The Biological Treatment of Metalworking Fluids: Insights into Carbon Removal Mechanisms and Integration with Biocide Toxicity Mitigation Strategies.

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Statement of Originality

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I. Abstract

The biological treatment of metalworking fluids (MWFs) is a cost effective alternative to conventional waste disposal processes. While research has proven that this process is capable of treating large volumes of wastes with high organic concentrations, there are uncertainties about the mechanisms by which the treatment occurs, and there are limitations that must be overcome.

There is a need to understand the importance of the mechanisms by which carbon (and hence COD) is removed from the wastewater. This will allow for waste practitioners to make better decisions for optimizing the process, and for disposing of waste (i.e sludge) that is generated.

The biological treatment process is also susceptible to biocides present within formulations. These compounds either need to be removed before the treatment process, or the bioreactors need to be made more resistant to them to ensure that their presence does not hinder the reactor functioning.

This study aims to answer the uncertainties about the carbon removal mechanisms involved in the treatment of oil-containing MWFs. In the first experimental chapter, it is shown that the predominant mechanism of carbon removal is oil/water separation induced by emulsifier degradation, and hence the bioprocess treatment rate is significantly affected by the biodegradability of surfactants and by the presence of cations found naturally in the water that used to prepare the emulsions.

The study then provides insights into the potential that coagulation and coalescence has for removing inhibitory components commonly found in MWFs. Coagulation and coalescence is shown to effectively remove biocides with low aqueous solubility

(iodopropynyl butylcarbamate) and those that partition themselves into the oil phase (o-phenyl phenate and its sodium salt).

Finally, to improve the resistance of reactors to inhibitory compounds, factors influencing the development of fixed-film reactors are investigated. A micro-cosmic system is used to study the both physico-chemical effects and nutritional factors on the development of biofilm reactors. It is shown that biofilm yields can be controlled through pH adjustment, and that these yields are maximized with phosphate stimulation and ammonium limitation. It is then shown that fixed-film reactors are able to treat metalworking fluids even under conditions deemed to be inhibitory.

In summary, this project provides insights into further understanding and enhancing the biological treatment of MWFs.

II. Contents

III. Glossary

CCC- Critical Coagulation Concentration

CFU- Colony Forming Unit

COD- Chemical Oxygen Demand

DGBE- Diethylene Glycol Butyl Ether

DI- Deionised

DIPA- Diisopropanolamine

EPS- Extracellular Polymeric Substances

HEPES- 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HPLC- High Performance Liquid Chromatography

IPBC- Iodopropynyl Butyl Carbamate

MWF- Metalworking Fluid

NMO- Naphthenic Mineral Oil

NRMSD- Normalised Root Mean Standard Deviation

OD- Optical Density

OPP- o-phenyl phenate

PBS- Phosphate Buffered Saline

PG- Propylene Glycol

TOC- Total Organic Carbon

TOFA- Tall Oil Fatty Acids

TPH- Total Petroleum Hydrocarbons

Chapter 1- Introduction

1.1 Background

Metalworking fluids are composite liquids that are used as coolants and lubricants for machining processes such as cutting, grinding and drilling (Bataller et al. 2004). Due to in-use contamination, and due to changes in the formulation caused by microbes and thermal deterioration, metalworking fluids used in a machining operation must be disposed of and replaced (Burke & Gaines 2006). In 2005, the annual global usage of metalworking fluids exceeded 2 billion litres, and considering that metalworking fluids are supplied in a concentrated form that must be diluted, the amount of metalworking fluid wastewater that is produced can be as much as ten times this amount (Cheng et al. 2005). Conventionally, metalworking fluids may be disposed of through incineration, however with tightening impositions by environmental protection agencies around the world, this means of disposal is no longer deemed acceptable (The European Parliament 2000b). There are a number of treatment processes which may be employed to ensure that the metalworking fluid wastewater meets acceptable standards before it may be disposed into sewage treatment works (Burke & Gaines 2006; Cheng et al. 2005). In comparison to other options, the biological treatment process offers the advantage of being cost-effective, and versatile in that it is capable of treating all types of metalworking fluids (Muszyński & Łebkowska 2005; Cheng et al. 2006; van der Gast et al. 2004). While much research has been shown that this process is capable of removing vast amounts of organics, there still are questions about the mechanisms of the process and challenges that must be overcome to improve its efficiency. One such challenge is the presence of anti-microbial agents which have

the potential to inhibit the biotreatment process (Jagadevan et al. 2012; Thill et al. 2016).

For metalworking fluids which contain mineral oil, there exist a number of carbon (and hence COD) removal mechanisms. This can be through biodegradation of the hydrocarbons and the organics, through adsorption or through oil/water separation induced by the biodegradation of oil emulsifiers. Understanding the mechanism by which the carbon is removed from the water is important since it determines the type of waste product that is produced from the treatment process. If adsorption is the main mechanism, then further considerations need to be made for sludge disposal since it would now contain a large fraction of recalcitrant carbon. A similar line of thinking applies for the oil/water removal mechanism. As Cheng et al. noted in their review of treatment processes of metalworking fluids, an understanding of the relative removal contributions of adsorption and degradation mechanism will assist in making better decisions for solids disposal (Cheng et al. 2005).

A second uncertainty with the biological treatment of metalworking fluids is its susceptibility to anti-microbial agents. This can usually be overcome by diluting the wastewater to a point that the anti-microbial agents are no longer inhibitory, but dilution either increases process capital requirements or the time that would be taken in order to process a given volume of wastewater. Emerging techniques have been applied to detoxify metalworking fluids before the biological treatment, and these have been successful in not only reducing toxicity, but also in removing recalcitrant components (Jagadevan et al. 2012; Jagadevan et al. 2013; Jagadevan et al. 2011; Thill et al. 2016). However, these techniques are expensive and are non-selective. A conventional cost-effective technique is required as a pre-treatment step in order to improve the efficiency of the biological treatment process

However, since metalworking fluid formulations contain different types, and different amounts of biocides, it is difficult to implement a universal technique for detoxification. If the pre-treatment does not remove the biocide, then improving the resistance and the resilience of the bioreactor to inhibitory chemicals is the next best alternative. Using fixed-film reactors as opposed to suspended reactors is a well applied technique for treating hazardous waste streams (Dong et al. 2011; Borghei & Hosseini 2004; Kargi & Eker 2005) and there are a few investigations that have applied such reactors to metalworking fluids (Muszyński & Łebkowska 2005). However, there are no investigations which look at the factors that influence biofilm development in metalworking fluids. Understanding the physico-chemical and nutritional factors influencing biofilm development can reduce start-up times for the reactor, and can also help in recommissioning the reactor after it has received an inhibitory shock load.

This project is thus focussed on improving both the understanding of the mechanisms of pollutant removal involved in biological treatment process, as well as integrating it with toxicity mitigation strategies.

1.2 Chapter Summaries

1.2.1 Chapter 2

Chapter 2 begins by introducing the reader to metalworking fluids. It explores what they are, why they are useful, and the chemistry involved in the formulation of metalworking fluids. It then proceeds to introduce the reader to the concepts behind the disposal of metalworking fluids, by examining why this needs to be done, and how it is done. A specific focus is given to the biological treatment. The chapter explores previous research that was done on the biological treatment of

metalworking fluids, and critically examines the advantages and limitations of the process. The chapter ends by also examining physical and chemical treatment strategies that have been applied to metalworking fluids, and how these treatments have been incorporated in the biological treatment process. After examining all of the research that has been conducted, a discussion on opportunities for further research is given, and the research objectives of this project are formally stated.

1.2.2 Chapter 3

The first experimental chapter of the project is aimed at investigating the importance of different mechanisms of carbon removal within soluble oil metalworking fluids. The concept of emulsion destabilisation and oil/water separation is introduced. It is shown the spontaneous demulsification and oil/water separation may occur within bioreactors as a result of dilution with relatively hard water. Since dilution is necessary for the treatment of soluble oil metalworking fluids, spontaneous demulsification may be a contributory mechanism to carbon removal. In examining the biological mechanisms for removal in reactors treating soluble oil metalworking fluids, it is found that oil/water separation is the predominant mechanism, contributing as much as 68% of the total carbon removed. Since the predominant mechanism for carbon removal is the biologically induced mechanism of oil/water separation, the rate of carbon removal is influenced by both the surfactant degradability and the presence of divalent cations within the wastewater. It is thus shown that the biological treatment process is dependent on both the hardness of the water that is used for dilution, as well as the type of surfactant package that is used within the formulation.

1.2.3 Chapter 4

Within this chapter, the concept of using coagulation, coalescence and oil/water separation as a means to remove biocides from metalworking fluids is explored. The chapter begins by determining the critical coagulation concentration of conventional coagulants used in wastewater treatment ($CaCl_2$, $MgCl_2$, and $FeCl_3$). It is first shown that the coagulation/coalescence process and the coagulants themselves do not possess any inhibitory effects to the micro-organisms that are used in the biological treatment process. Thereafter the ability of the coagulation/coalescence process to remove three conventional biocides (ortho-phenylphenol (OPP) and its sodium salt (Na-OPP), iodopropynyl butyl carbamate (IPBC), and benzisothiazolinone) is investigated. It is shown that the process is highly effective at removing biocides that have a high octanol/water partition coefficient, as well as from formulations that have the biocide concentrations much higher than its aqueous solubility. Furthermore, it is shown the extent of removal is dependent on the amount of oil that is present within the formulation of metalworking fluid that is being treated. The coagulation coalescence procedure was successful in detoxifying formulations which had up to 1g/L sodium ortho-phenylphenate and up to 1g/L of IPBC (which is the maximum recommended dosage concentration). However, coagulation/coalescence was not effective at reducing the toxicity of the concentrations of benzisothiazolinone investigated. This was because this biocide had a relatively low octanol/water coefficient. The results of this chapter show that coagulation/coalescence has the potential to detoxify metalworking fluids that contain oil/partitioning and water-insoluble biocides.

1.2.4 Chapter 5

Within this chapter, the influence of physico-chemical factors such as temperature, pH and airflow on both the development and the carbon removal performance of fixed-film reactors are investigated. It is shown that biofilm yield and biofilm dry weight is dependent on all three of these factors. Increasing the temperature of the bioreactors from 20 degrees to 30 degrees ℃ resulted in significant increases in carbon removal efficiencies and biofilm yields. In terms of pH, it is shown that the bioreactor carbon removal performances could be optimized by having a starting pH which ranged from 7-9, but biofilm yield is specifically optimized at a starting pH of 8. Furthermore, adding a pH buffer into the system resulted in significantly more biofilm yield. This suggests that pH may be used to control the amount of biofilm present in the reactor, and thus can be used to stimulate growth during start-up, or to prevent biofilm overgrowth. The flowrate of air into the system allowed for significantly more biofilm to be produced than having no airflow. This is likely due to a combination of the mixing and the oxygen content that the aeration provides. This chapter showed that physico-chemical factors can be used to optimize biofilm growth within fixed-film reactors.

1.2.5 Chapter 6

In this chapter, the influence of biostimulation, using ammonium and phosphates, on both the development and performance of fixed-film reactors is provided. When phosphates are present in excess, it is shown that stimulating the reactor with ammonium chloride results in a decline in biofilm dry weight and in reactor performance, even though this stimulation resulted in a greater amount of suspended cells. When ammonium is present in excess, stimulation with phosphates significantly increased carbon removal performance, but decreased

biofilm yield. These results suggested that having one nutrient being provided in a limited amount results in increased biofilm yields.

HPLC analysis showed that when the reactors are stimulated with ammonium chloride, removal of saturated fatty amides and of diethylene glycol butyl ether is accelerated. However, removal of diisoproponolamine (DIPA) is inhibited, and unsaturated fatty amides are re-dispersed into the system. The extent of demulsification in the ammonium stimulated system is less than that observed in that with limited stimulation. A possible explanation could be that the ammonium stimulated conditions result in the production of biosurfactants. Amine inhibition suggested that microbes utilised DIPA as a nitrogen source, and that this utilisation was not necessary in the presence of excess ammonium.

The chapter also shows that saturated fatty amides present within the metalworking formulation were primarily responsible for biofilm growth. Thus, metalworking fluid wastewaters containing saturated fatty amides should be used for the commissioning of fixed-film bioreactors. It was also shown that unsaturated fatty amides accumulated within the biofilm structure, introducing the possibility of surfactant recovery from this system.

The fixed-film reactors developed using the information gained from this chapter and in chapter 5 were then applied to the treatment of metalworking fluids containing high concentrations of Na-OPP. Even though these concentrations were found to be inhibitory to the micro-organisms, the fixed-film reactors were able to continuously remove the organic constituents from the metalworking fluid. However, the presence of Na-OPP did result in a lower rate of removal of these organics as compared to systems which did not contain Na-OPP. While the removal of other

organic components ranged between 80-90%, Na-OPP removal varied between 70-50%, suggesting that the component is recalcitrant, and that further treatment options would need to be implemented for its complete removal.

This chapter provides valuable information regarding the nutritional requirements for enhancing fixed-film reactor development and performance. Practitioners may be able to use this information for the commissioning of fixed-film reactors within industry. This chapter also shows that these reactors are capable of operating in the presence of high concentrations of the biocide Na-OPP, which suggests that the reactors have a high degree of resistance to anti-microbial agents.

1.2.6 Chapter 7

The final chapter of this thesis provides concluding remarks on Chapters 2 to 6, and assesses the findings against the objectives of the project. The chapter ends by providing the author's recommendations of future work that can be conducted in the field of the biological treatment of metalworking fluids.

Chapter 2- An Overview of Metalworking Fluids and the Techniques for their Treatment and Disposal

2.1 An Introduction to Metalworking Fluids

2.1.1 What are Metalworking Fluids?

Metalworking processes can be grouped under two different categories (McCoy 2006): metal deformation (such as drawing, stamping and rolling etc.) or metal removal (such as cutting, grinding etc.). The modern global market requires that these processes be done rapidly and efficiently, but since these processes require continuous and intimate contact between a work-piece and a machining tool, friction and heat generation create limitations to achieving this.

Lubrication and heat dissipation can be provided through the application of a fluid, called a metalworking fluid, at the point of contact between the work-piece and the tool (Bataller et al. 2004). Metalworking fluids are thus chemically composite liquids that consist of both lubricating and cooling properties so as to mitigate the damage between the work-piece and the machining tool. Furthermore, they serve to flush away metal chips that are produced within the process, and provide a desired finish to the work-piece. Their application to machining processes ensures that it is optimized by reducing operation down-time, reducing the amount of reject parts, and by extending tool-life.

Metalworking fluids are typically sold as a concentrate which is diluted with water prior to use. According the United Kingdom Lubricants Association, there are approximately 80 000 metal machining companies using metalworking fluids within the UK, creating a market sector valued at £100 billion (Chipasa n.d.). In 2005, the

annual usage of metalworking fluids was estimated to exceed 2 x 10^9 L (Cheng et al. 2005). These statistics provide an indication of the global usage and importance of metalworking fluids within the metalworking industry, which creates a demand for research into improving the sustainability of metalworking fluids in terms of cost effectiveness, and impact to health, safety and the environment.

2.1.2 A Brief History of Metalworking Fluids

There are a large number of historical accounts of man using lubricants in his works, tracing back even to early civilizations (McCoy 2006). Humankind first worked with metal to fashion weapons, ornaments and jewellery, and then later through hot-work as the blacksmithing developed. Unfortunately, the use of lubricants as metalworking fluids is not readily described within early documentations, possibly because the trade of craftsmanship was not viewed as scientific at the time. Nevertheless, by examining artefacts and weapons used by ancient Egyptian and Mesopotamian, and later Greek and Roman societies, one can ascertain that that forging and wire-drawing are amongst the oldest metalworking processes in existence, and one can infer that a lubricant would have been needed to ease the process (McCoy 2006).

It was only after the industrial revolution that friction became an important field of study and the importance of lubrication and cooling was fully appreciated (Brinksmeier et al. 2015; McCoy 2006). Animal fats and oils (tallow oil, lard oil, whale oil), and vegetable oils (olive oil, castor oil) were readily used in machinery for the purpose of lubrication. At this time, the lubricants were added directly to the mechanical parts of the work-piece.

The advent of the 19th century saw much development on machining tools, and metalworking fluid delivery systems (Brinksmeier et al. 2015). Furthermore, the increased availability of mineral oil led to its inclusion within metalworking fluid (MWF) compositions as a replacement for natural oils. This is because the oil, which was a by-product of kerosene refining at the time, was a cheap alternative (McCoy 2006). The 19th century also saw the development of scientific theories of lubrication, as well as the first systematic publications addressing the lubricating effects of metalworking fluids as well as approaches regarding its supply and re-application (Thurston 1885; Mallock 1881)

The 20th century saw the rise of many new mechanical inventions which prompted more need to understand the mechanics of friction, as well as how applications of metalworking fluids could be used to mitigate its effects (Brinksmeier et al. 2015). Further industrialization and the boom of the automobile and the aircraft industry led to a demand for high-performance metalworking fluids. Phosphorus, chlorine, boron and sulphur additives were included into metalworking fluid compositions since research had shown that these imparted improved lubrication properties at high pressures (Spikes 2004). Rising oil prices occurring during the middle of the 20th century created the drive to reduce the amount of lubricating oil that was used within metalworking fluids (Brinksmeier et al. 2015), thereby increasing the importance of water-based metalworking fluids. The creation of oil-in-water emulsions, which consists of both hydrophilic and lipophilic components, represents the first approach to combining cooling and lubrication within a single metalworking fluid combination. The need for greater performance also saw the rise of MWF formulations containing as many as 300 additives within the recipe (Brinksmeier et al. 2015).

The advent of the 21st century saw a greater drive for sustainability within industrial and engineering operations (McCoy 2006). This led to greater emphasis on the health and safety of the employees using metalworking fluids regularly, as well as on the environment which would receive the metalworking fluid waste products (Brinksmeier et al. 2015). In order to promote health, safety and environmental protection, legal protection agencies have introduced legislation banning or limiting the use of hazardous chemicals which were commonly used within formulation (such as formaldehyde-based biocides, amines and chlorinated additives)(The European Parliament 1998). Today, the focus is thus on creating cost-effective metalworking fluids which are friendly to employees, not amenable to rapid degradation, and that produce a waste that would be easy to treat.

2.1.3 Types of Metalworking Fluids

Metalworking fluids can be classified as either being oil-based or water-based depending on the amount of lubricating oil that is used within the formulation. Bienkowski et al. defined four categories of metalworking fluids based on their compositions (Bienkowski 1993). These are Neat Oils, Soluble Oils, Semi-Synthetic and Synthetic.

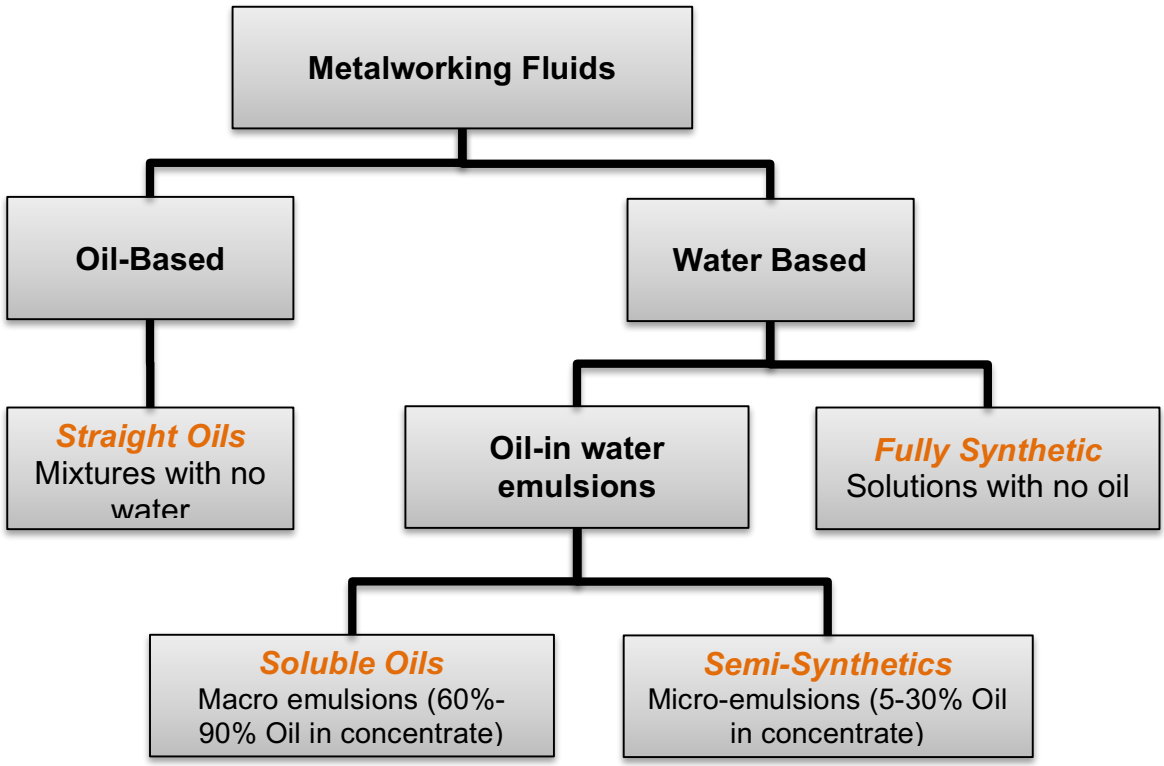

Figure 2-1: Classification of metalworking fluids based on amount of oil used within formulations.

Neat/Straight Oils: These are highly refined or reprocessed oils that are used for difficult machining and forming operations (Childers 2006; Anderson et al. 2003). The base is typically made up of petroleum mineral oils which can be used in isolation or in combination with other oils such as lard oil, vegetable oils, or synthetic oils. These metalworking fluids are suitable for processes where the delivery system has a lot of downtime, or when manual application is sufficient. Straight oils are stable to degradation, have excellent lubrication abilities, and provide good rust protection. Associated problems include higher cost, smoke and fire hazards, operator health problems due to oil misting, and tool damage due to a lack of cooling ability (Childers 2006).

Soluble Oils/Emulsifiable Oils: These metalworking fluids are supplied as a concentrate which may be added to water to form an oil-in-water macro-emulsion

(Typically 1 to 20% by weight, depending on the application). They thus consist of base oil, which can be petroleum oils, synthetic oils or vegetable oils (typically making up 60-90%of the weight of the concentrate), as well as an emulsifier package, typically petroleum sulfonates, fatty amides or fatty acid soaps (Childers 2006; Bienkowski 1993). The inclusion of water and oil into the formulation provides improved cooling properties without a large sacrifice to lubrication properties, which is an important requirement for operations involving high-speed machinery. However, the largest drawback of this type of metalworking fluid is emulsion breaking caused by emulsifiers binding or interacting with divalent ions within hard waters, or by being biodegraded by microbes present within the metalworking fluid (Anderson et al. 2003). Value additives may also be incorporated into the formulation to allow for pH buffering, resistance to microbial degradation, improved rust inhibition, or extreme pressure applications (Childers 2006).

Semi-Synthetic: In comparison to soluble oils, semi-synthetic formulations contain a much smaller fraction of oil within the formulation (typically the concentrates contain 5-30% by weight). Semi-synthetic formulations are typically translucent since they form micro-emulsions due to the high ratio of surfactant to base oil. Semi-synthetics offer enhanced cooling capabilities over soluble oils, but have lower lubrication properties. They are much more stable in hard water systems, but this also plays to a disadvantage since it is more difficult to treat.(Silliman 1992; Irani et al. 2005; Anderson et al. 2003)

Fully Synthetic: Synthetic formulations contain replacement chemicals which serve to provide the lubrication properties that would be imparted by oil. Their formulations thus contain no oil additives at all. Chemical additives for lubrication could be sulfated castor oil, polyethylene glycol or polybutenes. Synthetic

formulations are the most bio-resistant type of metalworking fluids, and are not susceptible to water hardness at all. They have excellent cooling properties, but disadvantages include lowered lubrication properties, foaming tendencies, and waste treatment difficulties. (Anderson et al. 2003; Childers 2006)

As can be ascertained, each metalworking fluid type has associated advantages and disadvantages, and thus the optimum metalworking fluid to be used for an application would depend on the process requirements.

Table 2-1 presents an example of ratings for each metalworking fluid type in each of the key performance indicators for grinding applications.

Table 2-1: Ratings of each metalworking fluid type for a grinding application. Rated 1 for worst, and 4 for best (Irani et al. 2005; Webster et al. 1995).

	Grinding Fluid Characteristics			
	Synthetics	**Semi-Synthetics**	**Soluble Oil**	**Straight Oil**
Heat Removal	4	3	2	1
Lubricity	1	2	3	4
Maintenance	3	2	1	4
Filterability	4	3	2	1
Environmental	4	3	2	1
Cost	4	3	2	1
Wheel Life	1	2	3	4

2.1.4 The Chemistry of Metalworking Fluids

Metalworking fluids are composite liquids that may be made up of both lipophilic and hydrophobic components. In addition to having oil and water within the formulations, metalworking fluids may contain various other chemicals that serve particular functions for the enhancement of the metalworking process. Examples of

the typical components that are used within metalworking formulations are given here.

Base Oils: Base oils are included within straight oil, soluble oil and semi-synthetic formulations. They are the primary components for providing lubrication characteristics and serve as a base for lipophilic components. Base oils used are typically petroleum mineral oils, and these may be either paraffinic (straight chain alkanes) or naphthenic (cycloalkanes). While paraffinic oils may offer better oxidative stability and less smoke during some metalworking processes, most formulations make use of naphthenic oils since a greater number of additives are soluble and compatible with it (Decrean 2005).

In recent times, vegetable oils are being investigated as an alternative to petroleum components due to the poor biodegradability of mineral oils, and due to increasing concerns over the adverse health effects that it poses to workers (Lawal et al. 2012; Shashidhara & Jayaram 2010).

Emulsifiers: The next major components that are included specifically in soluble and semi-synthetic formulations are emulsifiers. Emulsifiers are needed to allow for both water and oil to be incorporated within metalworking fluid recipes since they create micelles which allow for the dispersion of oil and lipophilic components within water. Typical emulsifiers include petroleum sulfonates, synthetic sulfonates, fatty amides, fatty acid soaps and ethoxylates (Anderson et al. 2003; Childers 2006). The major emulsifiers used within soluble oils were natural petroleum sulfonates, but due to them becoming scarce resources (Anderson et al. 2003) manufacturers are now utilizing synthetic alternatives such as synthetic sulfonates, and fatty amides.

Rust Inhibitors: When water became a component within metalworking fluids, it created a need for anti-corrosion chemicals. These chemicals create a protective film on the metal surface to prevent damage from peroxides and acids (Anderson et al. 2003). Rust inhibitors could be organic polar additives such as fatty acid salts, sulfonates, amines, and amides, or can be inorganic oxides such as borates and silicates(Anderson et al. 2003; Childers 2006). Rust inhibitors such as fatty acids and amines are the preferential carbon source which microbes use for growth and energy (Bienkowski 1993; Byung R. Kim et al. 1994), and thus biocides are needed when these are used within formulations.

Boundary Lubricants: These are additives that increase the wetting ability and the penetrating ability oil by producing a monolayer between the metal and the oil phase (Childers 2006). They are compounds that contain a small polar group which is attracted to the metal surface, and a larger hydrophobic group which is attracted to the oil phase (Anderson et al. 2003).

pH Buffers/Alkaline Reserves: These additives are required to maintain the pH of the metalworking fluid at an optimum value –typically between pH 8.8-9.2. This is done to prevent microbial contamination (with many micro-organisms preferring neutral environments), to prevent corrosion of the metal pieces, and to maintain emulsion stability. Typical additives that achieve this purpose are ethanolamines such as monoethanolamine, and diisoproponolamine (Anderson et al. 2003; Childers 2006).

