were investigated. The test compounds selected were monoethanolamine (MEA), triethanolamine (TEA) and benzotriazole (BZT) with concentrations similar to those found in operationally exhausted concentrate MWF (COD>120 g $L^{-1}$). Previous studies have demonstrated that alkaloamines are biodegradable at varying concentrations (Rabenstein *et al.,* 2009, Seo *et al*., 2007, West and Gonsior, 1996). Several attempts to isolate microorganisms that can degrade benzotriazole (BTZ) as a C or N source have reported failure (Matamoros *et al*., 2010, Giger *et al*., 2006, Weiss *et al*., 2006, Rollinson and Callely, 1986). Furthermore, BZT is an active nitrification inhibitor, thus curtailing complete oxidation of the amines. Co-existence of amines and BZT in the same formulation might therefore have led to inhibition of biodegradation of the former, owing to the presence of a nitrification inhibitor like BZT in the mix. The effect of ozone gas on the decomposition of these model pollutants were also analysed in this study.

Ozonation of these compounds proved that all three compounds were completely oxidised by the addition of ozone. Almost complete degradation of all three compounds were achieved in less than 30 minutes of ozonation. However, various by-products of ozonation were observed at different treatment times. By assessing the toxicity and biodegradability of chemically treated effluent and linking that information with chemical analysis of MWF

components such as MEA, TEA and BZT degradation, significantly helped understanding of the interactions between the main components of the effluent with ozone. Independent studies, employing selected model pollutants, did not however provide an accurate analysis of the reaction mechanism. This is because identification of these by-products ( by GC-MS/LC-MS) generated during ozonation is the only effective means to assess the cause of the toxicity during the process of ozonation and this was not available to us in this study.

## 6.5 Future Work and Recommendations

In the context of urban water systems, sustainability might be described as "to meet the needs and aspirations of the present generation without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). From this it follows that the magnitude and quality of water resources needs to be managed carefully in order to maintain the adequacy of such resources for future generations. In many countries, at present, the demand for water continues to rise and the quality of water resources is deteriorating through inadequate control of point and non-point pollution sources. In addition, stringent regulatory requirements are being applied to the quality of drinking water supplied to the consumer in response to increasing knowledge of health effects and potential threats (e.g. waterborne pathogens). Such pressure is leading to more extensive and complex treatment systems requiring greater use of chemicals and energy.

With technologies such as bioremediation and phytoremediation gaining popularity, nanotechnology offers a number of emerging technologies that could work to treat contaminants. The ability of nanotechnology to abate pollution production is just beginning to be explored and could potentially catalyse the most revolutionary changes in the environmental field. Iron is one of earth's most plentiful resources, making up at least

five percent of the earth's crust. As seen in this study, nano-Fe possess novel properties such as oxidising power, selective reactivity, stability as salt forms, and non-toxic decomposition by-products of ferric iron. In an environmental remediation process, Fe has been proposed as green oxidant, coagulant, disinfectant, and antifoulant, therefore a promising multipurpose water treatment chemical. The iron needed for such treatment could be extracted from environmental waste streams rich in minerals of iron such as acid mine drainage. The high concentration of iron combined with the extremely low pH in the mine drainage would generate iron powders that could be beneficial for advanced oxidation processes at increased rates. Harnessing iron from a waste stream (such as acid mine drainage) to treat other industrial waste and to recycle these minerals presents a sustainable solution.

The efficiency of biological oxidation techniques are often compromised by the presence of bio-refractory materials. In contrast, though advanced oxidation processes promise degradation of almost all the contaminants, their use is hampered by their associated costs. In the present study it was established that a hybrid method consisting of advanced oxidation processes to reduce the toxicity of the effluent up to a desired level followed by biological oxidation is required for the future. The results and observations made from the present study propose the development and implementation of a sustainable treatment approach for remediation of high pollution load industrial wastewater, such as used undiluted high strength MWF. Operationally exhausted concentrate semi-synthetic MWFs fluids are characterised by high COD (ranging from 120 g/L - 200 g/L), pH (8.5-9.5) consisting of an emulsion of 5-7% oil in water. In addition, MWF consists of a large array of different chemicals in the mixture, the exact compositions of which are proprietary

information, seldom divulged by the manufacturers. This makes the efficient treatment and disposal of such wastes increasingly difficult.