Extreme Pressure Additives: Metalworking fluids used for heavy duty applications that take place at high temperatures and pressures contain components which react with the surface of the materials to prevent micro-fusion between the surface and

the work tool. Examples of such components are chlorinated, sulfurized, and phosphate-containing organic constituents such as sulfochlorinated fatty acids and esters, and phosphate esters. (Childers 2006; Anderson et al. 2003)

Biocides: Metalworking fluids are an ample source of nutrients for micro-organisms such as yeast, fungi, mould and bacteria. Micro-organisms may utilise the organic components within metalworking fluids as a carbon source for growth and energy leading to severe bio-deterioration. Bio-deterioration results in reduced performance (Passman 2006), and the presence of micro-organisms poses a health risk to workers and hence chemicals are added within the metalworking fluids to prevent microbial growth. Typical types of biocides used are phenols (such as o-phenylphenol and sodium phenate), formaldehyde releasers (such as N,N′-methylenebismorpholine), isothiazolinones (such as benzoisothiazolinone) and chlorinated components (Aalto-Korte et al. 2008; Brinksmeier et al. 2015; DOW Microbial Control n.d.). Anti-microbial agents were initially manufactured with the intent of preserving the metalworking fluid (Rossmoore 1981); however, with tightening regulations due to an increase in environmental and worker health concerns, a number of common biocides are no longer being approved for usage. As an example, in 2014 the European Union banned the usage of glutaraldehyde and triclosan in metalworking fluids (The European Commission 2014). The modern day challenge for metalworking fluid manufacturers is thus to find a biocide that can be easily removed from wastewaters, that does not cause adverse effects to the health of workers, and that is effective in prolonging the life of metalworking fluids.

2.1.5 The Need for Periodic Disposals

In the duration of its service life, metalworking fluids undergo changes within their chemical composition and become contaminated with foreign entities which

ultimately lead to a severe deterioration in service performance. Changes in the chemistry of the metalworking fluid could be a result of aging due to the thermo-mechanical loads, and with regards to water-based metalworking fluids, microbial metabolism. Foreign chemicals, such as tramp oils, hydraulic fluids, solid particles and heavy metals accumulate within the fluid when it makes contact with the work piece (Eppert et al. 2003; Childers 2006).

For oil-based metalworking fluids, the main chemical change is due to oxidation and polymerization of components when the fluid is subjected to extreme temperatures and pressures (Brinksmeier et al. 2015). Furthermore, volatile components within the formulation may evaporate when subjected to high temperatures.

Water-based metalworking fluids experience similar deficiencies as oil-based fluids, but the addition of water to the formulation introduces a further problem- microbial contamination (Brinksmeier et al. 2015; Passman 2006). Biocides used within formulations may deteriorate with time leading to microbial proliferation. The build-up of micro-organisms within metalworking fluids leads to severe deterioration of the performance of the metalworking fluid since key performance components, such as emulsifiers and pH buffers are metabolized (Brinksmeier et al. 2015; Passman 2006). Moreover, microbial colonization of metalworking fluids poses a health hazard to workers using the metalworking fluid. Occupational exposure to aerosols generated through the use of metalworking fluids is known to lead to Hypersensitivity Pneumonitis (HP)(Rosenman 2009; Murat et al. 2012). While this may be attributed to chemicals within the metalworking fluids, recent research has shown that there is a causal relationship between the micro-organisms (especially *Mycobaterium Immunogenum*) contaminating metalworking fluids and the emergence of the condition (Thorne et al. 2006). Evidence of this is provided due

to the presence of specific precipitins in workers exhibiting HP, as well as *Mycobacterium Immongenum* existing in the occupational exposure environment.

Finally, foreign chemical accumulation within the system may result in reduced performance. Abanto et al. showed the effects that tramp oil have on biocide efficacy (Abanto et al. 1994). Zimmerman et al. demonstrated that ion accumulation may lead to emulsion destabilisation, and also result in the increase in the microbial loading of the metalworking fluid (Zimmerman et al. 2004).

This means that metalworking fluids have a shelf life, which varies amongst brands. Once performance decline is no longer within limits, the batch of spent metalworking fluid must be treated and disposed of in a manner that is consistent with environmental regulations.

2.1.6 An Overview of Treatment Processes for Spent Metalworking Fluids

Research into the treatment and disposal of metalworking fluids is driven by tightening regulations on environmental pollutant discharge. The common means of disposing of metalworking fluids would be through incineration, but since the advent of the European Union Directive (2000/76/EC) (The European Parliament 2000b) this method is no longer viable. The directive limits the amount of nitrogen oxides and sulphur dioxides that may be discharged into the air, and hence limits the amount of metalworking fluids that can be disposed via incineration (since they contain sulphur and nitrogen in their formulations). Furthermore, European Directives 2000/60/EC and 2010/75/EU has limited the amount of pollutants that can be discharged into the environment (The European Parliament 2000a; The European Parliament 2010). Limits on this list include discharge values for mineral oil, biocides and substances altering chemical and biochemical oxygen demands.

Such pollutants may be found within metalworking fluids (see Table 2-2). Within the United States, limits to the pollutants listed in Table 2-2 is provided by U.S. EPA 40CFR 433 (United States Environmental Protection Agency 2014). As regulations tighten, alternative and more cost effective treatment options must be explored.

Similar to the process for treating domestic sewage, the treatment steps for spent metalworking fluids can be categorized as being either primary stage treatments, secondary stage treatments and tertiary stage treatments. Figure 2-2 provides a schematic for the basic steps that are required for the treatment of metalworking fluid wastewaters.

Table 2-2: Conventional and non-conventional pollutants found in metalworking fluids (Burke & Gaines 2006).

Abbreviation	Pollutant Name
Conventional	
BOD_5	Biochemical Oxygen Demand - 5 day
COD	Chemical Oxygen Demand
SS	Suspended Solids
NH3-N	Ammonia as N
TKN	Total Kjedahl Nitrogen
O & GHEM	Oil and grease as hexane extractable material
Non-conventional	
As	Arsenic
Heavy metals	Heavy Metals
CN	Cyanide
SO_4	Sulfate
NO_3	Nitrate
NO_2	Nitrite
TTO	Total Toxic Organics as per U.S. EPA 40 CFR 433

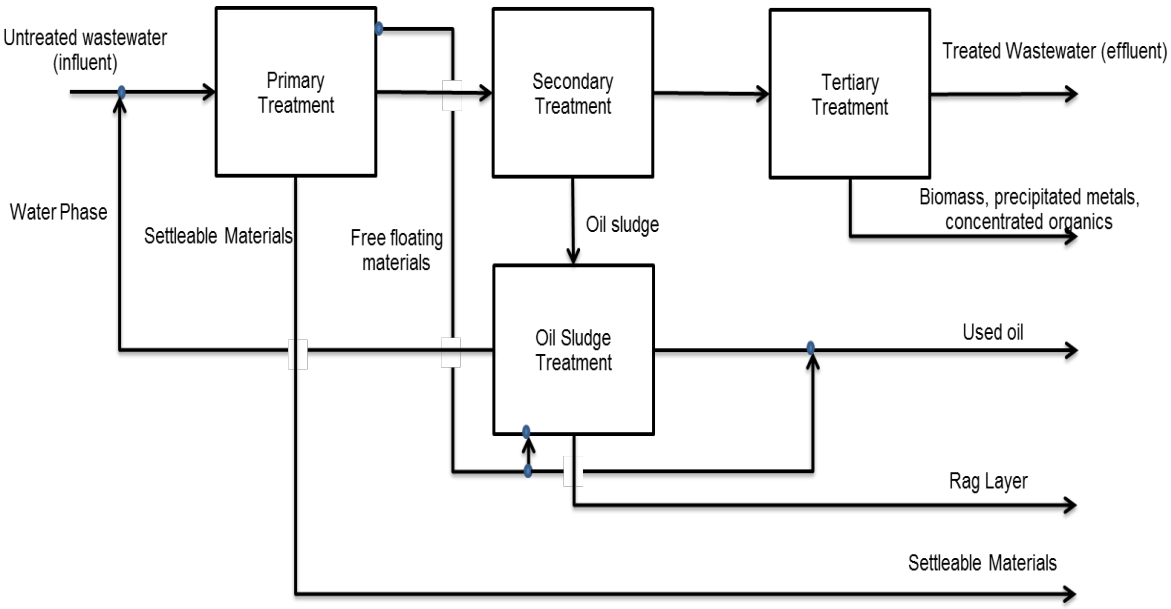

Figure 2-2: Basic treatment steps in metalworking fluid wastewater treatment (Burke & Gaines 2006)

2.1.6.1 Primary Stage Treatments

Influent wastewaters rarely enter treatment operations in uniform flows, and waste treatment facilities have storage units for the receiving wastewaters which act as a buffer between the flow of waste into downstream units and that entering into the facility (Burke & Gaines 2006). This buffer may also act as a means of controlling the concentration of organic pollutants entering into the facility to ensure that the process remains optimized. Influent wastes that contain large solids may need to be filtered before entering the storage tank, or the waste may need to be stored for a sufficient amount of time to allow these solids to settle. A final key purpose of the storage vessel is to allow for tramp oils and other low-density foreign chemicals to settle on the top of the wastewater (Burke & Gaines 2006). This can then easily be skimmed off the surface and thus removed from the wastewater.

2.1.6.2 Secondary Stage Treatments

The main purpose of the secondary stage treatment units is to remove the bulk of the chemical oxygen demand of the influent wastewater, and the choice of treatment unit is dependent on the type of metalworking fluid wastewater that is to be treated. The most common secondary treatment processes are Thermal Evaporation, Vapour Compression Distillation, Membrane Processes and Chemical Treatments. Details of the performance, advantages and disadvantages of each of these processes are provided in the Section 2.3

2.1.6.3 Tertiary Stage Treatments

While the secondary treatment units are able to remove the bulk of the COD, wastewaters may require a tertiary treatment step in order to meet discharge regulations. Tertiary treatments usually are applied to waste streams that have already undergone some form of treatment since the direct application to influent wastes can significantly increase costs. Examples of tertiary treatment units are: Reverse Osmosis, Adsorption Processes, Biological Processes and Advanced Oxidation Processes

2.2 Biological Treatment Options for Metalworking Fluids

The work done in this research project is primarily focussed on the biological treatment of metalworking fluids. Thus, in this section, a critical review on biotreatment studies is provided. An overview of the treatment process is provided, and the advantages and limitations are discussed. Possible solutions to the limitations are also provided. In section 1.3, physical and chemical treatment options are discussed, and literature which has coupled those treatments to the biological process in order to improve treatment efficiencies are reviewed.

2.2.1 An Overview of the Biological Treatment Process

The biological treatment of waste metalworking fluids is an increasingly popular system for the removal of organics and heavy metals (Burke & Gaines 2006; Adapa et al. 2016). Microbes such as bacteria are able to utilise the organic components within the waste metalworking fluid water as carbon and nitrogen sources for the production of new cells, biomass and energy (Alexander 1999). In utilizing these components, microbes break down organics into smaller, simpler organics which leads to a reduction in the COD and the BOD_5 of the waste metalworking fluid. Complete removal is attained when the organics are completely mineralised to form new biomass and/or carbon dioxide (Sperling 2007).

Biological systems can be classified as either being suspended or fixed, depending on the mode of growth (Burke & Gaines 2006; Sperling 2007). Suspended reactors consist of free-floating, dispersed cells and flocs of cells that are constantly circulated through mechanical stirring or aeration. There are no supporting structures for the bacteria to grow. Fixed-film systems contain a substrate upon which microbes may attach and form a biofilm (Sperling 2007). A number of different reactor configurations utilizing these two different modes of growth may be utilised. In the context of metalworking fluid treatment, there have been several studies which have considered different reactor configurations. A summary of the results obtained in each of these investigations is found in Table 2-3. These studies show that both the suspended and the fixed-film reactors are able to remove between 60-90% of the influent COD when the biological treatment process is directly applied to the metalworking fluid wastewater. Studies also show that the biological treatment of metalworking fluids can be applied to soluble oils, to semi-synthetics, and to synthetic metalworking fluids, making it an extremely versatile process

Thus, a summary of the advantages to opting to use the biological treatment process:

- It is a cost-effective treatment method that is applicable to large volumes of waste. It can be operated with minimal chemical addition (Cheng et al. 2005)
- Biological treatment methods may be applied to all types of metalworking fluids.
- Biological methods are robust and can be applied to varying influent conditions.

Table 2-3: Published works on the biodegradation of metalworking fluid waste.

Type of Metalworking Fluid	Microbes used	Concentration treated	Bioreactor Type	Mode	Performance	Notes	Reference
Semi-Synthetic (After emulsion breaking)	Not indicated	COD: 3000 mg/L (Diluted)	Fluidized Bed Reactor with Sand as Carrier	Aerobic	COD Removal : 90%	No details on biofilm development provided. Emulsion broken with H_3PO_4 before being fed to bioreactor.	(Schreyer & Coughlin 1999)
Synthetic + Soluble Oil (After Emulsion Breaking)	Activated Sludge	COD: 500-1500 mg/L (Diluted)	Continuous Flow Suspended Reactor	Aerobic	COD Removal : 70-84%	Emulsion broken for soluble oil.	(Polak 1986)
Soluble Oil	Defined Consortium	COD: 14 000 mg/L (slightly diluted)	Sequencing Batch Biofilm Reactor	Aerobic/Anoxic	COD Removal: 87%	Direct treatment of soluble oil. No mechanisms reported. No biofilm development data reported. Constant addition of microbes was necessary to achieve degradation	(Muszyński & Łebkowska 2005)
Soluble Oil/Semi-Synthetic	Activated Sludge	COD: 3 300 mg/L (Diluted)	Biological Granular Activated Carbon	Anaerobic	COD: 60%	Implied direct treatment of soluble oils/semi-synthetic. No mechanisms reported	(Kim et al. 1989)
Soluble Oil/Semi-Synthetic	Activated Sludge	COD: 3300 mg/L (Filtered)	Suspended Reactor	Aerobic/Anaerobic	COD: 88%	Samples filtered before analysis implying that oils would be removed from COD analysis. No mechanisms for COD removal reported	(Kim et al. 1992)

Type of Metalworking Fluid	Microbes used	Concentration treated	Bioreactor Type	Mode	Performance	Notes	Reference
Soluble Oil	Activated Sludge	COD: 560 mg/L (Very Dilute)	Suspended Reactor	Aerobic	COD: 26-78%	Investigated at different temperatures. No COD removal mechanisms reported	(Deepak et al. 1994)
Semi-Synthetic (After Ultrafiltration)	Defined Consortium	COD: 10000 mg/L	Suspended Reactor	Aerobic	COD: 50 to 75%	Investigated changes in COD removal with pH using defined consortium. Metalworking fluid was pre-treated with ultrafiltration before being subjected to the biodegradation	(van der Gast & Thompson 2004)
Synthetic	Defined Consortium	COD: 50000 mg/L	Suspended Reactor	Aerobic	COD Removal : 85%	Also showed the degradation of organics within the metalworking fluid	(van der Gast et al. 2004)
Soluble Oil	Single Species	TPH : 201 µg/L	Suspended Reactor	Aerobic	TPH Removal : 70%	No mechanisms reported- all attributed to biodegradation. Growth media required for process	(Moscoso et al. 2012)
Soluble Oil	Activated Sludge	COD: 2000 mg/L (Diluted)	Sequencing Batch Biofilm Reactor	Anaerobic	COD Removal : 80%	Direct treatment of soluble oil: No mechanisms reported	(Carvalhinha et al. 2010)

2.2.2 Choice of Microbes

In the context of metalworking fluids both single species and mixed consortiums have been applied to the biological treatment of metalworking fluids. Results that have been obtained vary.

Many researchers have shown that activated sludge from sewage treatment plants is capable of removing contaminants present within metalworking fluid wastes (see Table 2-3). However, in these studies, the influent metalworking fluids are diluted by several orders of magnitude. This is done to reduce the toxicity of the anti-microbial agents that are present in the metalworking fluid. van der Gast et al. demonstrated that following a set of protocols (van der Gast et al. 2002), a consortium of indigenous micro-organisms can be selected to provide superior performance to both a natural occurring consortium and the activated sludge micro-organisms (van der Gast et al. 2003; van der Gast et al. 2004). Their consortium was able to treat metalworking fluids at concentrations close to the undiluted waste concentration. They postulated that the consortium chosen was more effective since it consisted of micro-organisms that persisted both spatially and temporally within sites contaminated with metalworking fluid wastes (van der Gast et al. 2003).

Recently Moscosco et al. reported that a *Pseudomonas stutzeri* was capable of removing total petroleum hydrocarbons more effectively than other indigenous micro-organisms present in the wastewater (Moscoso et al. 2012). However, within their work, they were only able to achieve significant levels of removal after stimulating the waste with growth media- a practice that would not be feasible in industry.

Intriguingly, to the author's knowledge, there have been no reports in the use of fungi for the treatment and disposal of metalworking fluids, even though fungal control for pristine metalworking fluids remains to be a challenge (Passman 2008). The application of white rot fungi may prove to yield significant benefits over bacterial systems such as enhanced resilience, and the ability to degrade persistent pollutants (Barr & Aust 1994).

2.2.3 Anaerobic versus Aerobic

The first comparative studies on aerobic and anaerobic treatments for metalworking fluids were conducted by Kim et al. (Kim et al. 1989; Kim et al. 1992). Using a simulated wastewater, they showed that the aerobic treatment of metalworking fluids was able to remove more than 88% of the COD of the wastewater, while the anaerobic treatment could only remove 64%. While the efficiency of treatment was found to be better for aerobic processes, the anaerobic process results in biogas, a by-product which can be utilised as a source of energy. However, studies by Perez et al. (Perez et al. 2007) , Carvalhinha et al. (Carvalhinha et al. 2010) and by Teli et al. (Teli et al. 2015) showed that the yield of biogas attained is small. Thus, it appears that the anaerobic treatment of metalworking fluids offers no considerable advantage to the aerobic treatment (other than potential pumping costs of aeration, which may be offset by process time differences). Cheng et al. reasoned that the aerobic process was likely better because aerobic heterotrophs are the major organic pollutant degraders in the metalworking fluid reactors (Cheng et al. 2005).

2.2.4 Nutrients Required for Degradation

Carbon, Nitrogen and Phosphorus are essential nutrients that are required for the growth of micro-organisms (Alexander 1999; Cheng et al. 2005). Supplements for nitrogen and phosphorus are commonly added to metalworking fluid wastewaters

in order to promote the formation of these micro-organisms and to promote degradation activity (Moscoso et al. 2012; Cheng et al. 2006; Cheng et al. 2005; Schreyer & Coughlin 1999). In certain cases, carbon sources are also added to metalworking fluid waste streams in order to rapidly develop biomass. However, overstimulation using soft carbon sources can hinder microbial activity (Moscoso et al. 2012).

As Cheng et al. note, metalworking fluids have an abundance of carbon, nitrogen and sulphur, but there is a particular limitation in phosphorus (Cheng et al. 2005). Phosphorus within metalworking fluids usually only occurs as an additive for extreme pressure reagents, and hence may not be readily present, or readily bioavailable (Childers 2006). Schreyer et al. and Cheng et al. have demonstrated that phosphate addition to metalworking fluids have a positive effect on both reactor efficiency outcomes (Schreyer & Coughlin 1999; Cheng et al. 2005).

While there are many studies on the effects of nutrient addition on the performance of suspended bioreactors, there are limited studies on the effects of nutrients on the development of biofilms within metalworking fluid reactors. Optimization of both the nutritional conditions and the physico-chemical conditions required for biofilm development could reduce start-up times that are required to develop a mature reactor needed for the treatment of hazardous wastes.

2.2.5 Mechanisms of Pollutant Removal

Within industry, the biological treatment is typically applied as a tertiary treatment for emulsion waste (Burke & Gaines 2006; Cheng et al. 2005). Oils are typically removed before being subjected to the bio-treatment process since they have been reported to have a low biodegradability (Rabenstein et al. 2009; Aluyor & Ori-Jesu

2009; Novick et al. 1996), and since this reduces the organic load on the microbial reactor. However, as Table 2-3 shows, it is possible to apply the biological treatment directly as a secondary treatment step as well.

Thus, there is a contradiction in literature. Mineral Oil used within formulations of metalworking fluids does not have high rates of biodegradability (Rabenstein et al. 2009; Burke & Gaines 2006; Anderson et al. 2003). Bioremediation attempts and bioreactors treating mineral oils show that the removal efficiencies are varied, and that a relatively large amount of time is required for their mineralization. In fact, the actual test for biodegradability of oils is set for a standard time of 21 days due to the inherent low biodegradability of oils (Novick et al. 1996). Furthermore, within metalworking fluids, cyclic alkanes in the form of naphthenic mineral oils are used, further limiting the biodegradation potential (Anderson et al. 2003; Burke & Gaines 2006).

Even with the low biodegradability of oil, several researchers have reported relatively fast kinetics of COD removal in systems treating emulsified metalworking fluids (Moscoso et al. 2012; Muszyński & Łebkowska 2005; Kim et al. 1992; Deepak et al. 1994). This implies that there may be an alternate removal mechanism that is dominant within the treatment process. Carbon within the metalworking fluid may be removed through adsorption and through assimilation and dissimilation(Alexander 1999). For emulsions, a third possibility exists. Removal may occur through the biodegradation of emulsifiers within the metalworking fluid (Burke & Gaines 2006). Preferential degradation of emulsifiers may result in oil/water separation within the bioreactor and this oil may be adsorbed onto the microbes, onto the reactor walls or float on the surface of the reactor. Thus, while removal is observed, ultimate removal may not be achieved.

Table 2-3 shows that while there are many studies that have looked at the biodegradation of emulsified oils, none have investigated as to whether the mechanism for removal was destabilisation induced by degradation of the surfactants. For synthetic treatments, this is less of a problem since oil/water separation cannot be a removal mechanism (given that there is no oil).

As Cheng et al. notes, there is a need to understand the relative roles of biosorption and biodegradation within metalworking fluid reactors(Cheng et al. 2005). This needs to be done so that further considerations for sludge removal may be made. If indeed oil/water separation is the main observed removal mechanism, then oils accumulated on the top of the reactor, and that adsorbed on the side walls will need to be disposed of as hazardous waste once the reactor is cleaned.

2.2.6 Limitations to the Biological Treatment Process

The biological process is a cost-effective and highly versatile and adaptable process for treating metalworking fluids (Cheng et al. 2005). It is however, not without its limitations.

There are a number of limitations with the microbial treatment process of metalworking fluids.

1- Sludge Generation: The microbial treatment of metalworking fluids results in a sludge of microbes which must be disposed of either through incineration or through landfills (Wei et al. 2003). Adoption of biofilm technology may reduce sludge generation. (Nicolella et al. 2000).
2- Dilution requirement: Table 2-3 shows that for the majority of microbial treatment processes, there is a need for the dilution of metalworking fluids. This is necessary in order to reduce the anti-microbial effect of biocides (Thill

et al. 2016; Jagadevan et al. 2012). While dilution might be achieved through the recycling of process water, dilution would reduce the process time required to treat a given quantity of metalworking fluids, and will thus contribute to capital costs. While van der Gast's microbial consortium has the potential to withstand high organic loads (van der Gast et al. 2004), it may not be able to be universally applied to all metalworking fluid wastewaters. Recently, Thill et al showed that this defined consortium could not degrade a relatively dilute pristine metalworking fluid (Thill et al. 2016).

3- Related to the dilution problem is the general problem of susceptibility of microbes to anti-microbial agents. Introducing biocides into a bioreactor may result in an inhibition of reactor functioning (Cheng et al. 2005; Jagadevan et al. 2011), and may render the metalworking fluid resistant to bio-treatment.
4- Since the process is slower than that of conventional treatments, and since the capital costs are relatively high (due to dilution requirements), it is typically a process that is recommended for large producers of waste.

On point 3, a hybrid treatment approach is required so that metalworking fluids may be sufficiently treated. In section 2.3, common physical and chemical treatment techniques will be reviewed, and their potential for coupling with the biological treatment process to enhance treatments is presented.

2.2.7 Fixed-film Reactors for Treating Hazardous Wastes

There are two ways to overcome the limitation of biological system susceptibility to anti-microbial agents. The first means is to remove the anti-microbial agents via a pre-treatment technique. The second means is to improve the innate resistance of the reactor to anti-microbial agents. A natural means in which microbes are able to

protect themselves from anti-microbial agents is through the formation of a biofilm, and this innate trait can be exploited through the use of fixed-film reactors.

Fixed-film reactors, or biofilm reactors, are those that are designed to promote the growth of microbes in a biofilm (Alexander 1999; Gullicks et al. 2011). A broad definition of a biofilm is that it is a loose aggregation of cells that are associated and attached to a solid-liquid interface (Flemming & Wingender 2010). The cells that make up this aggregation are encased within a highly hydrated matrix of extracellular polymeric substances (which could include polysaccharides, proteins, nucleic acids, and lipids). The EPS matrix can contribute to about 90% of the biofilm dry weight, and has a number of functions such as to maintain the structural integrity, to act as storage for food sources, and to protect the cells from the harsh environment of the bulk liquid (which could be due to anti-microbial agents).

The formation of a biofilm allows for a reactor to develop a number of resistance mechanisms to anti-microbial agents (Flemming & Wingender 2010).

There are several advantages of employing fixed-film reactors for the treatment of hazardous waste. These are provided in both in Figure 2-3 and the list below:

1- Biofilms are structures with considerable thickness and cellular density. Being porous in nature, anti-microbial agents would need to diffuse from the bulk to the inner layers in order to inhibit cells encased within the EPS matrix (Das et al. 2012). This diffusion barrier allows for the protection of the cells, and can also increase the potential for the degradation of inhibitors since the concentration of inhibitor within the inner parts of the biofilm would be relatively dilute in comparison to that within the bulk (O ’toole et al. 2000; Mah

& O'Toole 2001). Retarded diffusion of anti-microbials may also afford more time for adaptive stress responses to be implemented (Stewart 2002).

2- The components of the EPS are able to adsorb anti-microbial agents, especially cations such as heavy metals. This binding would mean that the anti-microbial agents do not come into contact with the cells (O 'toole et al. 2000; Dunne 2002; Mah & O'Toole 2001).

3- Since the biofilm offers a micro-environment that is different to that of the bulk liquid (Dunne 2002; Mah & O'Toole 2001). This can alter the activity of anti-microbial agents, and can create a more diverse population of microbes in a bioreactor.

4- Cells within a biofilm behave as a community rather than as an individual. This behaviour increases the fitness of the community and thus makes the cells more capable of surviving harsh environment. (Jefferson 2004) Recently, Liu et al. demonstrated how metabolic co-dependence within a biofilm increased its resistance to hydrogen peroxide (Liu et al. 2015)

5- Microbes can impart anti-microbial tolerances and degradation characteristics to each other within the biofilm through a mechanism known as horizontal gene transfer (Jefferson 2004).

6- Biofilm reactors have a higher cellular density than that of suspended reactors, and thus can withstand a higher concentration of inhibitory components (Hosseini & Borghei 2005; Davies 2003; Liu et al. 2015).

7- Cells within the biofilm have slower growth kinetics than those in the suspended form. This means that anti-microbial agents that specifically target growing cells are not effective against cells within the biofilm (O 'toole et al. 2000; Lewis 2005; Dunne 2002).

8- Biofilms offer protection for “persister cells”, which are cells that have a phenotypic distinction that allows them to be resistant to anti-microbial agents. (O ’toole et al. 2000; Lewis 2005). This protection increases the likelihood of survival within harsh environments so that they may regenerate a cell population that has been subject to anti-microbial attack.

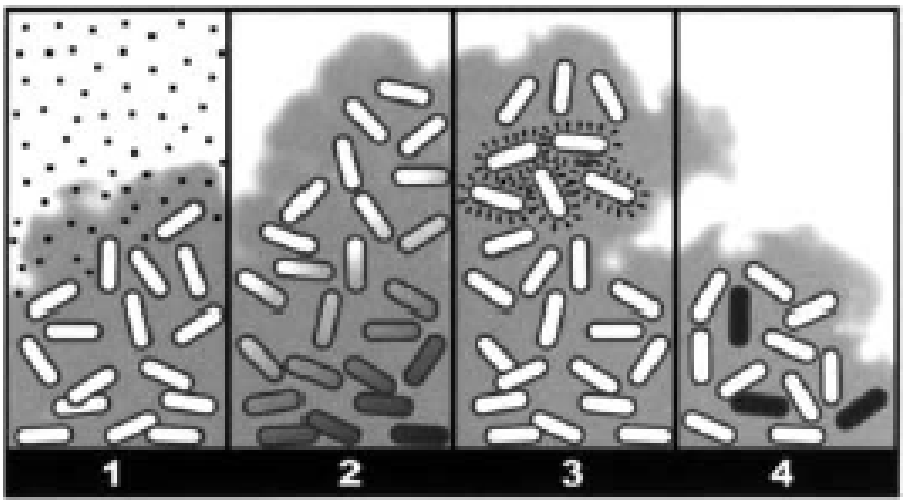

Figure 2-3: Four hypothesized biofilm resistance mechanisms. 1- The antibiotic (dots) penetrates slowly or incompletely; 2- a concentration gradient of a metabolic substrate or product leads to zones of slow or non-growing bacteria (shaded cells); 3- an adaptive stress response is expressed by some of the cells (marked cells); 4- a small fraction of the cells differentiate into a highly protected persister state (dark cells). (Stewart 2002) – Reused with permission.

Beyond having significantly more resistance mechanisms in comparison to their suspended counterparts, biofilms also offer several other advantages as well. Biofilm reactors retain 5 to 10 times more biomass per unit volume of the reactor. This increases treatment rates, and reduces capital costs (Nicolella et al. 2000; Ercan & Demirci 2015). Furthermore, since the cells are retained, biofilm reactors are particularly suited to processes which have slow treatment kinetics, or when dilution is significantly required (such as metalworking fluid wastewaters). This is because treatment using a suspended reactor in continuous mode may result in cell wash-out (Ercan & Demirci 2015; Vanloosdrecht & Heijnen 1993). For batch systems, retention implies that there is no need for re-inoculation (Vanloosdrecht &

Heijnen 1993). Biofilm systems are much more resilient and resistant to organic and hydraulic shock loads. Finally, biofilm reactors allow for increased sludge age and less sludge production, thereby reducing disposal costs.

It is due to these advantages that biofilm reactors have been applied, in various forms, for the treatment of hazardous waste (Kargi & Eker 2005; Katsivela et al. 1999; Lai et al. 2009; Borghei & Hosseini 2004; Dong et al. 2011). Biofilms have been applied for the remediation of sites contaminated with heavy metals, toxic hydrocarbons, plastic wastes and synthetic dyes (Das et al. 2012). Studies have also directly compared suspended and biofilm reactors for the treatment of both hazardous wastes (Dong et al. 2011) and for recalcitrant waste (Falås et al. 2013; Falås et al. 2012) and have shown the biofilm reactors exhibited greater removal rates per unit of biomass. Finally, studies have also compared the performances between microbes existing as suspended cells, and those within a biofilm, and have shown that biofilms are more efficient at treating toxic wastes. Shen et al compared the performances of suspended sludge against biofilm cells and showed that biofilm cells could tolerate higher concentrations of free ammonia in highly concentrated aniline water (Chen et al. 2007). Tziotzios et al. showed that packed-bed reactors were more efficient at treating phenolic waste streams than suspended growth reactors (Tziotzios et al. 2005). Similar results were obtained by Li et al. when considering the removal of phenols, ammonium and cyanate (Li et al. 2011).

While there are clear advantages to the use of biofilms for wastewater treatment, there are limitations with regards to long start-up and development times (Nicolella et al. 2000; Ercan & Demirci 2015). Slow development of biofilms may also lead to slower recovery times within the bioreactor. Several studies have shown that attachment rates and biofilm development may be influenced by substratum type,

nutritional factors such as carbon, nitrogen and phosphorus sources and physico-chemical factors such as temperature and pH (Donlan 2002). While there have been several studies that have looked at these effects for different micro-organisms, and different bulk liquids, there are no studies which have examined the factors which influence the development of biofilms within metalworking fluid wastes.

Further disadvantages could stem from the choice of reactor system employing biofilms. Packed beds and trickling filters may become blocked with biomass. Overgrowth of biofilms on particulates could lead to stratification of particles. Thus, careful selection of the correct bioreactor type would need to be made to ensure that the disadvantages do not outweigh the advantages of adopting biofilms.

2.3 Physical and Chemical Treatment Options for Metalworking Fluids

Having considered a broad overview of the common treatment processes for metalworking fluids in section 2.1.6, this section provides a more detailed analysis of work that has been done in applying and improving each process for the treatment of metalworking fluids. In this section, the physical and chemical treatment options are considered. Section 2.2 provides a dedicated analysis of the biological treatment process, which is the prime focus of the work.

2.3.1 Thermal Processes

Thermal processes are secondary treatments which involve providing thermal energy to boil waste metalworking fluids so that water and volatile components are evaporated and discharged from the remaining non-volatile components (Burke & Gaines 2006). The exhaust vapours may be vented into the atmosphere or they may be returned via a vapour compression cycle so that their latent heat may be reused. This process has the advantage that it may be applied to all types of

metalworking fluids and that it eliminates sewer discharge. While this process is simple and convenient, it is highly energy intensive and is thus only economical for users of small volumes of metalworking fluids (Burke & Gaines 2006). Furthermore, with tighter restrictions being imposed on acceptable discharge of pollutants into air (Cheng et al. 2005), it is likely that the application of this process will be limited in the future. Finally, the process still results in an oily sludge that needs to be reclaimed or disposed of.

Recently, Sanchez-Oneto et al. researched the application of hydrothermal oxidation to reduce pollutant loads (Sánchez-Oneto et al. 2007). This method utilises the unique physical-chemical properties of water above its critical pressure and temperature to oxidize organic contaminants. While they achieved more than 95% removal of COD and TOC, they could only do so at a temperature and pressure of 773 K, which would be unfeasible for large users. Portela et al. showed that the addition of hydroxyl promoters can make the process more efficient at lower temperatures (Portela et al. 2001), but this leads to an additional cost to the process. No cost analysis was done to determine if the cost of chemicals offsets the operating cost of the hydrothermal oxidation process.

To the author's knowledge, there are currently no studies which have combined thermal processes with biological treatment processes. Cheng et al. demonstrated that the thermophillic biological treatment of metalworking fluids may be an effective means of reducing pollutant loads (Cheng et al. 2006). In order to achieve the high temperatures that are required for this process, it may be possible to use the vent gasses from the process as a pre-heat to the biological reactor. Coupling with the biological process may reduce the amount of volatile organics that are discharged through the thermal treatment, and may reduce the amount of residual organics in

the left-over sludge. However, if microbes are made to enter into the evaporative process, there will now be a non-volatile suspended solids concentration in the residual sludge.

2.3.2 Ultrafiltration

Membrane processes are a common and versatile form of treatment that has been applied to waste metalworking fluids (Burke & Gaines 2006; Hilal, Busca, Hankins, et al. 2004; Hu et al. 2002). Membrane processes used in the secondary treatment stage are usually ultrafiltration units. These are pressure driven processes which result in the separation of the wastewater into two phases, namely a clean permeate consisting of predominantly water, and a concentrated retentate, containing emulsified oils and oil-partitioning organics (Hu et al. 2002). Soluble inorganic components and water partitioning organics are not effectively removed by ultrafiltration and thus this form of treatment is not effective for synthetic wastes (Burke & Gaines 2006; Chipasa n.d.).

Ultrafiltration units typically have a pore size of about 0.02 to 0.07 micron, but can reject components that have an effective diameter that is greater than 0.01 micron, since a gelatinous layer, acting as a secondary membrane, forms on the membrane surface (Burke & Gaines 2006). Typical operation pressures range from 2-10 bar (Mulder 1996).

In the context of metalworking fluids, ultrafiltration is able to sufficiently treat both soluble oils and semi-synthetics. Hilal et al. showed that ultrafiltration of a semi-synthetic formulation using a Polysulfone Nadir membrane was able to achieve 87.8% TOC removal (Hilal, Busca, Talens-Alesson, et al. 2004). Lipp et al. achieve more than 96% carbon removal for emulsified oils (Lipp et al. 1988). Similar results

were obtained by Hu et al. (Hu et al. 2002). Several factors, such as pH, transmembrane pressure, cross-flow velocity, oil concentration, inlet temperatures and pressures, affect membrane performances. These parameters were investigated by Hesampour et al. (Hesampour et al. 2008).

An important design parameter for membrane processes is the concentration factor. This is the factor by which the retentate is concentrated in the process. Typically, ultrafiltration units are designed to concentrate waste metalworking fluids from about 1% MWF by volume to about 30-50% by volume(Burke & Gaines 2006). Any further concentration is difficult due to flux limitations caused by fouling. It is important to realize that the brine that is produced from membrane processes must be further treated and disposed of. Since there is still a large amount of water within the brine, it is difficult to make use of this stream as a potential fuel source (Hilal, Busca, Talens-Alesson, et al. 2004).

Key advantages of membrane processes include high removal efficiencies, no extraneous chemicals (other than that required for cleaning), smaller equipment sizes, and lower energy costs as compared to thermal processes.(Cheryan & Rajagopalan 1998)

However, fouling is one of the greatest limitations to membrane processes (Cheryan & Rajagopalan 1998; Burke & Gaines 2006). It is a term that is broadly used to describe anything that causes a flux decline. In metalworking fluid wastes, fouling could be caused by dissolved components, emulsified components, solid particulates, or precipitants. Periodic cleaning may assist in the reduction of reversible fouling (Lindau & Jonsson 1994), but there are components within

metalworking fluids, such as silicates and cationic polymers, which can permanently damage metalworking fluid membranes.

The need for periodic cleaning and the propensity for irreversible fouling to occur limit the applicability of membrane processes to only small volume processes. Furthermore, since scale-up costs are approximately linear, large capital costs are required for the treatment of large volumes of wastewater (Cheryan & Rajagopalan 1998).

Ultrafiltration processes have been combined with biological processes for the treatment of metalworking fluids. This can be done as a pre-treatment to the biological process, so that poorly degradable organics (such as mineral oil) are removed prior to the treatment, or through its incorporation in a membrane bioreactor. The coupling of the process ensures that organics that pass through the membrane process are subjected to a further treatment for removal. However, in the case of the membrane bioreactor, microbial fouling becomes a new limitation which could result in significant flux declines.

No studies have investigated the capability of ultrafiltration as a pre-treatment for reducing the toxicity of metalworking fluid wastes. Since the treatment results in the separation of oil and water phases, it is likely that oil-partitioning biocides would not pass into the permeate, making it more amenable to biodegradation.

2.3.3 Nanofiltration and Reverse Osmosis

Nanofiltration and reverse osmosis are two pressure driven processes which contain even smaller pore sizes than that of ultrafiltration, and operate at much higher pressures (typically 10-50 bar) (Graff 2012). The molecular weight cut off for NF membranes are typically 250-400 Da, while it is less than 150 Da for Reverse

Osmosis. These membranes have pores that a small enough to retain ions and large molecules, and are thus able to remove organic material and heavy metals that are dissolved within the metalworking fluid waste-streams. Both nano-filtration and reverse osmosis membranes foul easily, and thus it is necessary for a pre-treatment to follow this main process. Hilal et al. investigated showed that ultrafiltration (Hilal, Busca, Hankins, et al. 2004) and coagulation (Hilal, Busca, Talens-Alesson, et al. 2004) can be used as an effective pre-treatment to nano-filtration, but showed that there is still residual carbon left in the permeate. Frequent cleaning is required to maintain a steady flux through these membranes, and since they are not as durable as ultrafiltration membranes, care must be taken when applying the cleaning since over-aggressive application may lead to complete disintegration of the RO/NF membrane (Burke & Gaines 2006).

2.3.4 Coagulation, Coalescence and Flocculation

Chemical treatment methods for the secondary stage involve coagulation, coalescence and flocculation (Burke & Gaines 2006). This method is most effective at treating soluble oils and semi-synthetics which have anionic emulsifiers within their formulations.

During the treatment of these metalworking fluids, inorganic coagulants resulting in positive mononuclear (such as $AlCl_3$ or $CaCl_2$) or polynuclear cations (polyaluminium chloride), or organic polyelectrolytes (such as poly-DADMAC), are added to destabilize the oil-in-water emulsions (Burke & Gaines 2006)(Teh et al. 2016). This leads to coalescence of that the oil droplets which then separate from the water via gravity. During the coagulation process, which involves rapid and vigorous mixing, the ions of the coagulant diffuse through the electrical double layer surrounding the oil droplets, and neutralise the anionic surfactants adsorbed on

them. This results in a significant decline of the repulsive double layer forces keeping the oil droplets in suspension, to the extent that the van der Waal forces of attraction are now sufficient to allow for collision and coalescence of the oil droplets (Teh et al. 2016). Furthermore, some coagulants precipitate after the coagulation process, and the solids that form are able to adsorb oil and organic species (Hilal, Busca, Talens-Alesson, et al. 2004).

Once coagulation has been completed, a slow stirring mechanism is employed to promote aggregation by both orthokinetic (bulk) and perikinetic (molecular) mechanisms (Teh et al. 2016). If flocculation also occurs, an anionic polymer may be added to better agglomerate the colloidal solids formed (Burke & Gaines 2006).

Within literature, there are a number of studies which have shown that coagulation and coalescence is an effective treatment to reduce turbidity, chemical oxygen demand, oil content and total organic carbon of metalworking fluids (Ríos et al. 1998; Hilal, Busca, Talens-Alesson, et al. 2004; Canizares et al. 2008). Coagulation and coalescence has also been a widely adopted technique for the improvement of successive treatment processes (Bensadok et al. 2007; A. Cambiella et al. 2006). Hidal et al. showed that nano-filtration processes could be effectively employed after coagulation/coalescence treatment (Hilal, Busca, Talens-Alesson, et al. 2004). Similarly, Gutierrez et al. showed that it can be used to improve efficiencies of ultrafiltration and vacuum evaporation (Gutiérrez et al. 2011).

While inorganic coagulants have been widely and historically used, cationic polymers are beginning to emerge as an alternative (Burke & Gaines 2006; Teh et al. 2016). This is because the precipitation of the inorganic coagulants results in a further sludge that needs to be disposed or reclaimed. Furthermore, if too much

precipitation occurs, then the solids become occluded within the oil to form a “rag layer” which lowers separation efficiency and quality (Burke & Gaines 2006). Cationic polymers are able to reduce sludge production and require lower dosages of chemicals.

The advantages of coagulation/coalescence are that it is a relatively simple process which is cost-effective for treating large volumes of wastewaters. It is also very effective at attaining high removal efficiencies for metalworking fluids with anionic surfactants (Burke & Gaines 2006). While it may be applied to those with non-ionic surfactants or those with no surfactants, the removal efficiency is much lower since the predominant mechanism would be precipitation and adsorption of organics (Hilal, Busca, Talens-Alesson, et al. 2004). A final advantage is the production of a distinct oil layer. If produced in sufficient quantities, the oil may be reclaimed and recycled- thus being a potential source of income for waste practitioners (Cheryan & Rajagopalan 1998).

Disadvantages of the coagulation process is that it is ineffective at removing water partitioning organics, that it is highly variable and thus the dosage requirements would change as the influent does, that it requires a skilled operator, and that it produces a chemical sludge (unless the more costly cationic polymers are used) (Cheryan & Rajagopalan 1998). Furthermore, it is not feasible for treating small volumes of waste. Dang et al. found that for a treatment process handling 500 gallons of waste a day, the coagulation process cost more than the ultrafiltration process (Dang et al. 1992).

Since the biological treatment of metalworking fluids is also a process that should be applied to large volumes to be feasible, a coupling of this process would be ideal.

While there is literature on the coupling of coagulation/flocculation processes for other waste streams (Al-Mutairi 2006; Liu & Chang 2004; Amuda & Amoo 2007; Karthik et al. 2008) there is a lack of literature detailing the coupling of the process to metalworking fluids. Within Chapter 3 of this thesis, the effect that coagulation has on the microbes involved in the biological treatment process in terms of toxicity reduction is provided.

2.3.5 Electrocoagulation

As an alternative to directly adding coagulants into a reaction vessel, coagulation and coalescence may be initiated through the use of an electric current (Emamjomeh & Sivakumar 2009). Electrocoagulation is the electrochemical production of destabilizing agents through the consumption of "sacrificial electrodes". For example, the electrolytic consumption of aluminium anodes will result in the production of Al^{3+} ions, which would behave similar to those produced through the direct addition of chemicals. The main advantages to electrocoagulation are ease of operation, no requirement for chemical usage, and reduced sludge production (Emamjomeh & Sivakumar 2009; Canizares et al. 2008; Chen 2004). Furthermore, the application of an electrical current promotes the higher sedimentation velocities as compared to the conventional treatment (Kobya et al. 2011), thus improving efficiencies as compared to conventional processes (Chen 2004).

Several studies have shown that electrocoagulation is able to achieve high COD and TOC reduction efficiencies when it is applied to metalworking fluid wastewaters (Bensadok et al. 2008; Kobya et al. 2008; Canizares et al. 2008; Sangal et al. 2013).

The major drawback of this technology is the high-investment costs and severe competition with the chemical treatment process, which is cheaper to implement (Bensadok et al. 2008). However, with research into the improvement of electrochemical processes and the implementation of more stringent environmental policies has seen this technique regain importance and it's cost has become comparable to that of conventional technologies (Chen 2004).

2.3.6 Oxidation Processes

As a tertiary treatment step, chemical oxidants may be utilised to react with organics and nitrogenous components still present in the wastewater (Burke & Gaines 2006). This is usually done in order to bring the discharge water to be within environmental regulations (Macadam et al. 2012). Oxidation processes utilise direct oxidants for the reaction, or are designed to produce highly reactive, non-selective radicals which are capable of driving the oxidation and mineralization of pollutants (Andreozzi et al. 1999; Jagadevan et al. 2012). Conventional oxidation processes utilise sodium hypochlorite, hydrogen peroxide, ozone, Fenton's reagent, ultraviolet radiation with hydrogen peroxide and ultraviolent with ozone (Burke & Gaines 2006).

Oxidation Processes typically make use of expensive chemicals, so it is rarely used as a secondary treatment step. In the context of metalworking fluids, the waste stream is pre-treated using techniques such as ultrafiltration or biological treatment steps before being subjected to the oxidative treatment (Macadam et al. 2012). Painmanakul et al. investigated the use of advanced oxidation processes before and after coagulation/coalescence, and found that an unfeasible amount of chemicals was required for complete treatment without pre-treatment (Painmanakul et al. 2013)

However, in terms of the biological treatment of metalworking fluids, oxidation processes may be employed as a pre-treatment since it would result in the degradation of toxic components that would hinder the biological treatment process (Macadam et al. 2012). Jagadevan et al. investigated the potential of oxidation processes using Fenton's reagent (Jagadevan et al. 2011), and using zero-valence nano-iron (Jagadevan et al. 2012) for reducing metalworking fluid toxicity. They reported that the nano-iron treatment led to an 85% reduction in toxicity indicators, and that the Fenton's treatment process increased the BOD_5/COD ratio from 0.160 to 0.538). Recently Thill et al. showed that electron beam treatments can also improve metalworking fluid biodegradability (Thill et al. 2016).

The advantages processes are that it is highly effective at removing toxic and recalcitrant wastes (Oturan et al. 2014). However the processes involve a constant addition of chemicals and/or energy input, and thus may not be feasible for large scale operations. Furthermore, there are significant hazards to health and safety in storing and transporting highly reactive oxidants.

However, costs may be reduced when combined with biological treatment techniques, either as a pre-treatment to reduce toxic loadings, or as a finishing step to reduce recalcitrant components (Macadam et al. 2012).

2.3.7 Adsorption

Adsorption processes employ adsorbents such as activated carbon or ion-exchangers to remove low-concentration chemical contaminants such as heavy metals and recalcitrant organics from wastewaters (Burke & Gaines 2006). The chemical contaminants bind to active surfaces on the adsorbents due to a number of mechanisms such as chelation, ionic interactions or van der Waal's forces.

Adsorbents may be applied as a fixed granular bed, or in a powdered form that must be flocculated after addition.

Conventional adsorption treatments make use of activated carbon as an adsorbent to treat metalworking fluid wastewaters (Burke & Gaines 2006). However, since activated carbon is expensive, and since regeneration and reactivation costs may be applicable, it is typically applied as a tertiary treatment. Hilal et al. investigated a process utilizing ultrafiltration and biodegradation, and found that an effluent with large concentrations of residual COD had resulted (Hilal et al. 2005). The activated carbon treatment process allowed for more than 80% of this residual COD to be removed.

A trend for wastewater treatment via adsorption is to find low cost adsorbents which may be able to replace activated carbon (Pollard et al. 1992). Several studies have shown that low-cost adsorbents may be utilised as a secondary treatment step since regeneration costs no longer need to be considered. Studies have shown that natural materials such as agricultural waste (Katiyar et al. 2014; Ibrahim et al. 2010), un-activated coal (Li et al. 2010), chitosan (Naowanat et al. 2016), fungi (Srinivasan & Viraraghavan 2010) and sawdust (Á. Cambiella et al. 2006) have all been able to directly treat metalworking fluids. Challenges with the application of waste and natural materials include a need for pilot scale testing, a need for regeneration studies, and feasibility studies proving that the alternative component will be in sufficient supply to replace activated carbon. (Pollard et al. 1992)

Adsorption processes have great potential in being coupled with the biological treatment process. When following the biological treatment process, adsorbents are able to remove recalcitrant components that are not amenable to biodegradation

(Burke & Gaines 2006; Hilal et al. 2005). When being applied in the same unit as the biological treatment process, microbes may form on the surface of the adsorbent which may lead *in-situ* regeneration (Kim et al. 1989). Moreover, by coupling together adsorption and biodegradation, the bioreactor is made more resistant and resilient to organic and toxic shock loads (Skouteris et al. 2015).

Coupling adsorption to the biological treatment can have synergistic effects. Kim et al. have also demonstrated the synergistic effect of coupling adsorption and biodegradation by showing that the adsorption capacity of activated carbon increased five-fold after the bio-treatment (Kim et al. 1990). Hilal et al. showed that compounds that adsorbed onto the surface of activated carbon were more amenable to biodegradation (Hilal et al. 2005).

While studies have shown that activated carbon acts as a good substrate for biofilm formation and pollutant removal (Camper et al. 1986), not many have investigated the potential of low-cost substrates. There is a need to investigate the potential of low-cost adsorbents as a substrate for biofilms so that they can be used in the bioprocess.

Table 2-4: Summary of treatment techniques for waste metalworking fluids, and their potential to be coupled with the biological treatment process.

Treatment Type	Treatment Stage	Advantages	Disadvantages	Couplings with biotreatment process
Thermal	Secondary	Efficient at reducing volume of waste No need for sewage discharge Can be applied to all types of metalworking fluids Units are compact in size	Highly energy intensive Volatile organics may be discharged into atmosphere Produces sludge that must be disposed of	None reported. There exists potential for bioprocess to reduce volatile organic discharge, and amount of organics in sludge.
Ultrafiltration	Secondary	High COD/TOC removal efficiencies No need for chemical addition in process Less energy intensive than thermal, less costly than advanced oxidation Cost-effective solution for small waste treatments	Not effective for synthetic metalworking fluids Produces concentrated sludge that must be disposed of Mineral Oil, and other metalworking fluid components cause fouling Not suitable for large treatment processes	Studies show it may be used as a pre-treatment for biological treatment to remove poorly degradable mineral oils. May be incorporated into a membrane bioreactor.
Nanofiltration	Tertiary	Able to provide more effective TOC/COD removal efficiencies than Ultrafiltration	Lower fluxes and higher operation pressures required (3- 20 bar) Fouling is extensive unless a pre-treatment process is implemented	Biotreatment likely to occur after the treatment step to remove molecules that passes through membrane. NF membrane bioreactors are possible, but no studies have used metalworking fluids as a waste stream.
Reverse Osmosis	Tertiary	Greater treatment performance than NF (<150 MWCO as opposed to 250-400 MWCO)	Operates at even higher pressures than NF. (5-120 bar) Pre-treatment is essential. Membranes are not as durable as ultrafiltration	None reported, but reverse osmosis unlikely to be deployed on a stream with microbes in it due to fouling concerns.

Treatment Type	Treatment Stage	Advantages	Disadvantages	Couplings with biotreatment process
Coagulation/ Flocculation	Secondary	Simple process Cost-effective solution for treating large volumes of waste High TOC/COD removal efficiencies for formulations with anionic surfactants Allows for oil reclamation	Ineffective at removing water partitioning compounds Low effectiveness for synthetic metalworking fluids Highly variable- dosages for different waste streams need to be determined Chemical Sludge is produced as a by-product Not feasible for small waste treatment operations	Coagulation/Flocculation has been used as a pre-treatment for biological processes treating several types of wastewater. No research into coupling the processes for treatment of metalworking fluids exist.
Oxidation Processes	Secondary Tertiary	Highly effective at removing organic matter Effective at removing toxic and recalcitrant waste Can improve the biodegradability of waste-streams	Can be very costly, usually not feasible to apply as a secondary treatment Chemicals are dangerous, and thus care must be taken during use and storage	Extensive research has been done on using oxidation processes to improve the biodegradability of metalworking fluids, and to remove recalcitrant wastes. It has been applied both before and after biotreatment process. When being applied after, it may also act as a sterilization process.
Adsorption Processes	Secondary Tertiary	Can be applied for the treatment of toxic and recalcitrant waste A wide variety of low-cost adsorbents made from waste can be utilised	Requires regeneration or disposal once the adsorbent is spent Activated carbon is expensive Easily fouled by oil and suspended solids, thus not usually applied as a secondary treatment	Can be applied directly to the bioreactor to act as both an adsorbent and a substrate for biofilm growth. The synergistic effect of this combination has been investigated. Can be applied after biotreatment to remove recalcitrant compounds.

2.4 A Prelude Discussion to the Aims and Objectives of the Research Project

In this chapter, a discussion about metalworking fluids and their options for treatment was given. A particular focus was given to the biological treatment of metalworking fluids since it is a versatile and cost-effective process. In the review of the biological treatment research that has been conducted, a particular need for the relative roles of different removal mechanisms for processes treating oil-in-water emulsions was identified. Many studies, if not all, do not investigate how the total carbon (and hence COD) is removed from the waste-streams, and thus the viability of the process cannot be correctly ascertained. This is because if the carbon from the metalworking fluid is not assimilated or mineralised, then it would need to be disposed of as a waste by-product in the process. It is thus one of the objectives of this project to determine the relative roles of each mechanism contributing to removal of carbon in the waste.

Another limitation to the biological treatment of metalworking fluids is that the waste streams contain anti-microbial agents, which means that waste streams require dilution before the treatment becomes effective. Dilution leads to longer process times, and larger capital costs for equipment. Furthermore, metalworking fluid wastes with large concentrations of biocide may reduce reactor functioning. In this chapter, alternative treatment options and their potential to be coupled to the biological treatment process were given. It was found that the main means of reducing toxicity was through oxidation processes such as the Fenton's process, treatment with ozone, treatment with electron beams, treatment with TiO_2 etc. The problems with applying these processes as a pre-treatment is that it is expensive,

and that they are non-selective in the sense that they will target even the biodegradable components in the waste (which is not a cost-effective solution).

Couplings to membrane processes have been explored, and have been found to be effective as a hybrid treatment process, but these processes have the aim of removing oily components from the metalworking fluid. The synergistic effect of removing biocides while removing oils has not been investigated. Furthermore, membrane processes cannot be sustainably applied to large volumes of wastewater due to fouling propensities. A second common means of emulsion breaking is coagulation/coalescence. Surprisingly, to the author's knowledge, there are no published works looking at the effects on coagulation/coalescence on the bio-treatment process of metalworking fluids. Coagulation/coalescence would have the same benefit as membrane processes in terms of oil recovery and biocide removal.

Another means of overcoming toxicity limitations is to increase the resilience of the bioreactor through the implementation of biofilms. While there are studies which have utilised biofilm reactors for the treatment of metalworking fluids, there are none which have directly looked at the influence of physico-chemical and nutritional factors influencing biofilm development in such wastewaters. A study addressing this would be beneficial to practitioners who wish to minimize down-times during start-up or after shock recovery

2.5 Research Aims

Based on the discussion in section 2.4, the aims of this research project are as follows:

- To determine the relative contributions of carbon removal mechanisms in bioreactors that are directly treating emulsified metalworking fluids. Thereafter, to use the information obtained to enhance carbon removal efficiencies.
- To determine the effects that coagulation/coalescence has on the growth and activity of metalworking fluid acclimated micro-organisms.
- To determine if coagulation/coalescence can be an effective technique in removing biocides from metalworking fluids, and hence to determine if coagulation/coalescence may be applied as detoxification treatment step.
- To use a micro-cosmic system to study physico-chemical factors influencing the development and performance of metalworking fluid biofilm reactors
- To study the effects of bio-stimulation on the development and performance of metalworking fluid biofilm bioreactors. Furthermore, to understand how carbon sources within the metalworking fluid may influence biofilm development.