At present, the treatment option currently adopted is dilution of the high strength wastewater to bring down the COD and then subjecting the diluted wastewater to aerobic or anaerobic biological treatment. Such a strategy of diluting the waste up-front increases the final volume of wastewater and does not present a sustainable solution for treatment of high strength toxic industrial wastewater. A more effective approach would be to develop a hybrid treatment integrating a chemical pre-treatment to reduce the concentration of toxic compounds followed by environment-friendly and sustainable post biological treatment, thereby avoiding the dilution and additional use of precious water.

The findings of this study demonstrate that highly toxic MWF constituents such as MEA, TEA and BZT (with concentrations similar to that found in operationally exhausted MWF) are amenable to ozone oxidation. In stage one of the proposed future work, high strength semi-synthetic wastewater would be subjected to ozonation (Figure 6.1). The alkaline pH (8.5-9.5) of this waste would aid the generation of molecular ozone and hydroxyl free radicals, leading to reduction in concentrations of toxic recalcitrant organic compounds (as seen from the results in the present study). The present study has helped to demonstrate that all three non-biodegradable test compounds (MEA, TEA and BZT) were completely degraded under ozonation with reaction times of 15-30 minutes. In addition, it was observed that ozonation led to the reduction in the amount of unsaturated carbon compounds ($A_{254}$nm) and colour ($A_{400}$nm).

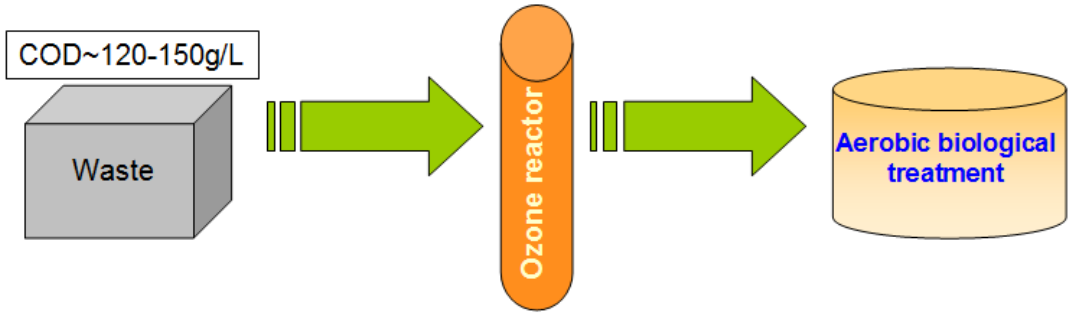



**Figure 6.1**: Proposed high-strength undiluted MWF wastewater treatment employing hybrid ozonation-biological degradation.

The primary aim of the proposed first stage ozone treatment is not to achieve significant removal of COD, instead it was employed specifically to deal with the recalcitrant components of the effluent. Decrease in the concentrations of aromatic, toxic refractory compounds and reduction of pH to neutral range will certainly stimulate second stage biological treatment whilst avoiding the need for any neutralisation or dilution step. Care should be taken to ensure that any residual ozone gas remaining in the system is flushed out with air, before being subjected to the second stage biological treatment. Such a hybrid approach avoids use of expensive and energy demanding physico-chemical methods, such as membrane filtration, incineration and offers a sustainable alternative to disposal into landfills.

In today's age of massive industrialisation and associated effluent generation, it is inevitable that technologies currently on the bench scale will gradually move into field-scale trials. However, the success of these technologies is reliant on a better understanding of their reaction and toxicity analysis; and to that effect the present study will hopefully open the door to more effective, less costly and more sustainable industrial wastewater treatments.