Chapter 3- Mechanisms for Organic Carbon Removal in Bioreactors Treating Soluble Metalworking Fluids

3.1 Introduction

3.1.1 Soluble Oil Metalworking Fluids, and Traditional Treatment Techniques

Soluble oils are the most widely used type of cutting fluids in the metalworking industry (Childers 2006). They are oil-in-water macro-emulsions that are used as lubricants and coolants for machining processes. A typical soluble oil formulation is supplied as a concentrate containing approximately 60-90% mineral oil (Bienkowski 1993), which is diluted with water to create an emulsion concentration suitable to its application, typically 3-5% by volume (Bataller et al. 2004). During usage, soluble oils may become contaminated with foreign chemicals such as hydraulic fluids and rust inhibitors (Abanto et al. 1994) or they may become biodeteriorated due to microbes feeding on the chemicals used in the formulation (Passman 2006). It is thus necessary to periodically dispose of spent metalworking fluids, and replace them with a fresh batch to ensure that their performance in enhancing the metalworking process is maintained. The treatment of spent soluble oil wastes is typically carried out with the aim of separating the oil and water phases (Knoblock et al. 1994) since the mineral oil that is used within the formulation is regarded as a hazardous material (The European Parliament 2010). This can be done through the application of ultrafiltration membrane processes for the purpose of retaining oil and other dispersed components (Benito et al. 2002; Benito et al. 2001; Milić et al. 2013), or through the use of chemical additives for the purpose of breaking the emulsion (Matos et al. 2016; Hilal, Busca, Hankins, et al. 2004; Ríos et al. 1998). While typical treatment processes are able to successfully separate oil and water phases, they

are ineffective at treating water-soluble organic contaminants. Thus, the treatments are typically coupled to a tertiary treatment such as reverse-osmosis, activated carbon adsorption or a biological treatment step in order to meet tightening waste disposal regulations (Burke & Gaines 2006).

3.1.2 The Biological Treatment of Soluble Metalworking Fluids

The biological treatment process offers a cost-effective means of simultaneously treating both oil and water phases within soluble oil metalworking fluids (Cheng et al. 2005). Recent studies have investigated the direct application of bacteria to the treatment of soluble oils, either by having the process coupled to a membrane treatment process (Sutton et al. 1994; Anderson et al. 2009) or as a stand-alone treatment unit (Deepak et al. 1994; Muszyński & Łebkowska 2005; Moscoso et al. 2012; Cheng et al. 2006). However, the mechanism for the treatment of soluble oils within bioreactors is not reported in publications looking at the direct treatment of soluble oils (i.e without any pre-treatment). Cheng et al. used a mixed consortium of bacteria and studied the thermophilic aerobic treatment of a mixture of metalworking fluid wastes, including soluble oils, and achieved 97.27% COD removal in 77.5h at a temperature of 50℃ (Cheng et al. 2006). They attributed all removal to biodegradation without investigating the removal mechanism. Muszynski & Lebkowska achieved more than 96% removal of the hydrocarbons present in a soluble oil waste in 21 days, and achieved 89% removal in just 7 days of operation (although the bioreactor took approximately 70 days to start-up)(Muszyński & Łebkowska 2005). No removal mechanisms were reported, and no explanation was given regarding the removal observed in their control reactor. Similarly Moscosco et al. evaluated the total petroleum hydrocarbon removal of a process utilising *Pseudomonas stutzeri* for treating soluble oils (Moscoso et al. 2012). They achieved

70% removal in less than 2 weeks of operation, but once again, no mechanism was investigated.

3.1.3 Possible Mechanisms by which Microbes Treat Soluble Oil Wastes

The biodegradation rate of mineral oils is dependent on the structure of the hydrocarbons. Micro-organisms are not able to easily degrade constituents with cyclic and branched molecular structures, hence paraffins are more biodegradable than naphthenes and aromatic chains (Haus et al. 2001). Extents of degradation of different mineral oils over 21 days range from 15-75% (Aluyor & Ori-Jesu 2009; Haus et al. 2001). Specifically, naphthenic mineral oil, a common base component in soluble metalworking fluids is known to have limited biodegradability (Haus et al. 2001; Novick et al. 1996). Rabenstein examined the degradability of metalworking fluid components and found that mineral oil was not degraded at all within the time investigated (Rabenstein et al. 2009). This suggests that mechanisms alternative to biodegradation could be responsible for the relatively high and fast removal rates that are observed in bioreactors treating soluble metalworking fluids.

Organics may be removed in a number of ways: a) through direct microbial utilisation for growth and respiration, b) through adsorption on the microbe surface, c) Through emulsion destabilisation due to the interactions with the surrounding cations within the bulk and d) through destabilisation due to microbial degradation of emulsifiers present in the metalworking fluids. In this chapter, the relative roles of each mechanism in the treatment of a representative soluble metalworking fluid waste are reported. Many microbial treatments require that wastes be diluted to reduce toxic effects. Hence, the emulsion stability of metalworking fluids when diluted with water of varying degrees of hardness is investigated to ascertain if spontaneous destabilisation may play a role in organic removal. Thereafter, the

relative quantities of each mechanism in the microbial treatment are presented. It is shown that emulsion breaking due to the degradation of surfactants is a predominant mechanism in the treatment of soluble oils. This is then validated by applying the microbial treatment to defined metalworking fluid formulations made up of different ratios of bio-stable (i.e Petroleum Sulfonate) and bio-supportive (i.e Tall Oil Amide) surfactants. Emulsions made up of bio-supportive surfactants are shown to be treated to a greater extent than those made up of bio-stable surfactants, thus providing further evidence that emulsion breaking is the predominant mechanism for carbon removal. By knowing the mechanism for the treatment of soluble oil metalworking fluids, practitioners can make better decisions with regards to reactor designs, and sludge disposal.

3.2 Aims and Objectives

This chapter focusses on the following research aim:

- To determine the relative contributions of carbon removal mechanisms in bioreactors that are directly treating emulsified metalworking fluids. Thereafter, to use the information obtained to enhance carbon removal efficiencies.

To achieve this aim, the objectives of the investigations presented are:

- To demonstrate that diluting soluble oil emulsions creates the possibility for spontaneous emulsion destabilisation to occur within bioreactors treating dilute soluble oil wastes.
- To demonstrate that the main mechanism for treatment within the time-frames investigated is destabilisation, due to the interaction with cations present within wastewaters and due to the degradation of emulsifiers.

- To demonstrate that since oil/water separation is the main mechanism for treatment, carbon removal efficiency is related to the biodegradability of the surfactant package.

3.3 Materials and Methods

3.3.1 Metalworking Fluids

Castrol CoolEdge BI was a commercially available soluble oil containing 60% mineral oil. It was used as the proxy metalworking fluid for the experiments since it was readily available, had a stable composition and was listed as being biodegradable (Castrol 2002). Castrol CoolEdge BI was provided in concentrate form. The concentrate was diluted with water, containing specified nutrient concentrations, to the metalworking fluid concentrations as described in the experiments presented in this chapter.

The concentrate for the custom soluble oil mixture was made according to the following modified recipe, adapted from Childers (Childers 2006):

Table 3-1: Recipes for soluble oil metalworking fluids used in experiments. Each recipe gives the ratio of the mass of each component to be added to the concentrate.

	Petroleum Sulfonate (emulsifier)	Tall Oil Fatty Amide (emulsifier)	Butyl Carbitol (Coupler)	Naphthenic Mineral Oil (Base Oil)
50% Sulfonates: 50 % Amides	1	1	0.8	6
75% Sulfonates: 25% Amides	1.5	0.5	0.8	6
100% Sulfonates: 0% Amides	2	0	0.8	6

To create the emulsion, Naphthenic Mineral Oil was first mixed with Petroleum Sulfonate using a magnetic stirrer in a 50 mL beaker until a uniform mixture was obtained. Thereafter, Butyl Carbitol was added, together with Tall Oil Fatty Amides

to the beaker. The concentrate was obtained upon mixing, and was used to create the solutions described in the experiments using the custom soluble oil. All emulsions created passed stability criteria in accordance to the DIN Method 51367 (Byers 2006).

3.3.2 Artificial Tap Water

The artificial tap water that was used for all experiments was made using a recipe provided online by the Joint Service Agent Water Monitor (JSAWM) program, on behalf of Dr Mark LeChevalier of the American Water Works Association. It is obtained by dissolving inorganic salts in DI water to obtain the following concentrations (in mg/L): $NaHCO_3$ -100; K_2HPO_4 –0.7; KH_2PO_4 – 0.3; $(NH_4)_2SO_4$ – 0.01; NaCl – 0.01; $FeSO_4.7H_2O$ – 0.001; $NaNO_3$ – 1000;

The amounts of $CaSO_4.2H_2O$ and $MgSO_4.7H_2O$ used in the recipe were varied so as to obtain varying degrees of water hardness. The amounts of each inorganic salt used, and the resultant hardness are given in Table 3-2.

Table 3-2: Concentration of Calcium and Magnesium salts used in artificial water recipes.

Water Recipe Name	**30 ppm**	**60 ppm**	**120 ppm**	**180 ppm**	**240 ppm**	**300 ppm**
$CaSO_4.2H_2O$ (mg/L)	30	60	120	180	240	300
$MgSO_4.7H_2O$ (mg/L)	30	60	120	180	240	300
Hardness Achieved ($CaCO_3$ equivalent)	29.6	59.2	118	178	237	296

A stock solution containing 4X the concentrations specified was made to create the actual solutions used in the experiments. The artificial water was autoclaved to create a sterile stock.

3.3.3 Microbial Consortia

A mixed consortium used for the industrial treatment of metalworking fluid wastes was supplied by Microbial Solutions Ltd. (a company specialised in the disposal of metalworking fluids). The mixed consortium was provided in freeze-dried form, and originated from an aged reactor treating spent metalworking fluid wastewaters. The seed consortium was a five-membered community consisting of *Agrobacterium radiobacter* (designated strain 5-BA-A*), Comamonas testosteroni* (1-BTZ-O), *Methylobacterium mesophilicum* (20-BTZ-N), *Microbacterium esteraromaticum* (15-BTZ-N), and *Microbacterium saperdae* (1-TEA-C). The method used to develop this reactor was described by van der Gast and Thompson (van der Gast & Thompson 2004). In order to resuscitate the freeze dried micro-organisms, 2 mL of phosphate buffered saline solution was added to the vials in which they were contained. The vial was left to stand for two hours, before the contents were transferred to a 250 mL flask containing 95mL of Luria-Bertani (LB) Media and 5 mL of 10% Castrol CoolEdge BI. The flask was incubated in an orbital shaking incubator set at 28 ℃ and 120 rpm for 18 hours. After incubation, the contents of the flask were centrifuged at 4100 rpm to collect the cell pellet and to discard the supernatant. The cell pellet was washed with DI water twice, before being re-suspended once more in DI water. This suspension served as the inoculum for all experiments described.

3.3.4 Bioreactor System

All experiments were conducted in 100 mL Duran bottles which served as bioreactors to remove the carbon within the metalworking fluids being treated. The bioreactors were maintained at a temperature of 27 ℃ in a water bath. Each bioreactor was agitated with airflow of 0.5LPM. The air was first passed through a 0.22 micron filter and a humidifier (made from a 50mL centrifuge tube) before

entering the reactors in order both to ensure sterility and to avoid bulk liquid evaporation during the experiment. A schematic of the bioreactor system is provided in Figure 3-1.

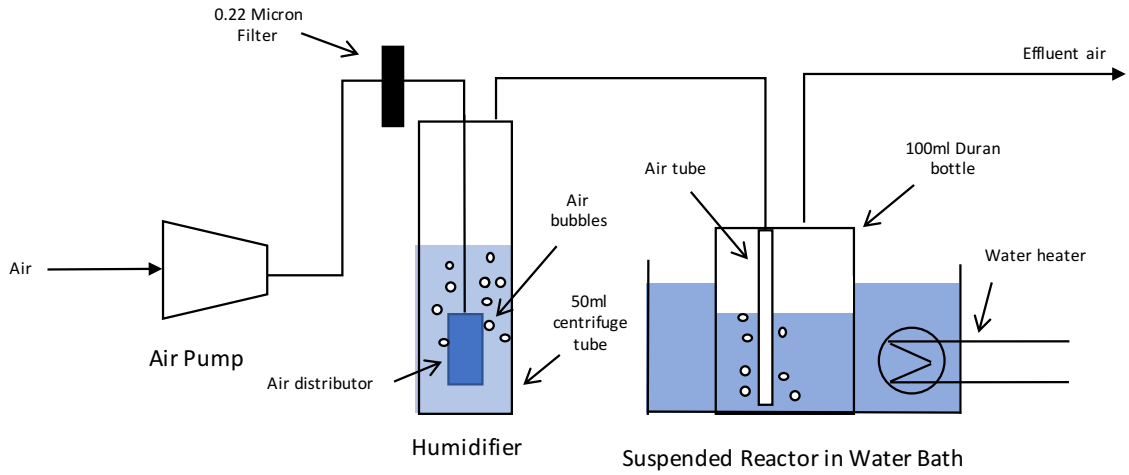

Figure 3-1: Schematic of suspended bioreactor system.

3.3.5 Measured Parameters

Total Carbon was measured using a Shimadzu TOC-VCPH. When measuring the total carbon within the bulk fluid of the reactors, samples were centrifuged to remove biomass. The resulting supernatant was diluted 10 times, and sonicated for 2 minutes using a Branson 3510 sonnicator set at 41.5 KHz before being injected into the instrument.

The optical density of the microbes was measured using a Shimadzu UV-Vis 1800 Spectrophotometer. Samples were centrifuged at 10000 RPM for 15 minutes to collect cell pellets. After the supernatant was discarded, the cell pellet was washed twice with DI water, and then re-suspended in DI water. The optical density of this re-suspension was measured at 600 nm using cuvettes with a 10mm path length.

3.3.6 Re-emulsification of Destabilised Oils and Carbon Balances

Destabilised emulsions and oily sludges were re-emulsified through the addition of Triton X-100 (an oil emulsifying surfactant used within metalworking fluids). A 10% (w/v) stock solution was prepared in DI water and was used for this purpose. Triton X-100 was found to be non-toxic to the microbes for the exposed dosage and time (as verified by achieving no significant difference in plate counts between a community exposed to Triton, and one exposed to a DI water control- data not shown). Triton also did not result in a significant increase in the bulk total carbon measurement in solutions containing only bacteria, and no oils, suggesting that lysis did not have any significant interference with the measurements (data not shown).

When calculating the relative mechanisms for carbon removal within a bioreactor, it was necessary to determine the amount of carbon recovered from the oily-sludge layer, and the amount that was recovered from the surface of the bacteria.

3.3.6.1 Carbon Adsorbed on Bacteria

To determine the amount of carbon adsorbed by bacteria in the bulk of the metalworking fluid being treated, 50 mL of the bulk liquid was drawn off the bottom of the reactor (to avoid re-emulsification of destabilized oils floating on the top of the reactor) and transferred to a 100 mL bottle. 0.5 mL of 10% Triton X-100 was added to this bottle and the solution was vigorously stirred for 10 minutes. This method was found to re-emulsify most adsorbed carbon on the bacteria (as validated by 96 % carbon recovery attained in an inoculated control- data not shown). The TOC of the solution was measured and the amount of carbon adsorbed on the bacteria was calculated as:

$$Total\ Carbon\ Adsorbed\ on\ Bacteria\ (mg) = \left(TC_{aft} - TC_{Triton} - TC_{bef}\right) * DF * V_2 * \frac{V_1}{V_2}$$ **...Equation 3-1**

Where TC_{bef} was total carbon of bulk before Triton addition (mg/L), TC_{aft} was total carbon of bulk after Triton addition, TC_{Triton} was the total carbon of the background Triton X-100, DF was the dilution factor, V_2 was the volume extracted from the reactor (L), V_1 was the total volume of the reactor (L).

3.3.6.2 Carbon in Destabilised Oil Layer

To determine the carbon concentration in the destabilised layer, the reactor bulk liquid total carbon was measured first. Thereafter, 1 mL of a 10% Triton X-100 solution was added to the reactor. After 10 minutes of vigorous stirring, a sample was drawn, diluted and measured for total carbon. The total carbon recovered from the oil layer was given as:

$$Total\ Carbon\ in\ oil\ layer\ (mg) = \left(TC_{aft} - TC_{bef} - TC_{Triton}\right) * DF * V_1 - TC_{adsorbed} \quad \text{Equation 3-2}$$

Where TC_{bef} was total carbon of bulk before Triton addition (mg/L), TC_{aft} was total carbon of the bulk after Triton addition (mg/L), TC_{Triton} was the total carbon of the background Triton X-100 (mg/L), V_1 was the volume of the reactor (L) and $TC_{adsorbed}$ was the total carbon adsorbed on the bacteria (mg).

3.3.6.3 Carbon in Bacteria

To measure the total carbon change in the bacteria, 30 mL of bulk fluid was drawn from a bioreactor. This sample was centrifuged to collect the cell pellet of bacteria and to discard the supernatant. The cell pellet was washed with 0.1 % Triton X-100, then with PBS three times. Thereafter, the cell pellet was resuspended in 30 mL DI water. The total carbon of this suspension was measured. The total carbon in the bacteria present in the bioreactor was given as:

$$Total\ Carbon_{Bacteria}(mg) = TC_{suspension} * \frac{OD_{reactor}}{OD_{suspension}} * V_{suspension} \quad \text{Equation 3-3}$$

Where $TC_{suspension}$ was the total carbon in the resuspension, $OD_{reactor}$ was the optical density of the microbes in the reactor and $OD_{suspension}$ was the optical density of the bacteria in the resuspended sample. $V_{suspension}$ was the volume of the suspension.

3.3.6.4 Carbon Mineralised

An estimate of the amount of carbon mineralised was given by:

$$TC_{mineralised}(mg) = TC_{initial} - TC_{final} - TC_{adsorbed} - TC_{oil\ layer} - TC_{bacteria\ final} + TC_{bacteria\ initial} \quad \text{Equation 3-4}$$

Where $TC_{initial}$ and TC_{final} were the initial and final amounts of carbon in the reactor (mg), $TC_{adsorbed}$ was the amount of carbon adsorbed on bacteria (mg), $TC_{oil\ layer}$ was the total carbon in the destabilised oil layer (mg), and $TC_{bacteria}$ was the amount of carbon in the bacteria. This was actually an estimate since the recovery of adsorbed carbon during measurement was not 100%.

3.3.7 HPLC Detection of Fatty Amides

A modified method given by Guarrasi et al. was used to detect Tall Oil Fatty Amides (Guarrasi et al. 2010). Tall Oil Fatty Amides were analysed using an Agilent Compact LC 1120 equipped with a UV-Vis detector. Samples were centrifuged to remove biomass before being placed in 1 mL sample vials. 10 µL of the sample was injected into the instrument equipped with an Agilent C18 Eclipse Plus column. An isocratic elution containing 90% Acetonitrile (acidified with 0.2% Acetic Acid) and 10% DI water, flowing at 1 mL/min was used to separate the peaks. Peaks were detected at 210 nm.

Individual peaks in the Tall Oil Amide used in this project were characterised using Mass Spectroscopy with the gracious assistance of Dr James Wickens (University of Oxford, Department of Chemistry). Analyses were performed using a Thermo

Exactive mass spectrometer equipped with Waters Acquity liquid chromatography system. Instrument control and data processing were performed using Thermo Xcalibur Software. The system was calibrated on the day of the analysis and its mass accuracy with external calibration (as used for these experiments) was better than 5ppm for 24 hours following calibration. Electrospray source conditions were adjusted to maximise sensitivity. Data was collected in positive and negative modes from *m/z* 120 to *m/z* 2000.

3 µL of each sample was injected onto the system. The column was a 2.1 x 50 mm (3 µm) ACE equivalence 3 C18. The oven was held at 40 °C throughout the analysis. A mixed eluent gradient was employed, and is described in Table 3-3.

Table 3-3: Gradient program used for LC-MS analysis of tall oil fatty amides

Retention [min]	Flow [mL/min]	%Acetonitrile	%Water + 0.1% Acetic acid
0	0.4	70	30
4	0.4	70	30
10	0.4	100	0
12	0.4	100	0
12.1	0.4	70	30

A UV absorbance chromatogram was simultaneously collected a 210 nm.

3.3.8 Models Used for Describing Biological Processes

The growth of microbes within the bioreactors may be described using the logistic equation, (Moscoso et al. 2012) as given by:

$$OD = OD_{max} * \frac{1}{1+e^{[\ln\left(\frac{OD_{max}}{OD_0}-1\right)-\mu t]}} \quad \text{.....Equation 3-5}$$

Where OD was the optical density of the microbes in the reactor at any given time t (h), OD_{max} and OD_0 are the maximum and initial optical densities and μ is the maximum specific growth rate (h^{-1})

Utilisation of carbon may be described using the logistic equation (Moscoso et al. 2012), or the Luedeking-Piret model together with the logistic equation for growth (Pazouki et al. 2008).

The logistic equation for carbon utilisation was given by:

$$D = D_{max} * \frac{1}{1+e^{[\ln\left(\frac{D_{max}}{D_0}-1\right)-\mu_D t]}} \quad \text{Equation 3-6}$$

Where D was the % of total carbon removal at time t, D_{max} and D_0 were the maximum and initial % removals respectively. μ_D was the specific maximum total carbon utilisation rate (h^{-1}).

The Luedeking-Piret equation was given as:

$$\frac{dD}{dt} = -m * \left(\frac{d(OD)}{dt}\right) - n * OD \quad \text{Equation 3-7}$$

Where m (dimensionless) and n (h^{-1}) are growth and non-growth utilisation parameters respectively.

The Luedeking-Piret model was modified in this work as follows:

$$\frac{dD}{dt} = -m * \left(\frac{d(OD)}{dt}\right) - n * Q * OD * D \quad \text{Equation 3-8}$$

$$Q = \frac{1}{1+e^{[\ln\left(\frac{1}{0.001}-1\right)-\mu_Q t]}} \quad \text{Equation 3-9}$$

Where Q was a parameter used to describe the state of the microbes to account for lag phases. μ_Q (h^{-1}) describes the rate at which the microbes transition from being in the lag phase to being active (Swinnen et al. 2004).

The error in each model was described using the normalized root mean standard deviation (NRMSD), given by

$$NRMSD = \frac{\sqrt{\frac{(D_{model} - D_{measured})^2}{n}}}{D_{max} - D_{min}} \quad \text{Equation 3-10}$$

Where n is the number of measured samples.

3.3.9 Reagents

Analytical grade inorganic salts ($CaSO_4.2H_2O$, $MgSO_4.7H_2O$, $NaNO_3$, NaCl, $FeSO_4.7H_2O$, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$) Triton X-100, Acetonitrile (99.9% purity) and Acetic Acid were purchased from Sigma Aldrich.

Samples of Naphthenic Mineral Oil were provided by Nynas Base Oils. Samples of Sodium Sulfonate were provided by Sonneborn. Tall Oil Fatty Amides were provided by Colonial Chemical.

3.3.10 MicroResp and Post Exposure Recovery Tests to Determine Relative Toxicity of Metalworking Fluid

A MicroResp™ system and post-exposure recovery test were used to determine the relative toxicity of the metalworking fluids investigated. The MicroResp™ method is a colometric technique that was used to measure CO_2 liberated from samples with microbes due to respirational activity. It involves the use of a 96-deep well plate coupled to a second micro-plate serving as a CO_2 trap. This may serve as a toxicity indicator since inhibitory substances within the samples will result in lowered respiration activity. A full description of the method was presented by Campbell et al. (Campbell et al. 2003). Seed micro-organisms originated from the suspension described in section 3.3.3. Into each well, 50uL of inoculum, together with nutrients and metalworking fluid was added to create a condition containing

200mg/L NH_4Cl, 100 mg/L KH_2PO_4, 5 g/L Glucose and a specified concentration of metalworking fluid (ranging from 0% to 4%) The deep well plate was then sealed with the CO_2 trap, and incubated in an orbital shaker set at 28℃ and 120 RPM for 48 hours. Absorbance values at 595 nm for the CO_2 trap were measured using a Tecan Microplate reader.

After taking the final absorbance reading, microbes from each micro-well plated were serially diluted and plated on LB Agar Plates containing 5g/L Glucose. Plating was done in accordance to the Miles and Misra technique (Miles et al. 1938).

3.3.11 Statistical Analysis

Student's t-test for unpaired samples, and single-factor ANOVA analyses were used to test for significance at 95% confidence levels.

3.4 Results

3.4.1 Dilution Requirements for Toxicity Reduction

Metalworking fluid formulations contain biocides to prevent in-use microbial contamination. The inclusion of biocides makes the end-of-life treatment of concentrated waste difficult, and thus metalworking fluids are usually diluted so that the microbial toxicity is not sufficient to hinder the end-of-life treatment process. Micro-respiration and post-exposure recovery tests were done to determine the suitable level of dilution needed for the microbial treatment process of the proxy soluble oil (CoolEdge BI) to be effective. Concentrations which resulted in complete inhibition would result in relatively low amounts of respiration and colony forming units (CFU) counts as compared to a non-toxic control. Figure 3-2 shows that tested concentrations of metalworking fluid above 0.50 % were inhibitory, while concentrations at 0.25% and 0.50 % stimulated growth as compared to the control

(containing only glucose and supplementary nutrients). Stimulation at low concentrations suggested that the microbial consortia was able to utilise the components within the metalworking fluid as a carbon source, and thus succeeding degradation experiments using CoolEdge were conducted at the concentration of 0.50%.

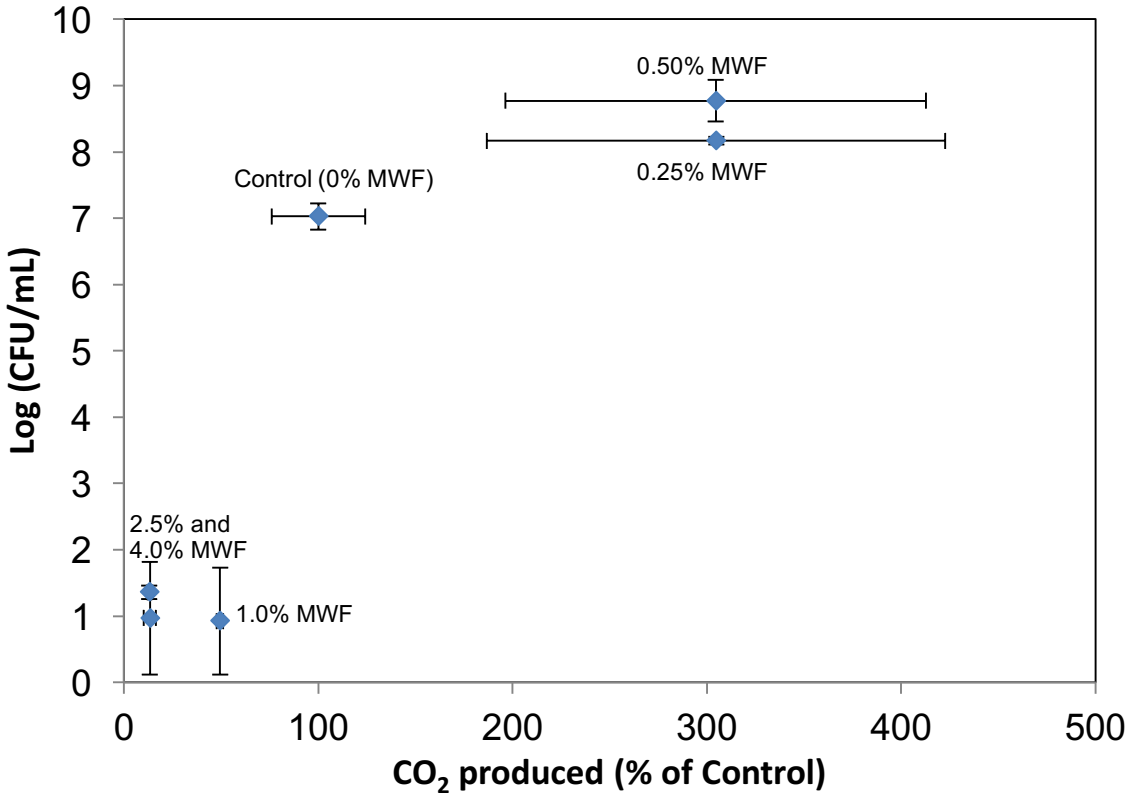

Figure 3-2: 24 hour MicroResp™ and post-exposure recovery results for CoolEdge BI Metalworking Fluid. Each point represents a well containing the specified concentration of metalworking fluid with 5g/L glucose, 500mg/L NH4Cl, 50 mg/L KH2PO4. Respiration activity is given relative to a control containing added nutrients with no metalworking fluid. Error bars are standard deviations of triplicate measurements.

3.4.2 Emulsion Stability Under Dilute Conditions

The colloidal stability of metalworking fluids may be affected by the presence of divalent cations that naturally occur within the waters used to make the emulsions, and to dilute them to treatable concentrations. Divalent cations, such as calcium

and magnesium, may destabilise emulsions by interacting with the anionic surfactants used in the formulation of soluble metalworking fluids. This interaction occurs either causing them to precipitate or by neutralising their charge and causing the oil droplets within the emulsion to coalesce (Childers 2006). In addition to neutralising the charge on the oil droplets, the divalent ions result in the compression of the double layer keeping the oil droplets in suspension. The compression of the double layer, and the charge-neutralisation effect resulted in the destabilisation of the emulsion.

In consideration of this, water hardness may have been a parameter that could influence the destabilisation kinetics of metalworking fluids, especially under dilute conditions where the surfactant concentration was relatively low. To assess the impact that water hardness had on diluted emulsion stability, artificial tap water was made with varying hardness degrees. The water hardness values were chosen to represent the range of values that can be found around the UK (Drinking Water Inspectorate 2011).

Figure 3-3 shows the impact that water hardness had on the carbon removal dynamics in a reactor treating CoolEdge BI. It can be seen that increasing the water hardness significantly ($p<0.01$) increased the extent of carbon removal. Reactors with a 60ppm $CaCO_3$ equivalent water resulted in a 2.3% decline in the bulk carbon, as compared to the 56.0% change observed for reactors with 240 ppm waters. As time progressed, a distinct oil layer appeared at the top of the reactors, indicating that emulsion destabilisation and oil/water separation had occurred. Furthermore, upon the addition of the non-ionic mineral oil emulsifying agent, Triton X-100, over 96.0% of the removed carbon was redispersed into the bulk liquid in all of the reactors (Figure 3-4). This suggests that the main mechanism for removal was due

to charge neutralisation of the oil emulsifiers in the metalworking fluids, which in turn lead to droplet coalescence and flotation due to the resulting buoyant forces. The increase in the removal rate due to the increase in the water hardness can be explained by the increased extents of coagulation and coalescence. The rate of coalescence was a function of the divalent ion concentration since this was well below the critical coagulation concentration for this metalworking fluid concentration (determined to be 10.5 mM of $MgCl_2$ at this concentration, data not shown).

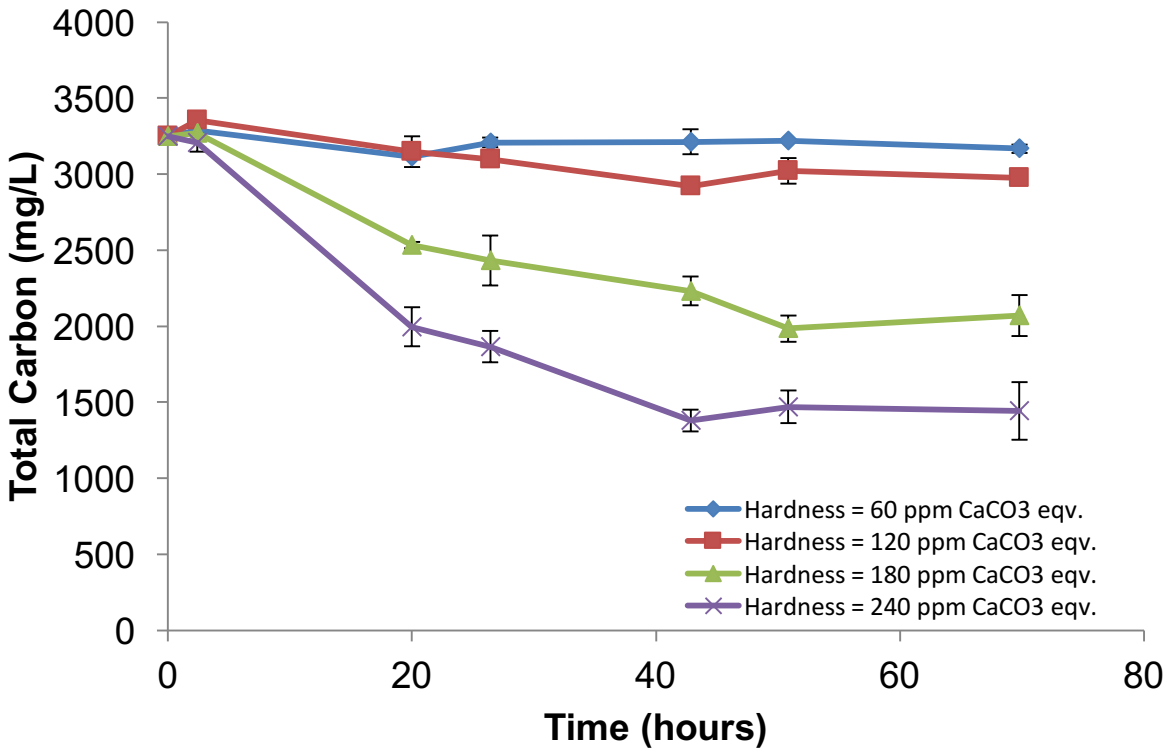

Figure 3-3: Increases in water hardness leads to increases in the rates of carbon removal from bulk liquid in reactors treating metalworking fluids. Concentration of metalworking fluid (CoolEdge BI) was 0.5%. Error bars represent standard deviation of triplicate measurements.

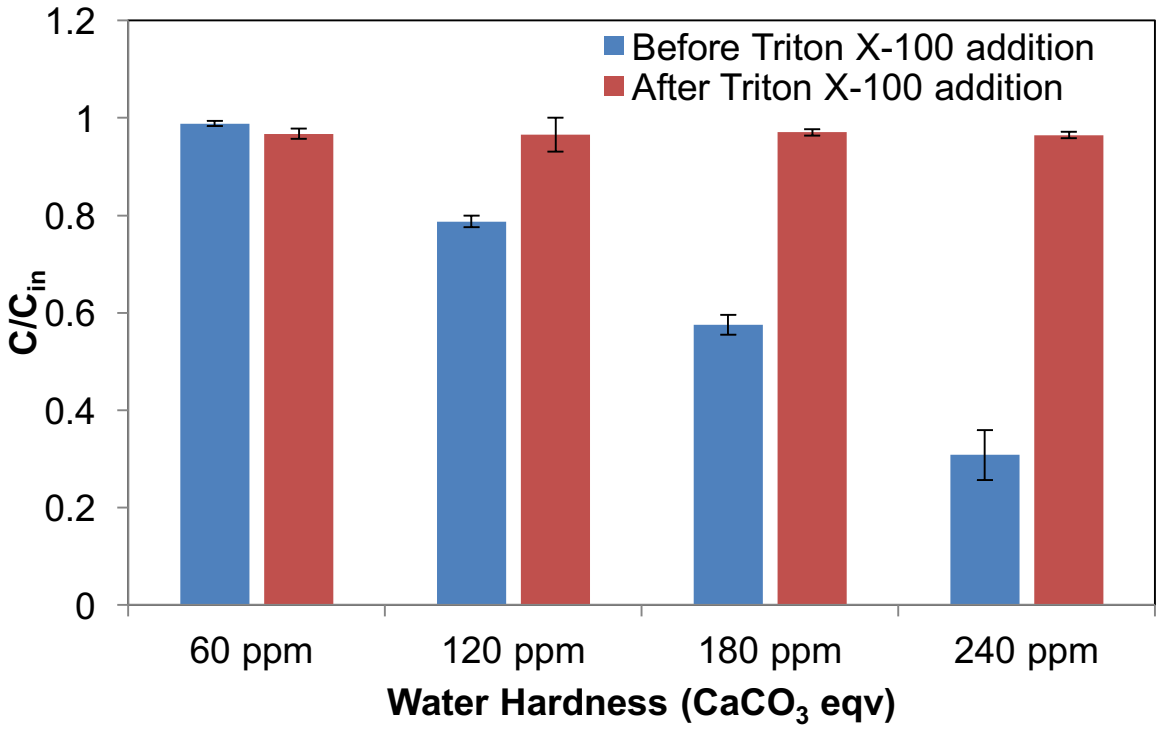

Figure 3-4: Results of carbon re-emulsification using Triton X-100. The addition of the surfactant results in the recovery of the initial amount of carbon within the system since the oil layer is re-suspended within the bulk.

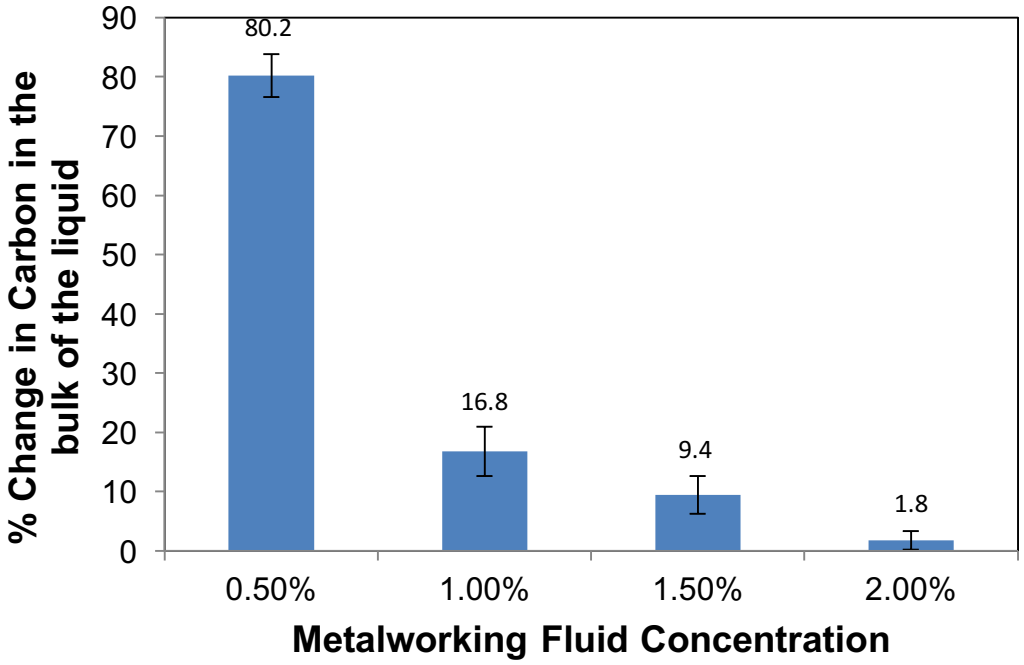

Figure 3-5: Effect of metalworking fluid concentration on extent of destabilisation (measured as % change of the carbon in the bulk liquid). CoolEdge BI was used as the metalworking fluid. Concentrations were made using water having a 240 ppm $CaCO_3$ equivalent water hardness value. Carbon measurement was made 5 days after the start of the experiment had started. Error bars represent standard deviations of triplicate measurements.

Figure 3-5 shows that the effect of hardness destabilisation significantly ($p<0.01$) decreased with an increase in the metalworking fluid concentration. The increase in metalworking fluid concentration increased the surfactant concentration present within the system. Since the concentration of divalent ions within the water remained constant, the extent of destabilisation was reduced since there was less charge neutralisation per adsorbed surfactant molecule at the oil/water interface. Thus, the rate of spontaneous destabilisation was a function of the metalworking fluid concentration, as well as the hardness of the water used for dilution. If soluble oil wastes are diluted with relatively hard waters, then oil/water separation may be observed in vessels containing the wastes.

The impact that water hardness may have on soluble oils is dependent on their formulation. Specifically, surfactant type and the oil to total surfactant ratio is important in determining the stability of the metalworking fluid within hard waters. Zimmerman et al. investigated the effects of surfactant types, and oil-to-surfactant molar ratios on hard water stability in 2500 formulations (Zimmerman et al. 2003). They found that twin-headed surfactants and total oil to surfactant molar ratios of 0.5 or less could impart improved oil stability. Thus, different metalworking fluid brands will have different levels of stability, and the level of dilution causing destabilisation in one type may not necessarily lead to destabilisation in another.

In the sections to follow, the effect that destabilisation has on the biological treatment of metalworking fluids is presented. Furthermore, results of an investigation into the extent to which microbes induce destabilisation and the relative contribution this makes towards the total treatment of soluble oil wastes are provided.

3.4.3 Stimulatory Requirements for the Degradation of Metalworking Fluids

Nutrient stimulants may be added to hazardous wastewaters in order to enhance the biological treatment process. Within this section of the chapter, results of the investigations into the effects of nutrient addition on the treatment performance for soluble oil metalworking fluids are presented. Metalworking fluid wastewaters are an ample source of carbon, nitrogen and sulfur- elements that are essential for the development of micro-organisms (Cheng et al. 2005).

However, components that have phosphorus within their chemical structures are limited to extreme hydraulic pressure applications (Childers 2006). It is thus common practice to stimulate metalworking fluids with inorganic phosphates in order to promote microbial growth (Cheng et al. 2006; Schreyer & Coughlin 1999). Thus, all treatments investigated contain 100 mg/L of KH_2PO_4. Further to the addition of phosphates, either ammonium chloride or Luria-Bertani broth was added to the reactors to facilitate rapid growth of micro-organisms. Figure 3-6 and Figure 3-7 show the effect of biostimulation on the performance of the microbial treatment in terms of its ability to remove carbon from soluble metalworking fluids. It can be seen that stimulation using low levels of growth media led to significantly higher removals of carbon as compared to stimulation using ammonium chloride ($p<0.01$), even though both stimulatory treatments resulted in rapid growth of cells. Stimulating the metalworking fluids with 150 ppm of LB resulted in 71.6% ± 1.6% of the carbon being removed from the system, which was significantly higher than that attained through NH_4Cl stimulation (the range attained being 48.2% ± 1.2%).

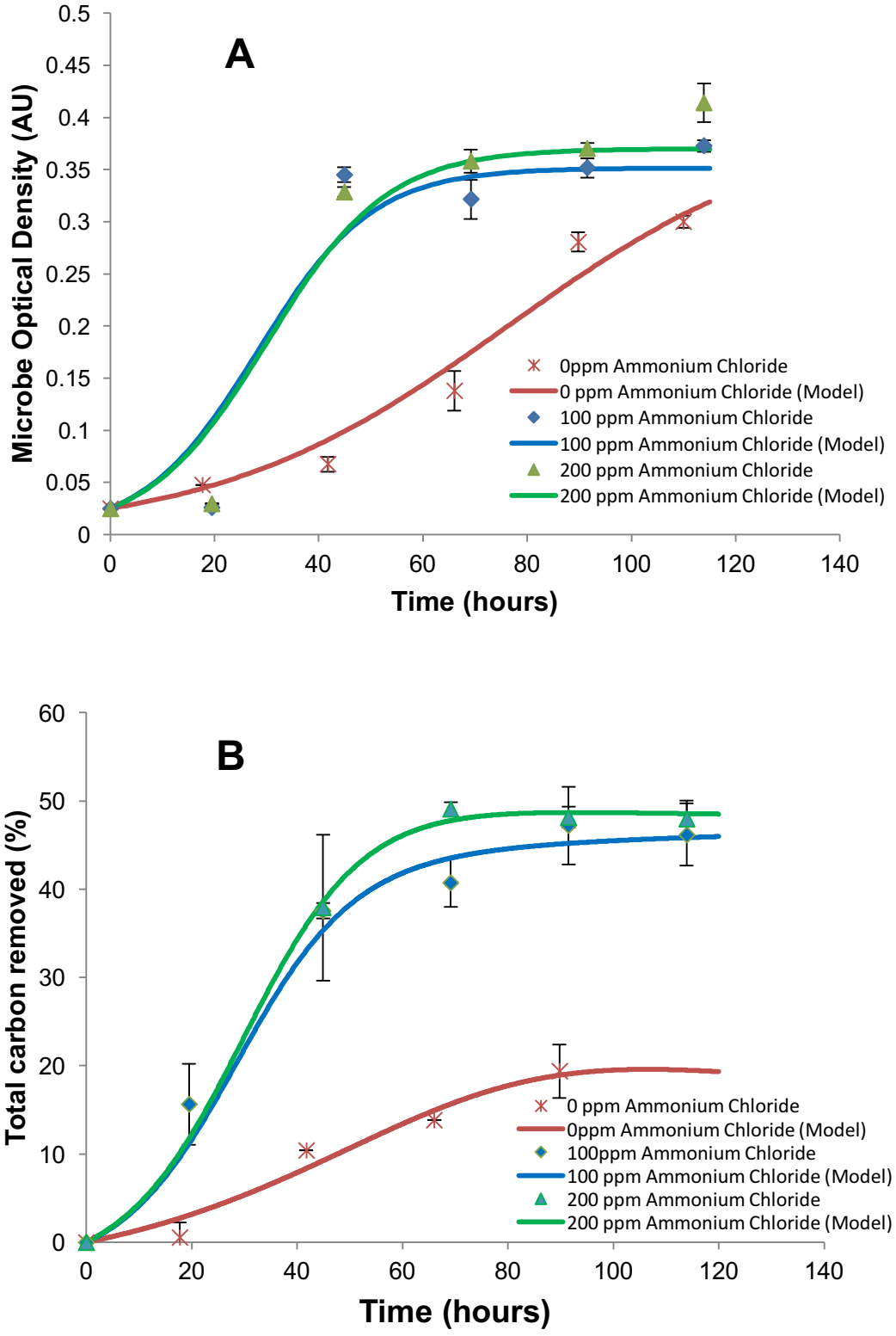

Figure 3-6: A) Optical density of microbes and B) Total carbon removal from the bulk fluid in bioreactors treating metalworking fluids. Each bioreactor contained 0.5% MWF with 60ppm hard water supplemented with 100 mg/L KH_2PO_4 and the specified amounts of NH_4Cl. Markers represent experimental measurements and solid lines represent modified Luedeking Piret Model. Error bars represent standard deviations of triplicate measurements.

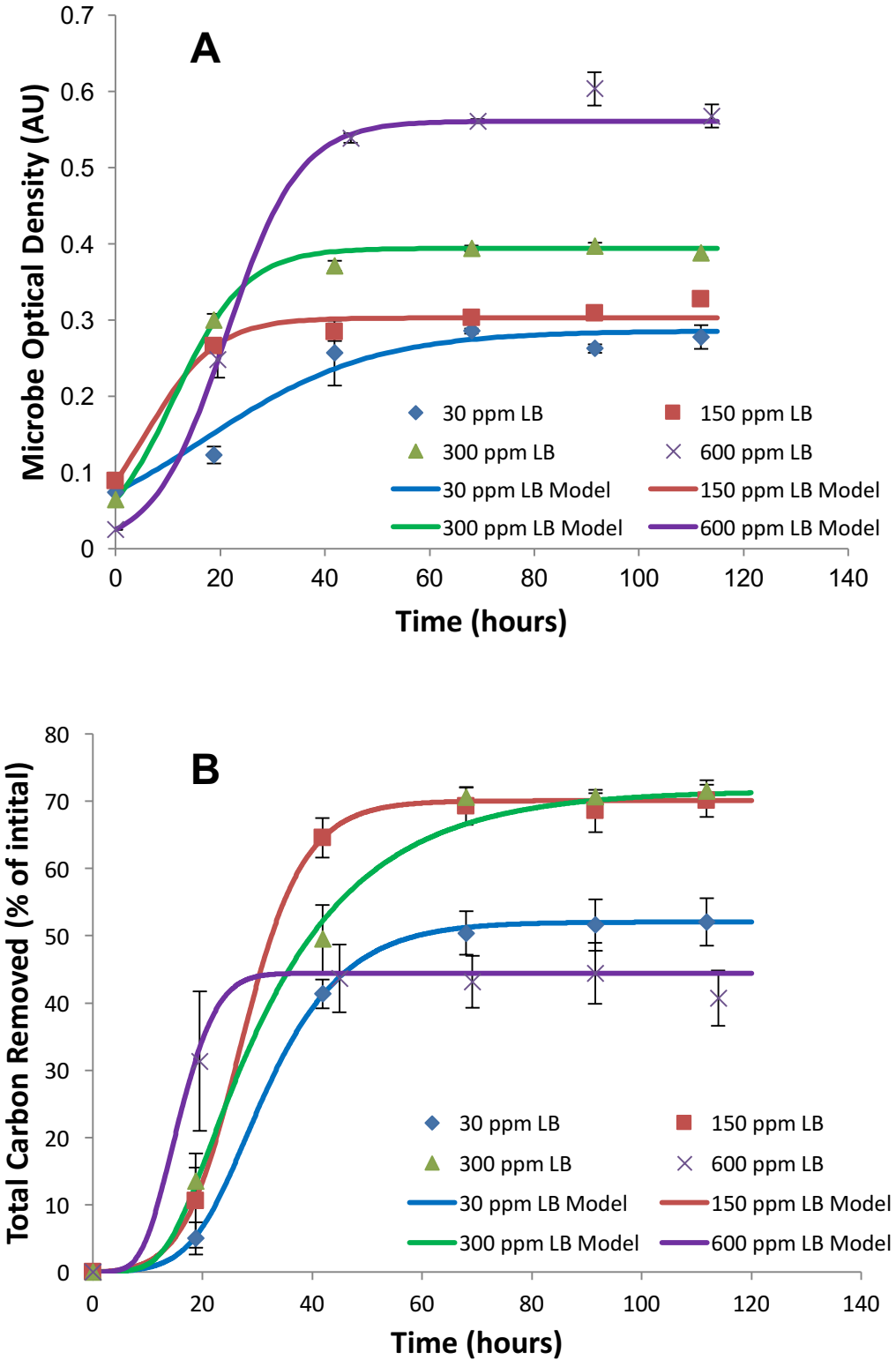

Figure 3-7 A) Optical density of microbes and B) Total carbon removal from the bulk fluid in bioreactors treating metalworking fluids. Each bioreactor contained 0.5% MWF with 60ppm hard water, supplemented with 100 mg/L KH_2PO_4 and the specified amounts of Luria Broth. Markers represent experimental measurements and solid lines represent modified Luedeking Piret Model. Error bars represent standard deviations of triplicate measurements.

Table 3-4: Parameters used in the logistic models for growth and carbon removal, the Luedeking-Piret model and the modified Luedeking-Piret Model. For each model, the normalized root mean square deviation to the model is given.

Data Set	Logistic (growth)		Logistic (carbon removal)		Luedeking-Piret Model			Modified Luedeking-Piret Model			
	μ (h^{-1})	NRMSD (%)	μD (h^{-1})	NRMSD (%)	m	n	NRMSD (%)	m	n	Q	NRMSD (%)
30 ppm LB	0.062	10.2	0.18	2.3	215	0.33	11.7	0	0.38	0.22	0.82
150 ppm LB	0.150	6.2	0.25	3.0	155	1.43	19.6	0	0.51	0.19	1.1
300 ppm LB	0.147	2.7	0.19	6.9	98	1.20	12.7	0	0.13	0.36	3.1
600 ppm LB	0.145	3.3	0.36	4.0	83	0.00	13.5	0	0.9	0.54	4.0
0ppm NH_4Cl	0.035	8.6	0.13	8.5	93	0.00	10.3	52	18.3	0.00	7.6
100ppm NH_4Cl	0.091	14.4	0.28	11.0	139	0.02	5.6	109	4.2	0.00	5.6
200 ppm NH_4Cl	0.087	12.4	0.17	1.2	142	0.00	2.2	105	7.3	0.00	1.7

Table 3-5: Calculated total carbon removal due to growth and non-growth microbial activity as predicted by the modified Luedeking-Piret model.

	Growth Contributions (%)	Non-Growth Contributions (%)
30 ppm LB	0.0	52.0
150 ppm LB	0.0	70.1
300 ppm LB	0.0	71.6
600 ppm LB	0.0	44.4
0 ppm Ammon	16.1	3.0
100 ppm Ammon	35.7	10.4
200 ppm Ammon	36.1	12.4

LB stimulation may have promoted removal due to the provision of amino acids in the form of tryptone and yeast extract. Similar results showing yeast extracts enhancing treatments of metalworking fluids have been reported (Muszyński & Łebkowska 2005; Moscoso et al. 2012). As expected, overstimulation using growth media resulted in lower removal efficiencies. When growth media was present in abundance, microbes began to preferentially utilise supplementary carbon instead of the carbon within the metalworking fluid. The lowered removal was likely due to

the catabolite repression of enzymes required for the degradation of organic components in the soluble metalworking fluids, a well-documented phenomenon (Duetz et al. 1996; Szőköl et al. 2014).

Moscosco et al. (Moscoso et al. 2012) demonstrated that equation 3-5 can be applied for bacterial growth in bioreactors treating metalworking fluids, and that both equation 3-6 and equation 3-7 may be applied to predict total petroleum hydrocarbon (TPH) removal from the reactors. These models can also be applied to the data presented in Figure 3-6 and Figure 3-7 with the fitted parameters given in Table 3-4. While the equation 3-6 was able to model removal in all stimulatory conditions with a fair degree of accuracy (NRMSD < 11% in all cases), equation 3-7 was only able to adequately model the ammonium stimulation reactors, and not LB stimulations. This was likely due to the fact that LB stimulation resulted in rapid growth of microbes without utilisation of the carbon within the metalworking fluid components (indicated by a sharp rise in optical density with minimal decrease in total carbon). While equation 3-7 did have a term for non-growth utilisation, this term was a function of solely the biomass in the reactor, and not of the concentration of the components in the metalworking fluid. Additionally, this term does not take into account the fact that the microbes would first utilise the components within the LB stimulants, and then proceed to utilise the carbon within the metalworking fluid components. In order to account for these deficiencies, the non-growth term in the equation 3-7 was modified to include the carbon concentration in the metalworking fluid bulk, and a lag-term included to account for preferential carbon utilisation.

The results of the application of equation 3-8 can be seen in Figure 3-6 and Figure 3-7 and the resulting NRMSD with the parameters used are given in Table

3-4. It can be seen that the modification of equation 3-8 resulted in an NRMSD that was smaller than that of equation 3-6 and equation 3-7 for all stimulatory cases observed, and that this NRMSD is < 8%.

Table 3-5 presents the contributions that the growth and non-growth terms have to the total carbon removal for each application of equation 3-8. It is shown that the model predicts that the carbon removal within the metalworking fluid was mainly growth associated for the ammonium stimulatory cases, and non-growth associated for the LB stimulatory conditions. This was the expected trend because there was no alternative source of carbon to utilise for growth in the ammonium chloride conditions and thus growth would result in simultaneous carbon decline. Luria-Bertani broth contained carbon sources which stimulated microbial growth, and thus the observed carbon removal from the metalworking fluid was due the respiration activity, rather than due to production of new biomass.

Moscoso et al. applied equation 3-7 to their data and concluded that since the growth parameter “m” was larger than that of “n”, all of the TPH removal observed was associated with the growth of the micro-organisms (Moscoso et al. 2012). In actuality, the parameters cannot be directly compared since they have different units. A more correct approach would be to compare the growth and non-growth utilisation terms in the equation. If this approach is used in the analysis of the data presented by Moscosco, it can be seen that there is a substantial contribution to removal which is non-growth associated. This is in line with what the observations in this work.

3.4.4 Mechanisms for Removal by Microbes in Bioreactors

As the treatment proceeded, an oily sludge began to emerge at the top of each of the reactors. This gives an indication of oil/water separation occurring within the reactors. Sludge did not appear in the controls, both inoculated and abiotic, suggesting that the appearance of the sludge was due to microbial activity (data not shown). Therefore, Triton X-100 was added to the reactors to see if this sludge could be re-emulsified.