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# APPENDIX

SEPPIC UK Ltd, PO Box 338, Hounslow, Middlesex TW4 6SH
Tel: +44 (0)20 8577 8800 - Fax: +44 (0)20 8570 2106
www.seppic.com

## • Suggested formulations of "micro-emulsions"

- *Formulation 1: non foaming formulation which can be used in soft water; Simulsol® OC72 brings detergent properties that can prove useful when working cast-iron;*

| Ingredient | % Wt | |
|---|---|---|
| T 22 | 23 | < 3% aromatic [Ninas] |
| *Erucic acid* | *2* | *Stearinerie Dubois* |
| Na Sulphonate | 5 | PCAS [Arcot® 406] |
| Tall oil fatty acids | 3.5 | Ca. 30% resinic acids |
| Akypo RO 90 | 1 | Dispersant for Ca soaps [Chemy / CECA] |
| SIMULSOL OC 72 | 1.5 | |
| Butylglycol | 2.5 | Co-solvent; anti-foam |
| Fungicide | 0.5 | |
| Monoethanolamine | 8.5 | |
| Water | 38.2 | |
| Boric acid | 9 | Brings bio-stability; used in combination with MEA |
| Anticorrosion additive [yellow metals] | 0.3 | CIBA |
| SEPPIC 1050 | 5 | Corrosion inhibitor |

- *Formulation2: for use in harder water (less sulphonates) but anti-foam agent required; DEA-free formulation; low content in amines therefore less potential for skin irritation;*

| Ingredient | % Wt | |
|---|---|---|
| T 22 | 28 | < 3% aromatic [Ninas] |
| Boric acid | 3 | |
| EDTA [acid form] | 0.8 | Sequestrant; alternative to Akypo® |
| Monoethanolamine | 7 | |
| Tall oil fatty acids | 3.6 | |
| *Erucic acid* | *0.9* | *Stearinerie Dubois* |
| Anticorrosion additive [yellow metals] | 0.2 | |
| ORAMIDE ML802 | 5 | Brings alkalinity |
| Na Sulphonate | 2 | |
| Butylglycol | 2 | Co-solvent |
| Fungicide<br>Bactericide | 0.4<br>2 | IPBC or Na Omadine |
| Rewopol CL30 | 0.7 | Similar to Akypo®; dispersant of Ca salts |
| Simulsol OC 72 or OC 75 | 3.5 | |
| Triethanolamine | 8.5 | |
| Water | 9 | |
| SIMULSOL PG 711 | 0.9 | Anti-foam |

- *Formulation 3: for use with cast-iron; anti-rust agent required; incorporation of Simulsol® NW342 in order to improve the cleanliness of the metal surface;*

| Ingredient | % Wt | |
|---|---|---|
| T22 | 20 | |
| *DI-Isopropyl oleate* | *6* | *Stearinerie Dubois* |
| Tall oil fatty acids | 3 | |
| *Erucic acid* | *0.7* | *Stearinerie Dubois* |
| Akypo RO 90 | 1.4 | |
| SEPPIC 1050 | 5 | Alkalinity reserve |
| ORAMIDE ML 802 | 6 | |
| SIMULSOL NW 342 | 4 | |
| SIMULSOL OC 72 | 1.5 | |
| Water | 33.1 | |
| MEA | 7.7 | |
| Boric acid | 9.5 | |
| Anticorrosion additive [yellow metals] | 0.4 | |
| Bactericide | 1 | |
| Fungicide | 0.5 | |
| SIMULSOL PG 711 | 0.2 | Anti-foam |
| Anticorrosion for Aluminium | 0.3 | |

## • Suggested formulations of "emulsions"

- *Formulation 4: milky aspect; for difficult metal working & for use with Al; enhanced lubrication properties with TMP trioleate; good detergent properties with Montane® 80 & Simulsol® OC72; reduced Aluminium staining problem with Oramide® ML802;*

| Ingredient | % Wt | |
|---|---|---|
| T 22 | 44 | < 3% aromatic [Ninas] |
| *TMP trioleate* | *5* | *Stearinerie Dubois* |
| Tall oil fatty acids | 6.2 | *Stearinerie Dubois* |
| *Erucic acid* | *0.5* | |
| MONTANE 80 | 1.5 | Emulsion stabiliser |
| SIMULSOL OC 72 | 3.5 | |
| Butyl glycol | 2 | |
| ORAMIDE ML 802 | 5 | |
| Anticorrosion additive [yellow metals] | 0.2 | CIBA |
| Akypo RO 90 | 2 | Allows usage with hard water conditions |
| Revopol CL30 | 1 | |
| Bactericide | 1.2 | |
| Water | 9.1 | |
| MEA | 7.7 | |
| Boric acid | 6.5 | |
| TEA | 4 | |
| Fungicide | 0.4 | |
| Anticorrosion for Aluminium | 0.2 | |