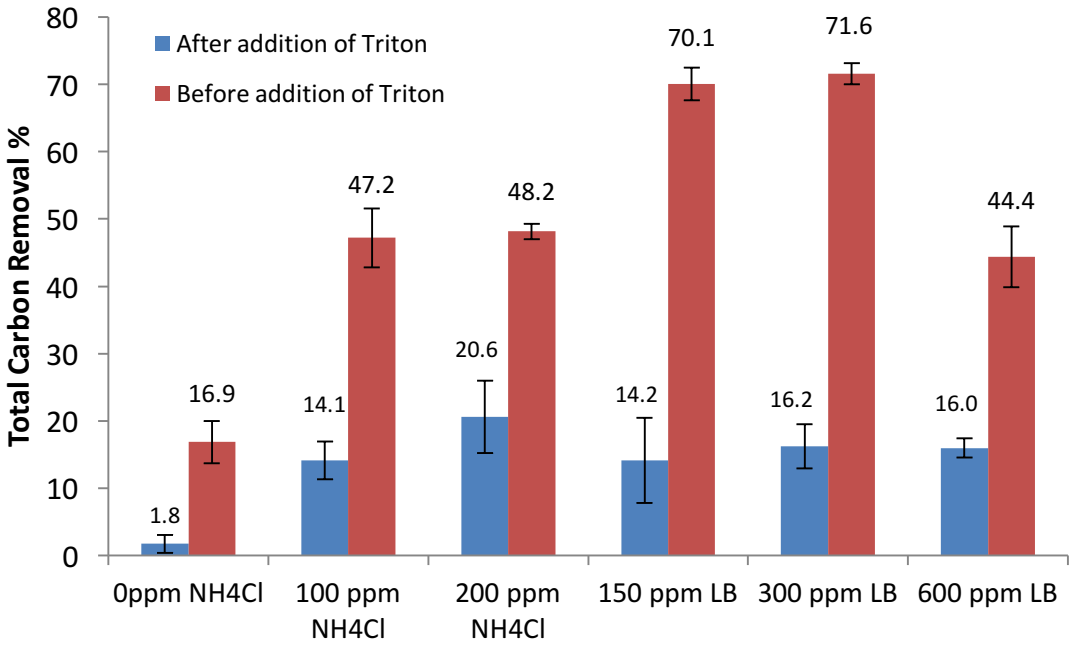

Figure 3-8: Calculated total carbon removal efficiency in the bioreactors, treating 0.5% MWF, before and after the re-emulsification of the oil sludge appearing during the process. Re-emulsification was performed through the addition of Triton X-100, and the total carbon readings were adjusted to account the latter's carbon contribution. Bio-treatment process was conducted for 5 days. Error bars represent standard deviation of triplicate measurements.

Figure 3-8 shows that the addition of 0.1% Triton X-100 resulted in the re-dispersal of a large fraction of the removed carbon. This suggested that microbes do not metabolise a vast majority of the carbon present within metalworking fluid. The removal mechanism could then be either due to adsorption, or due to oil/water separation through the degradation of emulsifying components. Figure 3-8 shows that even though LB stimulation resulted in higher removals than ammonium

chloride stimulation, it did not result in higher levels of mineralised carbon. Thus, it was possible that LB stimulation resulted in the degradation of emulsifiers, while ammonium stimulation resulted in the degradation of non-emulsifying components within the metalworking fluid. A second theory was that ammonium stimulation resulted in the production of biosurfactants. This concept is explored further in Chapter 6 where it is shown that ammonium addition had different impacts on the rates of degradation of different components within metalworking fluids.

The contributions of each mechanism of removal (adsorption, oil/water destabilisation, microbial growth, and microbial respiration) could be measured or calculated using the equations outlined in the methods.

Figure 3-9B shows the relative contributions of each of the removal mechanisms within the reactor. It can be seen that removal due to respirational activity, growth of microbes, and adsorption were significantly smaller than that removed due to oil water separation ($p<0.05$). This shows that the main mechanism for removal observed within this system was demulsification and oil/water separation. Since the inoculated control did not result in any observed removal, it was likely that the removal observed was due to the microbial degradation of the emulsifiers present within the proxy metalworking fluid.

An interesting observation made was that the average optical density of microbes present within the bulk liquid of the metalworking fluid increased significantly after the addition of Triton X-100 to the sludge-containing half of the reactor (an increase from 0.3 to 1.2) and that this increase did not occur in the reactor that did not contain sludge. It was likely that the addition of Triton resulted in the re-emulsification of the

oils floating on the top of the reactors, and that microbes that had been associated with the oil had become re-suspended.

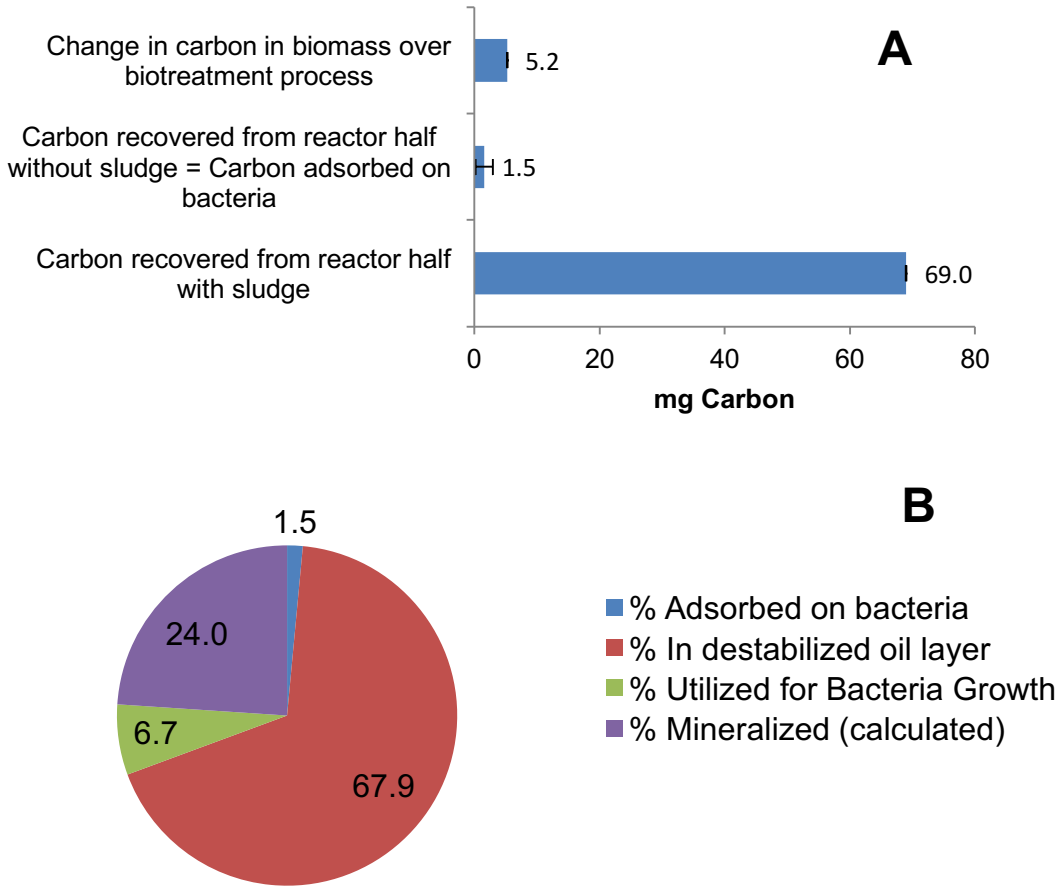

Figure 3-9: A) Carbon recovery using Triton X-100 in upper and lower partitions of a bioreactor stimulated with 150 ppm Luria-Bertani broth. Reactor treated 0.5% MWF over 5 days. Error bars represent standard deviation of triplicate measurements B) Relative contributions (%) of each removal mechanism to the total amount of carbon removed from the system (62.5% of initial amount of carbon was removed).

3.4.5 Effects of water hardness on carbon removal efficiency in bioreactors

Having shown that the major mechanism for removal was oil/water separation, it was thus possible that water hardness would have had a significant effect on removal rates of carbon in metalworking fluid bioreactors. It is shown in Figure 3-3 that water hardness could have a significant effect on dilute emulsion stability due to the interactions that the divalent ions have with the anionic emulsifiers present in

the metalworking fluid and their overall effect on electrical double layer interactions. It has also been shown in Figure 3-5 that, as the metalworking fluid becomes concentrated, this effect weakens. This was possibly due to the higher concentration of emulsifiers that would be present in the metalworking fluid. Since emulsifier concentration had a significant effect on emulsion stability, then as emulsifiers became biodegraded, the tendency of an emulsion to remain stable within a given hardness concentration would decline. In theory, emulsions that were stable at the given water hardness will gradually become unstable as biodegradation proceeds. For emulsions that were unstable at the given water hardness values, the rate of destabilisation would increase as biodegradation proceeded. This is illustrated in Figure 3-10. It can be seen that the final percentage of the total carbon removal achieved in a bioreactor with 60ppm $CaCO_3$ water hardness was 41.5% ± 1.7%. This was significantly larger ($p< 0.01$) than that of the abiotic 60ppm $CaCO_3$ control which only achieved 3.3% ± 0.5% removal. Similarly, the bioreactor containing 240 ppm $CaCO_3$ hardness achieved 85.7% ± 0.2% removal, which was significantly higher ($p<0.01$) than the abiotic removal figure of 54.2% ± 2.9%. Not only was the extent of removal higher for the 240 ppm bioreactor when compared to the abiotic counterpart, but the removal rate was much greater as well. Within the first day of operation, the bioreactor achieved 80.5% ± 8.8% removal when compared to 39.5% ± 2.8% achieved by the abiotic control. This suggested that the presence of the microbial community accelerated destabilisation. The acceleration can be attributed to microbial activity since microbes did not adsorb significant amounts of oil (as proven in Figure 3-9), and since it was unlikely that the physical presence of microbes resulted in destabilisation. The latter was

known since bacteria, like the oil droplets, carry a negative charge on their surface (Dickson & Koohmaraie 1989)).

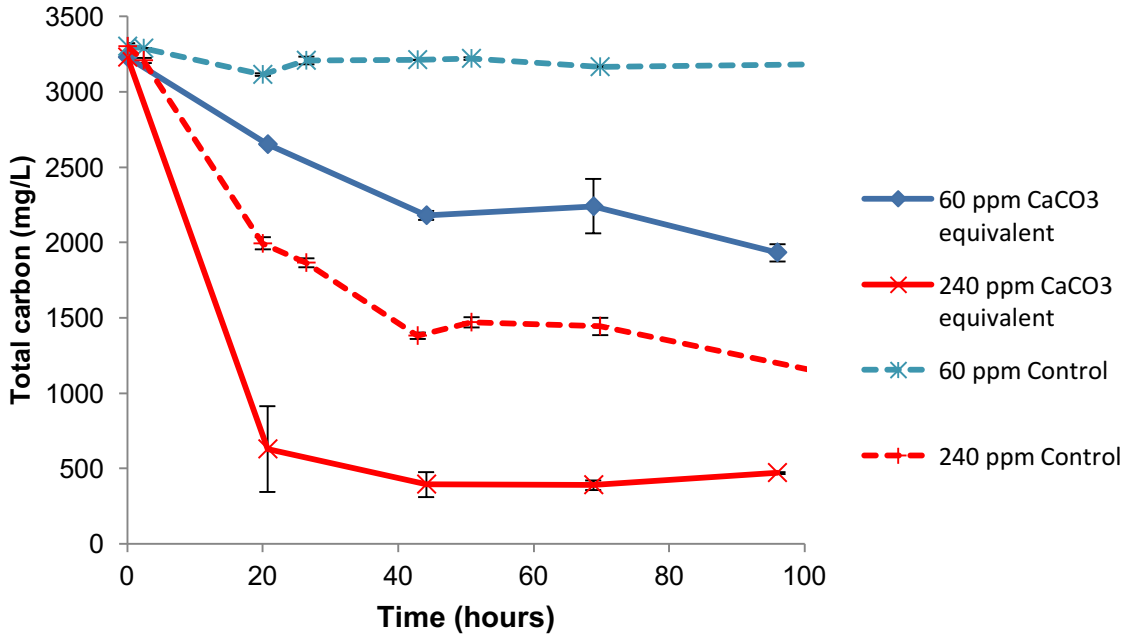

Figure 3-10: The effects of water hardness on the bio-treatment process of 0.5% MWF. Solid lines represent the live biological reactors, while dashed lines represent abiotic controls. Error bars represent standard deviation of triplicate measurements. Reactors were stimulated using 0.5% LB

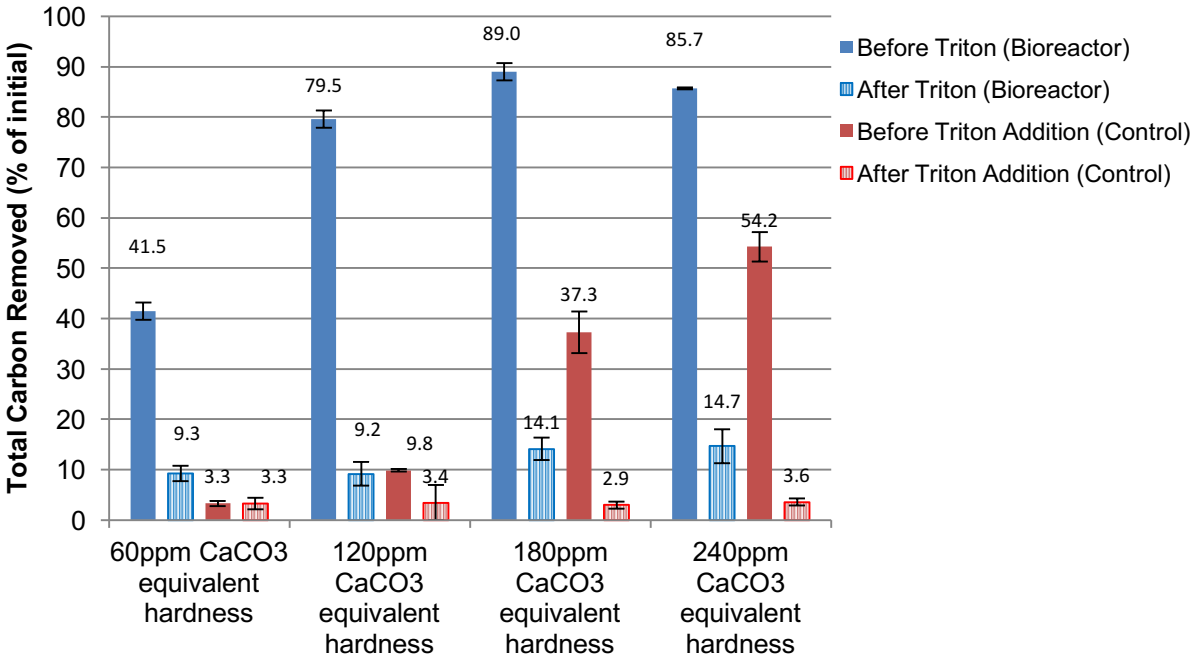

Figure 3-11: Calculated total carbon removal from the bulk liquid before and after Triton X-100 addition for reactors treating 0.5% MWF with varying degrees of water hardness. Controls represent abiotic reactors. Addition of Triton X-100 allows for re-emulsification of destabilised oils floating at the top of

each bioreactor, which reveals the total carbon removed through microbial activity. Measurements were taken after 4 days of treatment. Error bars represent standard deviation of triplicate measurements.

Figure 3-11 shows that the addition of the emulsifier Triton X-100 to reactors treating 0.5% MWF with different water hardness concentrations. It can be seen that although bulk removal increased with increasing hardness (from 41.47% at 60 ppm, to 85.75% at 240ppm), ultimate biodegradation, which was the calculated total carbon removal after the oil/sludge layer has been re-emulsified, was found to vary from 9.26% at 60 ppm to 14.67% at 240 ppm. The differences in the removal extents were not significant ($p= 0.157$). Furthermore, the amount of biomass present in each reactor after re-emulsification was found to be similar in each water hardness concentration investigated. This suggested that while increasing water hardness does promote bulk removal, it does not promote growth of the micro-organisms, nor does it increase the level of metabolic activity in the reactors. This was consistent with the observations that the bulk removal was due to oil/water separation induced by the biodegradation of emulsifiers.

In order to validate the observation that the trends observed were not exclusive to the choice of proxy soluble oil, the effects of hardness experiment was repeated using a generalised formulation containing Naphthenic Mineral Oil, Tall Oil and Sodium Sulfonate. Figure 3-12 shows that the trend of increased carbon removal within increasing water hardness was observed with the generalized formulation as well. Similar to the biodegradation of CoolEdge, an oily sludge began to appear in reactors with live biomass. Abiotic reactors did not develop this sludge.

Furthermore, since the formulation was defined, the extents of surfactant degradation were determined in Figure 3-13 and in Table 3-6. Figure 3-13 shows that, like total carbon removal, Oleamide DIPA (oleic acid that has undergone

amidation with diisoproponolamine) removal also increased with increased water hardness. Oleamide DIPA (an anionic surfactant) was the main component found to be in the Tall Oil Amide that was used in the experiments, and thus it was chosen as the representative emulsifying component to report (although the trend of increased removal with increasing water hardness was observed for the other emulsifying components as well- data not shown). The increase in the removal of this component was thought to be due either to more favourable osmotic pressure effects (the pressure on the cell membrane of the bacteria due to differences in the concentrations of components inside and outside the cell) with increasing hardness, or due to greater partitioning to the oil phase, which separates from the water during the process. However, osmotic effects were not responsible since stimulating soft waters with sodium chloride had no effect on the total carbon removal, or the surfactant removal extents (data not shown). Therefore, the increased removal was likely due to the oil/water separation. This is confirmed in Table 3-6, where it is shown that the re-emulsification of the destabilised oil resulted in the re-emergence of unsaturated surfactants within the bulk liquid of the reactor.

It was interesting to note that the saturated fatty amide surfactants (surfactants with no double bonds such as Palmitamide-DIPA) did not re-disperse upon the re-emulsification of the oily-sludge, which indicated that these surfactants have been either completely mineralised or utilised by microbes for growth. The unsaturated fatty acid surfactants such as $C_{24}H_{46}O_3N$ (Oleamide DIPA) and $C_{24}H_{48}O_3N$ (Linoleamide DIPA) had an absolute removal of 22.5% ± 6.5% and 25.3% ± 7.2% respectively, when compared to $C_{22}H_{46}O_3N$ (Palmitamide DIPA) and $C_{24}H_{50}O_3N$ (Stearamide DIPA) which had an absolute removal of 98.9% ± 0.8% and 95.7% ± 1.9% respectively.

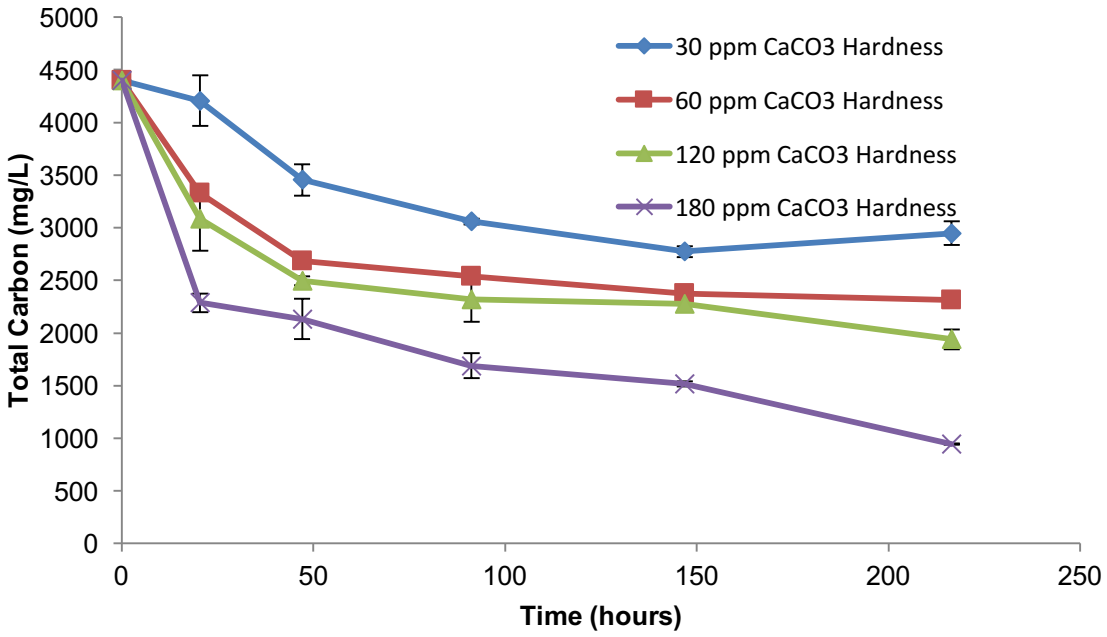

Figure 3-12: Increasing water hardness increases the total carbon removal in bioreactors treating 0.5% of the defined soluble oil. Error bars represent standard deviations of duplicate measurements. Reactors were stimulated with 100 ppm NH_4Cl and 100 ppm KH_2PO_4.

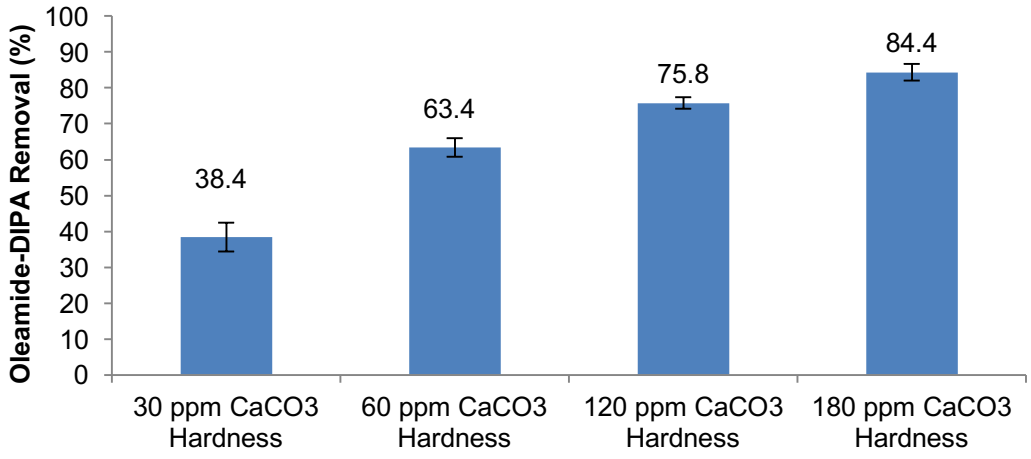

Figure 3-13: Increasing the hardness increases the extent of Oleamide-DIPA removal, which is a main fatty amide emulsifying component in the metalworking fluid. Error bars represent standard deviations of duplicate measurements. Samples were extracted from reactors 9 days after treatment. Reactors were stimulated with 100 ppm NH_4Cl and 100 ppm KH_2PO_4.

Berg et al. found that unsaturated fatty acids were more difficult to degrade compared to saturated fatty acids since the double bond required additional steps for degradation (Berg et al. 2002). Nevertheless, there was a net percentage of degradation of the emulsifier package present in the soluble oil waste. It was this net degradation that resulted in the emulsion becoming more susceptible to

oil/water separation due to coagulation and coalescence. This explains the relatively small amount of mineralisation of total carbon in bioreactors (approximately 20%) even though the observed removal was relatively large (approximately 70%). This observation also explains the apparent contradiction that was observed in publications regarding the treatment of soluble oils; in these publications it was claimed that carbon removal in a matter of a few days even though the time-scale for mineral oil degradation occurs over weeks.

Table 3-6: Extents of Tall Oil Fatty Acid surfactant removal before and after re-emulsification of the oil sludge in a bioreactor treating 0.5% of a defined soluble oil. The emulsifier package contained 50% Tall Oil and 50% Sodium Sulfonate. The bio-treatment process was conducted for 7 days. Standard deviations of duplicate measurements are given. Water hardness was 120 ppm $CaCO_3$ equivalent, and reactor was stimulated with 100 ppm NH_4Cl and 100 ppm KH_2PO_4.

Component	% of initial in bulk of liquid before re-emulsification	% of initial in bulk of liquid after re-emulsification	% of initial concentration removed from bulk	% of initial concentration removed from reactor (through microbe growth and mineralization)
Total Carbon	31.7 ± 0.9	76.7 ± 3.2	68.3 ± 0.9	23.3 ± 3.2
$C_{24}H_{44}O_3N$	17.7 ± 3.8	44.8 ± 6.1	82.2 ± 3.8	55.2 ± 6.1
$C_{24}H_{46}O_3N$	27.0 ± 3.2	77.5 ± 6.5	73.0 ± 3.2	22.5 ± 6.5
$C_{22}H_{46}O_3N$	0.3 ± 0.1	1.1 ± 0.8	99.6 ± 0.1	98.9 ± 0.8
$C_{24}H_{48}O_3N$	25.6 ± 3.0	74.7 ± 7.2	74.4 ± 3.0	25.3 ± 7.2
$C_{24}H_{50}O_3N$	1.7 ± 0.7	4.2 ± 1.9	98.3 ± 0.7	95.7 ± 1.9

The implications of these findings suggest that the fast kinetics of the removal of the total organic carbon (TOC) associated with soluble oils was due to demulsification and oil/water separation, and this resulted in an oily sludge that floats at the top of the reactor. Practitioners that employ the microbial treatment should be aware that this may be the predominant mechanism in their treatment process, and thus make arrangements for the disposal of this sludge. Since mineral oils used within metalworking fluids are biodegradable, the sludge can be digested for longer

periods of time to reduce the oil content. Alternatively, it may be treated as concentrated hazardous waste that may be incinerated. Practitioners may also wish to consider removing the oil from the emulsion before the microbial process as a means of reducing sludge and recovering the oil from spent waste streams.

It should be noted that whilst the predominant mechanism for carbon removal observed in the experiments was oil/water separation, certain microbes (such as Acinetobacter) are capable of re-establishing the emulsion through the production of biosurfactants (Chen et al. 2012). Should re-emulsification occur, oil removal via microbial activity will be enhanced, but the overall removal rate of carbon will decline.

Treatment processes for soluble oils were effective in carbon removal for formulations made up with hard water, as compared to that made up with soft water. Artificial hardening of soluble oil wastewaters can be used as a strategy to improve the performance of bioreactors.

3.5 Effects of Surfactant Type on the Degradation of Metalworking Fluid

Oil/water separation, induced through the microbial degradation of emulsifiers, was found to be the main mechanism for carbon removal within bioreactors treating soluble oil metalworking fluids. This implied that the inherent biodegradability of the surfactants would determine the rate and extent to which the treatment process was able to occur. To investigate this, the recipe for the emulsifier package used for the defined soluble oil was varied to contain different ratios of Sodium Sulfonate and Tall Oil Fatty Amide. Sodium Sulfonate is a biostable surfactant, and hence it was not readily amenable to biodegradation (Childers 2006). Conversely, Tall Oil Fatty Amide is a biosupportive surfactant which could be readily biodegraded (Childers

2006; Rabenstein et al. 2009). Thus, as the ratio of Sodium Sulfonate to Tall Oil Fatty Amide increased, the extent of carbon removal through biological activity would decrease. The naphthenic mineral component, which was the major component in soluble oils, was kept constant. Thus, the total carbon of each of the recipes does not vary greatly.

Figure 3-14 shows that after 1 week of the biotreatment process, the reactors containing a 1:1 ratio of amides to sulfonates (by weight) had the greatest carbon removal efficiency (68.3%); this was significantly ($p<0.01$) higher than the 1:3 formulation, or the pure Sulfonate formulation (achieving 36% and 0.8% removal efficiencies respectively). The very low removal efficiency of the formulation for sulfonates did not improve upon further stimulation with ammonium chloride, which suggests that nitrogen limitation due to the absence of Tall Oil Amides was not the reason for the low removal observed (data not shown).

The results from Figure 3-14 imply that it was the surfactant type which controls the extent of carbon removal observed in bioreactors treating soluble oil wastes. Formulations that contain biostable or bioresistant surfactants would be less amenable to treatment as compared to those containing biosupportive surfactants. Thus, practitioners who make use of metalworking fluids with biosupportive surfactants would be able to treat their wastes using the biological treatment process much more easily than those who do not.

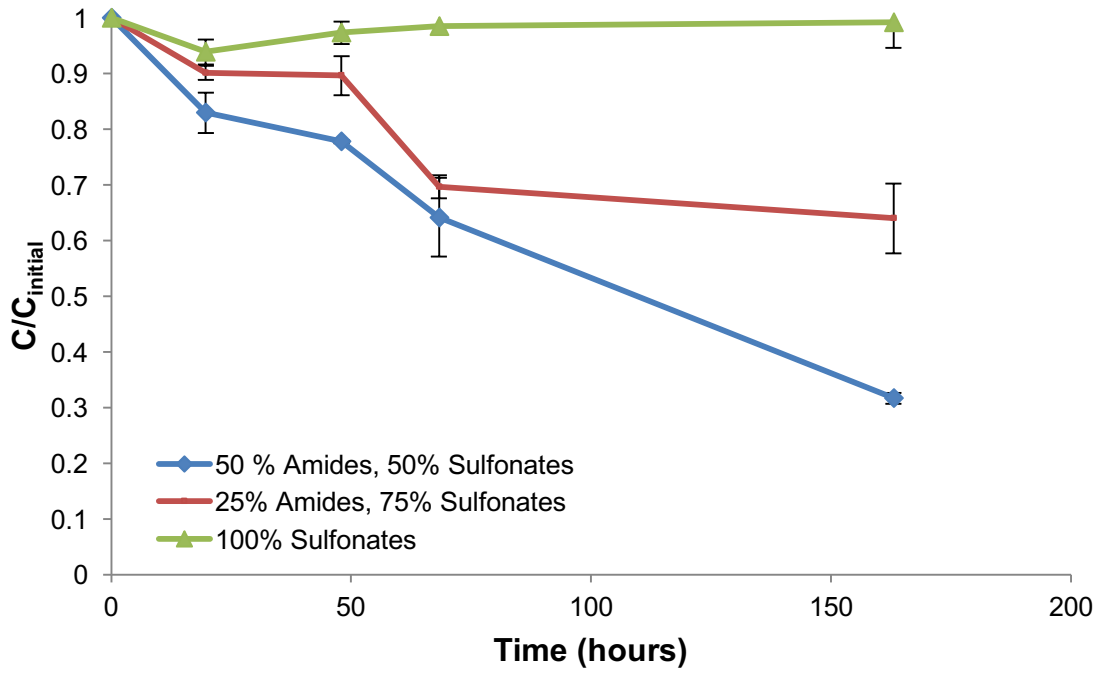

Figure 3-14: The type of emulsifier package used in formulation influences the rate of carbon removal during the bio-treatment process. Metalworking fluid concentrations were 0.5% (w/v). The emulsifier package was fixed to account for 23.8% of the weight of the concentrate. Dilution water hardness was 120 ppm $CaCO_3$ equivalent. Error bars represent standard deviation of duplicate measurements. Reactors were stimulated with 100 ppm NH_4Cl and 100 ppm KH_2PO_4.

3.6 Discussion

In this chapter, it is shown that the main mechanism for the removal of carbon in biological systems treating soluble oil emulsions was oil/water separation. It was shown that the carbon removal rate was dependent on both the surfactant biodegradability as well as the hardness of the water that was used for the dilution of the metalworking fluid. These two observations suggest that the main mechanism for removal was due to surfactant degradation, which leads to oil/water separation within the bioreactor. Furthermore, beyond the biological mechanism of removal, it was shown that dilution of metalworking fluid wastewaters with relatively hard waters led to spontaneous destabilisation of the emulsion and oil/water separation. Thus, wastewater treatment practitioners should take into account both surfactant type and water hardness when considering the efficiency of their process. A choice to treat metalworking fluids with biodegradable surfactants would lead to a much

more rapid and efficient treatment process than a choice to treat metalworking fluids with bio-stable surfactants. Thus, in order to create a metalworking fluid that is easily treatable via the biological treatment process, metalworking fluid practitioners should use formulations that contain easily biodegradable surfactants.

Moreover, choosing to operate the process in a region with relatively hard water would lead to more rapid and efficient processes than regions with softer waters.

It was also shown that, if the dilution extent was sufficient enough, then spontaneous demulsification was possible, leading to an additional physical removal mechanism of carbon within the bioreactors. The rate of carbon removal was once again dependent on the hardness of the water that was used for the dilution process.

The findings of this chapter have a number of implications. Firstly, since the mineral oil was not mineralised in the same time-scale in which it was removed, it is possible that it may accumulate at the top of the reactor, or adsorb onto the walls of the vessel. This will lead to an oily sludge which must be treated further or must be disposed of via incineration. In either case, this adds an additional cost to the biological treatment of metalworking fluids.

Secondly, since the main removal mechanism was found to be oil/water separation, it was possible that the process could be influenced by the geometry of the reactor. This was because the removal process involved agglomeration of oil droplets and their subsequent rise to the top of the reactor due to induced differences in buoyancy (Foltz 2006). It was thus likely that the rate of removal was dependent on the height of the reactor since increasing the height increases the mean distance that the oil droplets must travel before reaching the top of the reactor (Reddy et al. 1981). Thus, for a given volume of reactor, as the ratio of reactor height to reactor

width becomes smaller, the rate of carbon removal should increase (provided the agitation quality is kept constant). Further research will need to be done to confirm this hypothesis.

3.7 Conclusions

- Metalworking fluids require dilution in order to reduce the inhibitory effects of biocides present within the formulation. The extent of reduction may be such that spontaneous oil/water separation may occur due to naturally occurring divalent ions present within the water used for dilution, and within the metalworking fluid formulation. Such demulsification and oil/water separation may be a contributory mechanism for removal within bioreactors treating soluble oil wastes.
- Bio-stimulation can be an effective means of promoting carbon removal within reactors treating soluble oil wastes. Stimulations involving potassium phosphate, ammonium chloride and Luria-Bertani (LB) broth can be used to increase extents and removal rates of carbon in bioreactors. LB stimulation enhances removal to a greater degree than ammonium stimulation, although the ultimate degree of degradation was found to be similar.
- The Luedeking-Piret model can simultaneously describe the growth of micro-organisms, as well as the carbon removal from the reactors. The accuracy of the model can be improved by modifying the non-growth utilisation term to become first-order with respect to the concentration of carbon in the reactor, and by including a lag-term to account for the preferential use of soft carbon that may be provided during stimulation.
- The main mechanism for carbon removal in the biotreatment process was found to be demulsification and oil/water separation that is induced by

microbial activity. Bacteria are likely to degrade the emulsifying components within the soluble oil, which results in destabilisation of the oil-in-water emulsion. The emulsion droplets coalesce and become large enough to float. This results in an oily-sludge that contains a mixture of oil, organics and microbes that appear on the top of the bioreactor. This sludge would need to be treated as hazardous waste requiring further treatment.

- Since oil/water separation was the main mechanism for removal, the rate of carbon removal within the bioreactors increases with water hardness. This is because of the effect that divalent cations (such as calcium) have on the repulsive forces between electrical double layers on the surface of oil droplets; these droplets are initially negatively charged due to the presence of the surfactant emulsifier. Thus, by artificially hardening waste streams, the carbon removal in the bio-treatment process may be enhanced. The bio-treatment process lowers the stability of emulsions as microbes degrade emulsifiers, and thus the rate of destabilisation of emulsions is greater in the presence of microbes than it would be in abiotic reactors experiencing natural demulsification (provided that the emulsifiers are biodegradable).
- Since biodegradation of emulsifiers and oil/water separation are the main removal mechanisms, waste streams that contain biodegradable emulsifiers are more amenable to the biotreatment process than those with biostable surfactants. Practioners who make use of mineral oils in formulations should consider using biosupportive surfactants in the emulsifier package to facilitate cost-effective end-of-life treatment.

Chapter 4- Coagulation and Coalescence as a Pre-treatment Process for the Removal of Biocides from Metalworking Fluids

4.1 Introduction

4.1.1 The Need for a Hybrid Treatment Approach

The microbial treatment of metalworking fluids is a cost effective means of reducing pollutant loads in wastewaters (Cheng et al. 2005). Within industry, the direct microbial treatment is employed to soluble oils and semi-synthetics in a number of different ways. This could either be through a suspended (Moscoso et al. 2012; van der Gast et al. 2004) or fixed bioreactor (Muszyński & Łebkowska 2005), through a membrane bioreactor (Anderson et al. 2009), through fluidized beds (Schreyer & Coughlin 1999), or through biological activated carbon (Kim et al. 1989). In Chapter 3, it is demonstrated that the main carbon removal mechanism in bioreactors that are directly treating soluble oils was oil/water separation induced by the biodegradation of emulsifiers. This results in an oily sludge that contained a mixture of hydrocarbons and microbes which requiring further treatment for disposal. The microbial treatment of metalworking fluids thus incurs an additional cost since the carbon is not biodegraded in the same time scale as it takes to be removed from water. If one wanted to avoid this additional cost, one would need to increase the treatment time within the bioreactors since investigations have shown that minerals oils are biodegradable, but only in timescales of weeks (Aluyor & Ori-Jesu 2009; Novick et al. 1996). The disadvantage in doing this is that equipment sizes and hence capital costs would increase in order to process a defined capacity. A further disadvantage to employing a direct microbial treatment method is that oil

reclamation becomes more difficult since the oily sludge is contaminated with microbes (see section 3.4.4). Thus, the potential for recycling the oil within metalworking fluids is reduced (Burke & Gaines 2006).

A second point that is discussed in Chapter 3 is that metalworking fluids require dilution before any treatment efficiency can be observed. This is because metalworking fluids contain anti-microbial agents which inhibit the biodegradation process when they are present in water at high concentrations (Jagadevan et al. 2013; Thill et al. 2016) . Dilution of waste metalworking fluids results in the same disadvantages as increasing the time of the treatment process- larger equipment sizes and capital costs are required in order to meet defined target capacities. This chapter tries to address these two disadvantages with the microbial treatment process. A treatment technique that is able to reclaim a majority of the carbon (in the form of mineral oil) and simultaneously reduce biocide concentrations has the potential to be greatly beneficial to improving both process efficiencies and costs.

4.1.2 Oil Removal Processes as a Pre-treatment to the Biological Treatment of Metalworking Fluids

Oil from metalworking fluids may be removed abiotically through either physical or chemical techniques(Cheng et al. 2005).

Membrane treatment processes, such as ultrafiltration and nanofiltration are able to simultaneously remove oil and retain organic contaminants (Hilal, Busca, Hankins, et al. 2004). By employing a membrane which contains a pore size that is smaller than that of the emulsified oil droplets (2-50 μm) (Childers 2006), one is able to effectively separate both the oil and the water phases. Both ultrafiltration and nanofiltration processes do not completely reject all organic matter (Burke & Gaines

2006). Small, water soluble organics that pass through the membrane may then be utilised in a biological treatment process down-stream (Busca 2004).

Utilising the membrane process as a pre-treatment step has the disadvantage of being highly susceptible to fouling, and not being suitable for treating large volumes of waste (Hilal, Busca, Hankins, et al. 2004; Lindau & Jonsson 1994). Moreover, they result in brine that would be concentrated with water soluble organic contaminants (Burke & Gaines 2006; Milić et al. 2013). While the problem may be tackled through the utilisation of a membrane bioreactor, the problem of accumulating biocides would mean that dilution would still be a requirement in order to make the process feasible.

A second approach would be to consider the combinatorial approach of coagulation, coalescence and biodegradation. Through the addition of chemical coagulants, such as inorganic salts or polyelectrolytes, the anionic emulsifiers which stabilize metalworking fluid emulsions are neutralised and the zeta potential of the oil droplets are lowered (Ríos et al. 1998; Canizares et al. 2008). This leads to a collapse of the double layer forces which keep the oil droplets in suspension, thus leading to coalescence of oil droplets and oil water separation(van den Tempel 1953). Since this results in a distinct oil and water phase, the oil may be recovered and reclaimed, used as a fuel, or may be disposed of via incineration (Hilal, Busca, Hankins, et al. 2004; Busca 2004; Burke & Gaines 2006). In Chapter 3, it is observed that this oil/water separation also resulted in the removal of organic components that partitioned into the oil phase to a greater extent than the water phase. Thus, coagulation and coalescence has the potential for removing biocides which behave in a similar manner. Removing the biocides from the metalworking fluid means that the biological treatment process will become more efficient at

removing the water soluble organics that are not removed during the oil/water separation process. While the membrane based pre-treatment process would share this advantage, the chemical process does not suffer from the deficit of requiring periodic cleaning.

A disadvantage of the coagulation/coalescence process is that it requires an addition of chemicals, which may be costly (Fouad 2014). Also, depending on the type of coagulant that is used, sludge may be formed during the process which would either need to be reclaimed or disposed. One means of tackling this disadvantage would be to use coagulants which remain in solution after the coagulation/coalescence process, so that they may be recycled after the treatment process (by means of returning a portion of the treated effluent back into the coagulation/coalescence vessel). This would mean that the coagulants would then be present for the biological treatment process and hence their toxicity would need to be determined.

While there are numerous studies that have investigated the potential of coagulation in reducing oil content, COD and total carbon (Ríos, Pazos & Coca, 1998; Bensadok, Belkacem & Nezzal, 2007; Canizares et al., 2008; Kobya et al., 2008), there are few which look at the toxicity of coagulants, and the role that the coagulation process has in reducing biocide concentrations. Thus, there is a need to determine the potential of coagulation and coalescence has at reducing the toxicity of metalworking fluids. In this chapter, these concepts are explored using a defined soluble oil formulation, conventional inorganic coagulants ($CaCl_2$, $MgCl_2$ and $FeCl_3$) and common biocides that are found within metalworking fluid formulations (1,2-benzisothiazol-3(2H)-one, o-phenyl phenate and its sodium salt, and iodo-propynyl N-butylcarbamate).

4.1.3 Toxicity of Coagulants

When considering a hybrid process involving microbes, it is necessary to understand the inhibitory effects that one process has on the other. In the case of coagulation and coalescence, the effects of residual coagulant concentration on the succeeding bio-treatment process must be studied. Several researchers have shown that the residual concentrations of alum, iron sulphate and iron chloride can be toxic to downstream micro-organisms, and may even increase the toxicity of the final effluent itself (Stephenson & Duff 1996; Al-Mutairi 2006; Teh et al. 2016). These toxic effects are brought about through binding and disruption of cell membranes, DNA and cell walls (Pifia et al. 1996). While the precipitation of alum and iron chloride would reduce toxicity (Evuti & Lawal 2011), the solid sludge that is produced must then be further treated or reclaimed (Evuti & Lawal 2011).

While much research on the toxicity of alum and iron has been reported, there is limited research on the potential inhibitory effects of residual Ca^{2+} and Mg^{2+} concentrations. This may be due to the fact that these ions are nutrients that are required by micro-organisms to maintain enzymatic activity, cellular structure and membrane permeability (Lusk et al. 1968; Patrauchan et al. 2005). Nevertheless, the increase in the ionic strength of the effluent could lead to osmotic inhibition (Wood 2015), and hence this effect on metalworking fluid micro-organisms needs to be studied. In this chapter, the effects of these coagulants on the respiration activity and microbial growth of the metalworking fluid acclimated microbes are presented.

4.1.4 Determining the Critical Coagulation Concentration

The critical coagulation concentration (CCC) is the minimum amount of coagulant that is required to induce diffusion controlled aggregation within a process (Trefalt

2016; Serra et al. 1992; Verrall et al. 1999). It is an important design concentration as it represents the maximum amount of coagulant that should be utilised within a coagulation process (further additions might actually hinder the process due to re-stabilization). While coagulation/coalescence may occur at electrolyte concentrations lower than the CCC, it is optimized at the CCC since every collision of particles is guaranteed to result in agglomeration (Verrall et al. 1999; Reerink & Overbeek 1954). To determine the toxicity that coagulants have on the microbial process, their toxicity at the CCC must be determined.

Two common means to determine the critical coagulation concentration of emulsions involve turbidimetric methods (Kissa 1999; Serra et al. 1992; Lawrence & Parfitt 1971). The batch turbidimetric method is a quick and simple technique which involves subjecting a batch of influent samples to different coagulant concentrations (Burke 1991; Ríos et al. 1998). After a pre-determined amount of time, the batch is visually inspected, and the concentration of coagulant which results in a sudden rapid decline of turbidity is deemed to be the critical coagulation concentration. This method suffers from inaccuracies involved in visual inspection and the choice of an arbitrary amount of time for inspection.

A more accurate method, first developed by Reerink and Overbeek (Reerink & Overbeek 1954), involves determining the instantaneous slope of turbidity decline at the beginning of the coalescence process, which is proportional to the rate of aggregation. Once the slope $(\frac{d\tau}{dt})_{t\to 0}$ remains constant with respect to coagulant concentration, then the critical coagulation concentration must have been obtained. By considering the change in the rate of the turbidity decline with coagulant concentration, the critical coagulation may be determined at the point at which the

slope first reaches zero. However, accurately measuring $(\frac{d\tau}{dt})_{t\to 0}$ for metalworking fluids is difficult because they are concentrated emulsions which scatter all incident light.

Since agglomeration of particles is known to follow a second order process, the rate constant describing this process can be used as a means to estimate the critical coagulation concentration. This is because the point at which the rate constant becomes independent of concentration would also be the point where $(\frac{d\tau}{dt})_{t\to 0}$ becomes independent. This is the method used for this work.

4.1.5 Current Practices for Toxicity Reduction of Metalworking Fluids

Metalworking fluids contain anti-microbial agents which makes the biological end-of-life treatment difficult. Recently, there have been attempts to pre-treat metalworking fluid wastewaters with the aim of improving its biodegradability. Jagadevan et. al investigated the potential of using ozonation and Fenton's reagent as a means of removing both inhibitory and recalcitrant components (Jagadevan et al. 2013; Jagadevan et al. 2011). Thill et al. showed that both nano-iron and electron beam treatment is capable of improving biological treatment outcomes (Thill et al. 2016). More conventional treatments involve the use of activated carbon or adsorbents to remove biocidal components. While these treatments are effective, they either require costly chemical consumables or are highly energy intensive. Thus, it should be reserved as a finishing step to the treatment process.

Biocides that are used within metalworking fluids may be formulated within the concentrate or may be added to the tank side once the emulsion has been formed (DOW Microbial Control n.d.). Those typically formulated into the concentrate are highly soluble within the base oil, and hence have a greater partitioning tendency to

oil phase within the formulation. The coagulation/coalescence procedure thus has potential for removing these components, together with the biostable mineral oils which could thereby improve biological treatment outcomes.

Within this the chapter, the ability of coagulation to remove ortho-phenyl phenol and its sodium salt (OPP and Na-OPP), 2-iodo-3-propynyl N-butacarbamate (IPBC) and 1,2-benzisothiazol-3(2H)-one (benzisothiazolinone) from wastewaters were investigated. Each of these biocides is approved for usage within either the European Union (The European Commission 2015; The European Commission 2016) or the United States (EPA 2015), and is thus common within metalworking fluid formulations. Within the United Kingdom, their discharge concentrations are regulated by the European Directive 90/68/EEC (The European Parliament 1979) and thus treatment is required for effluent waste to meet regulations. Studies for the treatment of these three biocides are limited. Investigations show that OPP and Na-OPP is susceptible to photo-catalytic degradation (The DOW Chemical Company 2015c) that it can be biodegraded by bacteria and fungi at low concentrations (< 150mg/L) (Gonsior et al. 1984; Perruchon et al. 2016). However, in metalworking fluids, concentrations can exist in ranges from 0.5g/L to 5g/L. IPBC is hydrolytically and photo-catalytically stable. It is also not biodegradable according to the OECD test, but may be biodegraded in soil environments (The European Commission-Denmark 2013; The DOW Chemical Company 2015b). The lack of research on IPBC removal thus limits the treatment options for IPBC in metalworking fluids. Benzisothiazolinone has shown to degrade using ozonation, which, as mentioned previously, is a costly and energy intensive process (Li et al. 2016). Coagulation and coalescence could thus prove to be a cost-effective alternative for the removal of these anti-microbial agents.

4.2 Aims and Objectives

The research aim of this chapter are:

- To determine the effects that coagulation/coalescence has on the growth and activity of metalworking fluid acclimated micro-organisms.
- To determine if coagulation/coalescence can be an effective technique in removing biocides from metalworking fluids, and hence to determine if coagulation/coalescence may be applied as detoxification treatment step.

The objectives to obtain these aims are:

- To determine the concentration of conventional coagulants that are required to induce diffusion controlled aggregation within the defined metalworking fluid waste
- To determine the toxicity of the coagulants to the microbes involved in the biological treatment process
- To determine the toxicity of conventional biocides to the microbes involved in the biological treatment process
- To determine the role of the coagulation process in removing conventional biocides from metalworking fluid formulations, and hence to determine the role that coagulation and coalescence plays in reducing toxicity.

4.3 Materials and Methods

4.3.1 Metalworking Fluid

A modified soluble oil formulation, based on the recipe presented by Childers, was used for all experiments in this chapter (Childers 2006). For 100 g of concentrate, each of the following components were added: 73.6g napthenic mineral oil, 18.7g sodium sulfonate, 5.5g tall oil fatty amides and 2.2g propylene glycol. Napthenic mineral oil was first added to sodium sulfonate, and then mixed vigorously using a magnetic stirrer. Thereafter, tall oil fatty amides were added, followed by propylene glycol. All contents were mixed together with a magnetic stirrer to form a homogenous mixture. 25mL of the concentrate was added to 450mL of deionised (DI) water to form a 5% (v/v) stock solution, which was used to make the experimental MWF formulations in this chapter. Unless stated, all concentrations of metalworking fluid used in the experiments of this chapter were 2.5%.

Stock solutions of biocides were prepared as follows:

For OPP and IPBC a 100g/L stock solution was prepared in propylene glycol. This was added to the metalworking fluid formulations to make the specified concentrations. For Na-OPP, the 100g/L stock solution was prepared in DI water. For BIT, a 30g/L stock solution was made in propylene glycol.

4.3.2 Micro-organisms

The actual micro-organisms used, and the procedure for cultivation is the same as that in Chapter 3, with the exception that the CoolEdge BI is replaced with the metalworking fluid described in Section 4.3.1.

4.3.3 Reagents

Analytical grade inorganic salts ($CaCl_2$ anhydrous, $MgCl_2.6H_2O$, $FeCl_3$, Al_2SO_4, NH_4Cl, KH_2PO_4), propylene glycol, acetonitrile (99.9% purity) and acetic acid were purchased from Sigma Aldrich.

Biocide reagents o-phenyl phenol, sodium o-phenyl phenate, 2-iodo-3-propynyl N-butacarbamate and 1,2-Benzisothiazol-3(2H)-one were purchased from Sigma Aldrich.

Samples of naphthenic mineral oil were provided by Nynas Base Oils. Samples of sodium sulfonate were provided by Sonneborn. Tall oil fatty amides were provided by Colonial Chemical.

4.3.4 Coagulation and Coalescence Procedure

A simple and rapid coagulation and coalescence protocol was used for the experiments in this chapter. 1M stock solutions of $CaCl_2$, $MgCl_2$, $Al_2(SO_4)_3$ and $FeCl_3$ were prepared in DI water. To a 50mL centrifuge tube, stock solutions of metalworking fluid, coagulant and DI water were added to make a 30mL with specified experimental concentrations. The tube was immediately vortexed for 3.5 minutes to simulate the rapid coagulation process. The tube was then left to stand for 2 days, which was found to be the minimum amount of time to achieve a steady turbidity and total amount of carbon reading.

For the determination of rate constants of turbidity decline, the tube contents were emptied into a 30mL turbidimeter cuvette immediately after coagulation, which was then placed in a 2100N Turbidmenter. This instrument constantly gave turbidity readings with time.

4.3.5 Micro-Resp and Post Exposure Recovery Tests.

The MicroResp™ and Post-Exposure Recovery experiments conducted followed the protocol described in section 3.3.10, with the exception that propylene glycol was used instead of glucose and that the samples tested were either fixed concentrations of metalworking fluid with varying concentrations of biocides, coagulant solutions, or samples of metalworking fluid before and after the coagulation/coalescence procedure.

4.3.6 HPLC Detection of Biocides

Biocides OPP, NaOPP, BIT and IPBC were detected using an Agilent 1120 Compact HPLC equipped with a UV-Detector. Acetonitrile with 0.2% acetic acid was used as the eluent. The flowrate of the mobile phase was 1 mL/min, and the samples were detected at 210nm. The HPLC was equipped with a C18 Agilent Eclipse Plus column. Samples of NaOPP and OPP were diluted 10-fold before 2.5 µL was injected. Samples containing BIT had an injection volume of 1µL while samples containing IPBC had an injection volume of 20 µL. Concentrations in the samples were determined using calibration curves with R^2 values greater than 0.98.

For the detection of propylene glycol and of diisoproponolamine, samples were derivitized before 5µL was injected into the HPLC system (with the same settings as above).

A method for HPLC sample derivitization developed by Sinjewel et al. (Sinjewel et al. 2007) was modified and applied to samples with PG and DIPA.

Briefly, 100 µL of sample was added to an Eppendorf tube, together with 100 µL of ethylene glycol (which served as the internal standard). To this tube, 250 µL of a 300g/L NaOH solution was added with 15 µL Benzoyl Chloride. The tube was then

vigourously shaken for 15 mins using a TOC X-5 shaker. After this, 100 µL of 100g/L glycerol solution was added to terminate the derivitization reaction. The sample tube was then shaken for another 5 mins. After this, 400 uL of hexane was added to the tube, which was then shaken for another 5 mins. After shaking, the tube was centrifuged for 5 mins at 14 500 RPM. The hexane layer was extracted and dried under a stream of air. To the residue, 300 uL of Acetonitrile was added. This acetonitrile sample was injected into the HPLC for analysis.

Fatty Amide detection was conducted using the same procedure outlined in section 3.3.7.

4.3.7 Measured Parameters

TOC measurements were as described in section 3.3.5. pH measurements were done using a Jensen 3320 pH meter. Turbidity measurements were conducted using a Hach 2100 N Turbidimeter.

4.3.8 Statistical Analysis

Student's t-test for unpaired samples, and single-factor ANOVA analyses was used to test for significance at 95% confidence levels. P values less than 0.05 and 0.01 were deemed to be statistically significant.

4.4 Results

4.4.1 Critical Coagulation Concentration

The metalworking fluid chemical treatment process of coagulation and coalescence involved the addition of cationic electrolytes to destabilize the colloidal suspension of mineral oil, followed by the aggregation of the oil droplets and the subsequent oil/water separation (Hilal, Busca, Talens-Alesson, et al. 2004; Ríos et al. 1998).

For mononuclear inorganic salts, the coagulants were added to a reaction vessel (50mL tube) which was rapidly vortexed to ensure that they were able to neutralise the charge on the oil droplets and compress the double layer surrounding them (Teh et al. 2016). The coagulants moved through the diffuse layer, and penetrated into the stern layer so as to counteract the anionic surfactants adsorbed onto the oil. Both charge neutralisation and double layer compression caused a reduction in the double layer forces keeping the oil droplets in suspension, to the extent that the van der Waal forces between the droplets was strong enough to overcome the electrostatic repulsive forces, which led to coalescence. While coalescence within industrial processes occurs via both ortho-kinetic and peri-kinetic mechanisms (Teh et al. 2016; Kovalchuk & Starov 2012), only the movement by brownian motion was responsible for agglomeration in experimental protocols utilised. The separation process still occurred at an acceptable rate since sample sizes are relatively small. Coalescence led to the separation of both the oil and water into two phases.

Figure 4-1 and Figure 4-2 show that the rate of turbidity decline could be accurately approximated as a second order process (Equation 4-1) (Reddy & Fogler 1981).

$$\frac{d\tau}{dt} = -k_t * \tau^2 \quad \text{.....Equation 4-1}$$

Where k_t was the rate constant ($NTU^{-1}. s^{-1}$) and τ was the turbidity (NTU). This implied that the rate constant in this approximation could be used as parameter to estimate the critical coagulation concentration. Once this parameter remained constant, the turbidity change with time became independent of concentration, which would imply that $(\frac{d\tau}{dt})_{t\to 0}$ had become independent as well. This provided a

more quantitative and accurate estimate of the CCC as compared to the estimations obtained through visual inspection.

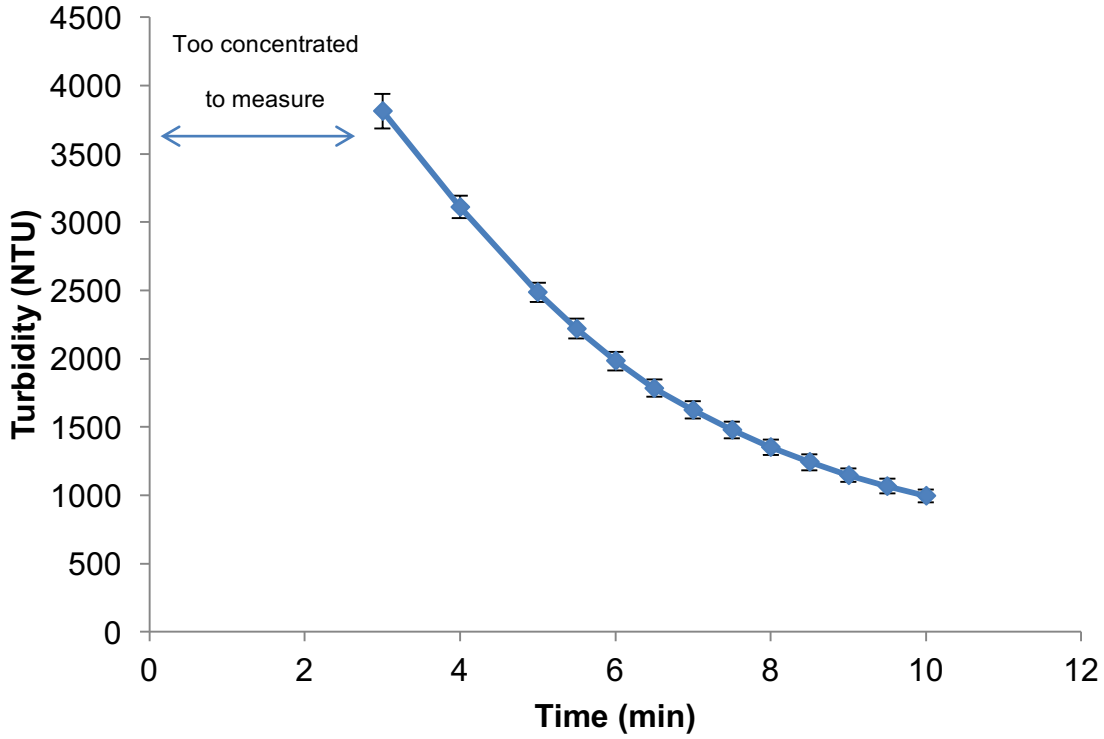

Figure 4-1: Change in turbidity of soluble metalworking fluid with time. Sample was coagulated with 17mM of $CaCl_2$. Error bars represent standard deviation of duplicate measurements.

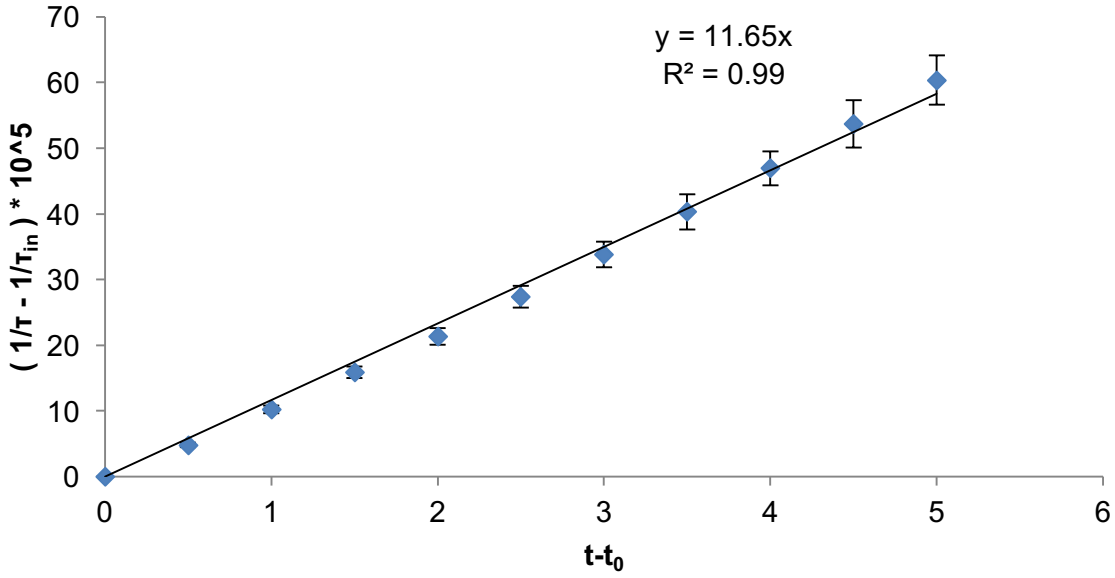

Figure 4-2: Fit of the second order rate equation to change in turbidity data. Slope of the curve is indicative of the rate constant for turbidity decline.

Figure 4-3 shows the rate of turbidity decline constant at different electrolyte concentrations of different coagulants. The intersection between the horizontal lines and the sloped lines gives an approximation for the critical coagulation concentration.

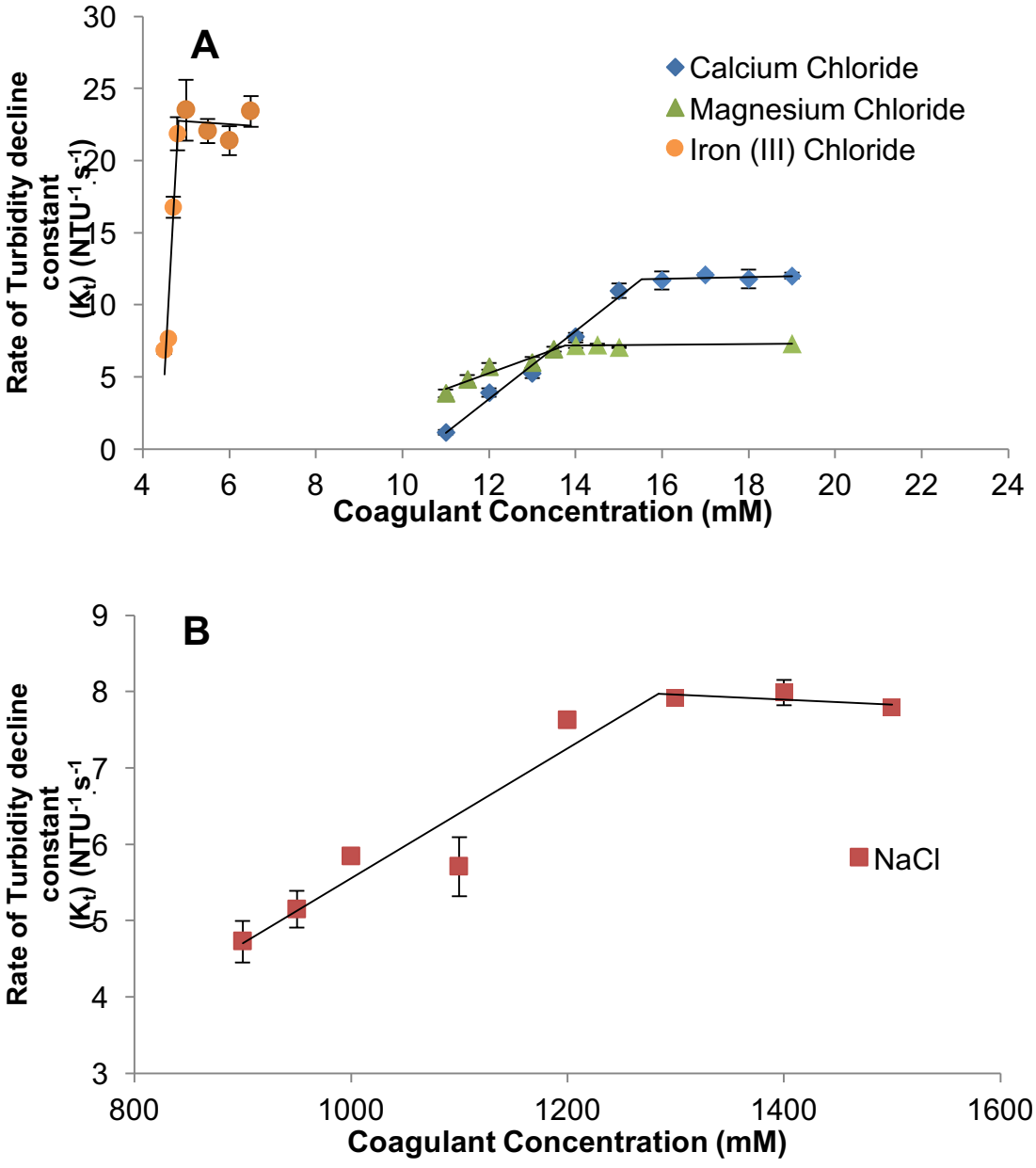

Figure 4-3: Changes in the rate of turbidity decline as a function of different coagulant concentrations. The intersections of the sloped lines with the horizontal lines represent the critical coagulation concentration for each coagulant. A) $CaCl_2$, $MgCl_2$ and $FeCl_3$. B) NaCl.

Table 4-1 shows the actual critical coagulation concentrations and the maximum K_t values attained for different coagulants used. It can be seen that as the valence coagulant cation increased, there was a large decrease its CCC value. This was in compliance with the Schulze-Hardy rule, which states that the destabilizing power of an electrolyte is primarily dependent on the valence of the counter ion (Verrall et al. 1999; Nowicki & Nowicha 1994; Trefalt 2016). The relationship between the CCC and the electrolyte concentration may be derived using DLVO theory, which states:

$$CCC_{coagulant} = \frac{K_{colloid}}{z^6} \quad \text{.....Equation 4-2}$$

Where $CCC_{coagulant}$ is the critical coagulation concentration of the coagulant, $K_{colloid}$ is a parameter that is dependent on the colloid (Busca 2004; Trefalt 2016), and z is the valence of the counter-ions in the electrolyte.

Table 4-1 shows the calculated values of $K_{colloid}$ using the experimentally determined values for the critical coagulation concentration. It can be seen that the Schulze-Hardy rule was obeyed for systems using NaCl, $MgCl_2$ and $CaCl_2$ since the $K_{colloid}$ parameter obtained in each case was roughly similar (± 20%). A similar variation in $K_{colloid}$ was given by Busca, who examined the coagulation and coalescence behaviour of a semi-synthetic metalworking fluid (Busca 2004).

Table 4-1: Critical Coagulation Concentration, Rate of Turbidity Decline Constant, Schulze-Hardy Rule Constant and pH of bulk solution for different coagulations used for the treatment of the defined metalworking fluid.

Coagulant	CCC (mM)	kt, max	K colloid	pH of supernatant after coagulation
Sodium Chloride	1281 ± 30	8.03 ± 0.002	1281	9
Magnesium Chloride	13.8 ± 0.02	7.36 ± 0.007	883	9
Calcium Chloride	15.5 ± 0.06	12.2 ± 0.054	992	9
Iron Chloride	4.83 ± 0.03	23.8 ± 1.66	3521	2.5
Aluminium Sulfate	6.10 ± 0.04	13.9 ± 0.23	4447	2.5

Furthermore, the similarity of the CCC of Ca^{2+} and of Mg^{2+}, and of Al^{3+} and Fe^{3+} provided a further indication that it was predominantly the valence that was responsible for large changes in the CCC value. However, it can be seen that $K_{colloid}$ which was obtained for the trivalent ions was vastly different than those predicted by the other salts (it is about 3.5-4 times larger). A possible reason for the deviation from the Schulze-Hardy rule was due to the change in pH that accompanied the addition of the salt. It was likely that the excess H^+ ions in the low pH solution hindered the adsorption of the trivalent ions onto the oil droplet. Similar results of increasing CCC with declining pH was observed by van den Hul and Vanderhoff, who observed that decreasing the pH of a latex A-2 colloid from 7 to 3 increased the CCC of $AlCl_3$ from 0.15 mM to 0.37 mM (van den Hul & Vanderhoff 1971). Goldberg and Glaubig also demonstrated that CCC of montmorillonite and kaolinite were pH dependent (Goldberg & Glaubig 1987). Increasing the pH to 9 resulted in the precipitation of the trivalent electrolytes. Thus, in industry, a compromise would need to be met to potentially lower the CCC for trivalent ions at the expense of producing a solid sludge within the coagulation/coalescence process.

An examination of Table 4-1 shows that for electrolytes with Cl^- as the common ion, the maximum value for the rate of turbidity decline constant ($K_{t,\,max}$) increased with increasing valence (with the exception of $MgCl_2$). This was likely due to the need of requiring less coagulant to achieve the CCC. Smaller coagulant concentrations resulted in less adsorbed entities on the oil, which led to more mobility. A possible explanation for the anomalous behaviour of $MgCl_2$ could be explained by considering the tendency of Mg^{2+} ions to form co-ordinate bonds with amine groups such as the ones present within the metalworking fluid formulation

(diisopropanolamine). The formation constant for bonding with the amine groups was found to be larger for Mg^{2+} than for Ca^{2+}. This means that within the $MgCl_2$ system (Weller et al. 2014), a large proportion of the cations responsible for destabilisation would be a part of the chelated structure containing magnesium and the alkanolamines, which are bulkier than the lone Mg^{2+} groups. It is possible that these bulky cations reduced the mobility of the oil droplets during the aggregation.

Table 4-2: Parameters of bulk fluid before and after coagulation/coalescence process. Coagulation was accomplished using 14mM of $MgCl_2$. Standard deviations are for duplicate measurements. n.d = not detectable with utilised methods. Parameters measured after 48 hours of standing time for coalescence. Standard deviations are given for triplicate measurements.

	Before Coagulation Process	After Coagulation Process
pH	9	9
TOC (mg/L)	3673 ± 126	729 ± 38.2
Turbidity (NTU)	> 4000	30.1 ± 2.4
Palmitamide-DIPA (% of Initial)	100	0 (n.d)
Oleamide-DIPA (% of Initial)	100	0 (n.d)
DIPA	100	95.2 ± 3.7
Propylene Glycol	100	94.5 ± 5.3

Table 4-2 shows the parameters of the bulk fluid before and after coagulation/coalescence using $MgCl_2$ (similar results were achieved for the other coagulants- data not shown). It can be seen that the coagulation/coalescence process was effective at removing 80.2 ± 1.0 % of the total carbon in the metalworking fluid in 48 hours. This was similar to the results obtained by Busca, who obtained a total carbon of approximately 87% in their studies with a semi-synthetic metalworking fluid (Busca 2004).

The formulation used for these studies contained a large amount of sodium sulfonate as the emulsifier, and in Chapter 3, it was shown that such formulations are more difficult to degrade biologically than those with softer emulsifiers. This

suggests that coagulation/coalescence was an effective pre-treatment technique that was able to remove components that the biological treatment process was unable to treat. Table 4-2 also shows the extent of removal of certain measurable organic constituents within the defined metalworking fluid. It can be seen that the coagulation/coalescence process was able to remove all of the detectable amounts of tall oil fatty amides (which acted as pH buffers and emulsifiers) present in the metalworking fluid, suggesting that these components had a large octanol/water partition coefficient. The coagulation process however, was ineffective at removing components such as propylene glycol (a metalworking fluid coupler) and diisopropanolamine (a pH buffer), which have a greater tendency to partition to water (GSI-Chemicals 2014; The DOW Chemical Company 2015a). Therefore, the biological process was still required in order to remove water partitioning organics from the waste. The ability that the coagulation/coalescence has for removing oil partitioning components from the metalworking fluid waste introduces the potential for it to remove oil partitioning biocides that may interfere with the biological removal of water partitioning components such as PG and DIPA. In the subsequent sections of the chapter, this potential is explored in terms of biocide removal efficiency and toxicity reduction.

4.4.2 Toxicity of Coagulants and the Coagulation/Coalescence Process

In order to accurately assess the toxicity of metalworking fluid biocides before and after the coagulation/coalescence process, it was first necessary to understand if the process had any inhibitory effect on the micro-organisms that used to treat the metalworking fluid waste. It was thus necessary to test both the toxicity of the coagulants that were used and the toxicity of the bulk fluid before and after coagulation/coalescence. Micro-Resp™ and Post-Exposure Recovery tests were

performed at CCC values of $MgCl_2$, $CaCl_2$ and $FeCl_3$. The pH of each well in the test was adjusted to 9 before the test had commenced. Since Fe^{3+} ions precipitate at this value, solids formed were removed by centrifugation. Thus, only the supernatant after Fe^{3+} precipitation was tested. Figure 4-4 shows the results for these tests. No significant inhibitory effects which impeded the micro-organisms' ability to utilise propylene glycol as a carbon source were observed ($p=0.25$). Furthermore, no significant inhibitory effects to the post-exposure growth of the micro-organisms were observed ($p=0.70$). This suggested that the presence of the coagulant within the wastewater, even after the coagulation/coalescence process has been completed, would not have any negative impacts to the succeeding bio-treatment process. Figure 4-5 also shows that there were no significant differences in both the respiration activities and post-exposure growths of the microbes in tests with and without the metalworking fluid. This implied that metalworking fluid formulation being tested had no significant inhibitory properties. This was expected since the formulations did not have any anti-microbial agents included within these tests.

The results also show that the coagulation/coalescence process did not significantly change the toxicity of the metalworking fluid, suggesting that there were no un-anticipated synergistic inhibitory effects between the remaining metalworking fluid components and the coagulant. These results are important for the next section of the chapter, as it can be ascertained that any inhibitory effects observed would have been due to the addition of anti-microbial agents, and any reduction in toxicity after the coagulation/coalescence process would have been due to the removal of that component.

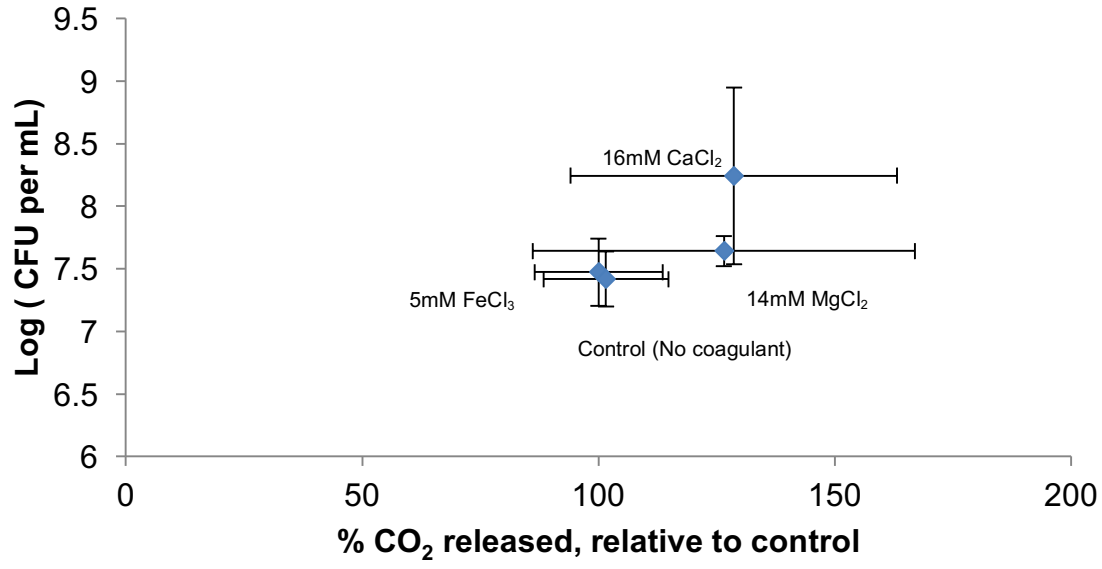

Figure 4-4: Respiration and post-exposure recovery results for $MgCl_2$, $FeCl_3$ and $CaCl_2$. Each well in the Micro-Resp® test contained 5g/L Propylene Glycol, 0.1g/L KH_2PO_4 and 0.2g/L NH_4Cl. Controls did not have any coagulant. Error bars represent standard deviation of triplicate measurement.

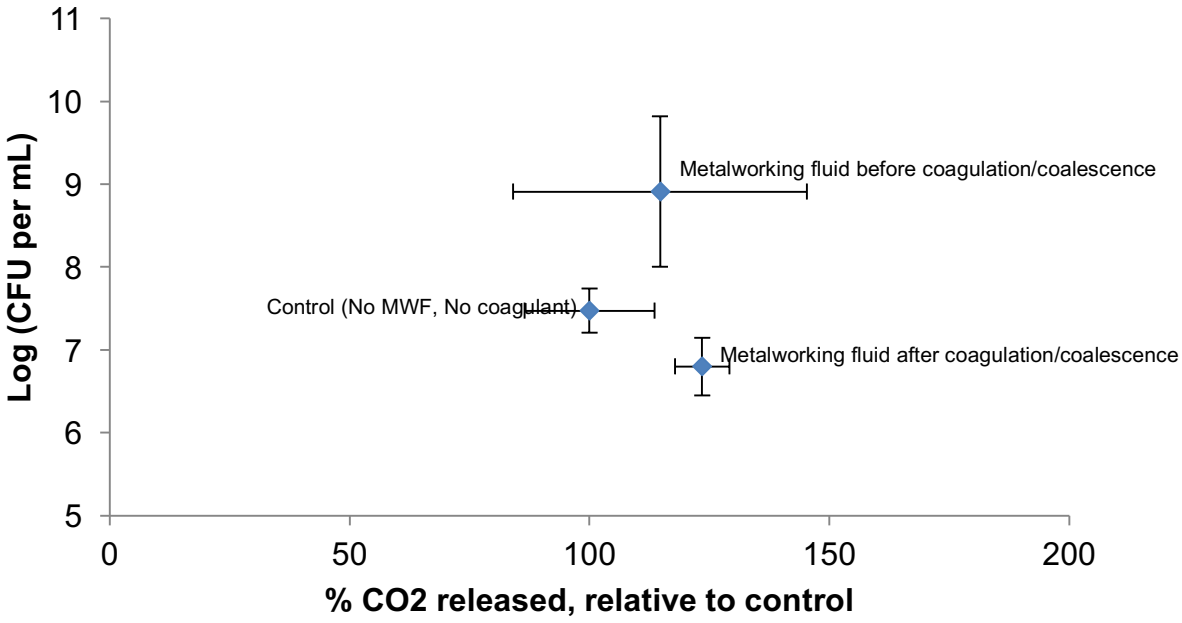

Figure 4-5: The impact of the coagulation/coalescence process on toxicity of 2.5% metalworking fluid solution. 14mM of $MgCl_2$ was used as the coagulant. Each well contained 5g/L Propylene Glycol, 0.2g/L NH_4Cl and 0.1 g/L KH_2PO_4. Controls did not have any coagulant, or metalworking fluid.

4.4.3 Biocide Removal and Toxicity Reduction

Having established that the metalworking fluid formulation did not have any inhibitory properties without the inclusion of any anti-microbial agents, the effect that coagulation/coalescence on formulations that do have such agents could then be assessed. Within this section of the chapter, the effects that coagulation/coalescence has on the removal of biocides and on the reduction of toxicity are presented.

4.4.3.1 O-Phenyl Phenate and its Sodium Salt

o-Phenyl phenate (OPP) and sodium o-phenyl phenate (sold commercially as DOWICIDE 1E and DOWICIDE A respectively) are broad-spectrum anti-microbial agents that can be added to the concentrate of the metalworking fluid (in the case of OPP) or through tank-side addition into the water emulsion (in the case of NaOPP) (The DOW Chemical Company 2003; The DOW Chemical Company 2006). OPP has a large octanol-water co-efficient, ranging from 3.09 – 3.36 (Kwok 2007) and thus it was expected that a large amount of OPP would be removed through the coagulation/coalescence process. Figure 4-6 shows the results for the coagulation and coalescence process. It can be seen that 76.0 ± 2.6% of the initial amount of OPP within the metalworking fluid formulation (1g/L or 5.9 mM) was removed from the bulk liquid through the coagulation/coalescence process. At the same molar concentration, a similar % removal was achieved for Na-OPP (72.8% ± 2.3%, p=0.181). This suggests that while Na-OPP was not soluble in the oil phase, the organic component of the salt was able to partition into the oil layer once it had been dissolved in water. Since OPP and NaOPP do have the same organic constituent, the similar removal behaviour is not surprising.

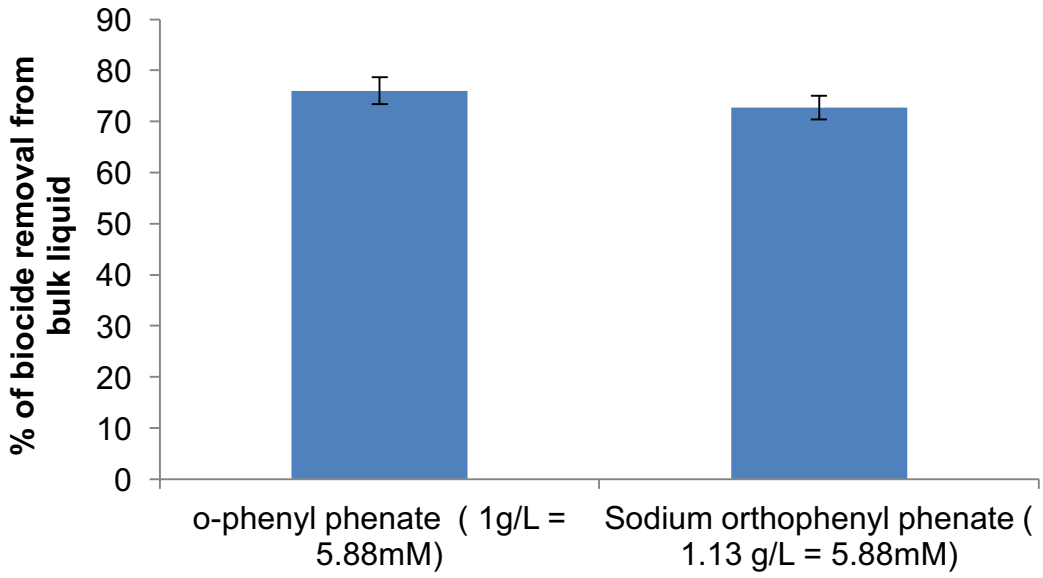

Figure 4-6: o-Phenyl phenate and sodium o-phenyl phenate removal through the coagulation/coalescence process using 15mM $MgCl_2$. Error bars represent standard deviation of triplicate measurements.

The results from Figure 4-6 provide the first indication that the coagulation/coalescence process was able to detoxify metalworking fluids. Guidance for practitioners suggested that the range of concentrations of Na-OPP used in metalworking fluids should be between 0.5 – 5 g/L.(The DOW Chemical Company 2006). Figure 4-7A shows how the removal % of Na-OPP changed with regards to the concentration present within the metalworking fluid. It can be seen that there was a statistically significant decline in the removal efficiency with increasing concentration (from 73.1% ± 1.4% at 0.5g/L to 61.1% ± 1.82% at 5g/L, $p<0.01$). This was likely due to the oil phase gradually approaching its saturation limit- as the oil became more saturated with the phenate group, the removal efficiency began to decline. It can be seen from Figure 4-7B that there was a fairly linear relationship ($R^2=0.99$) between the initial concentration of Na-OPP and the residual concentration after the coagulation process. This suggested that in the

concentration range investigated, both the oil and water phases approached saturation, but had not yet reached the limits. Also, the residual concentration and hence removal efficiency can be calculated using a partition coefficient.

Figure 4-8 shows that the removal of Na-OPP was affected by the concentration of metalworking fluid within the sample. The removal efficiency for 1g/L significantly increased with increasing metalworking fluid concentration (from 54.3% ± 0.98% at 1.0% MWF concentration, to 74.4% ± 1.24% at 2.5% MWF concentration, $p<0.01$). This was because the ratio of oil to Na-OPP increased with increasing metalworking fluid concentration. Hence, the oil phase resulting from treating concentrated metalworking fluids via the coagulation/coalescence process was larger and less saturated when compared to the resulting phase from treating dilute metalworking fluids. Thus, the coagulation/coalescence process was less effective at treating dilute metalworking fluids with relatively large amounts of biocides, when compared to concentrated metalworking fluids with smaller quantities of biocides.

Having shown that the coagulation/coalescence process was capable of removing a significant amount of OPP and Na-OPP from metalworking fluid formulations, the next point of discussion was the effect that this removal had on the toxicity reduction.

MicroResp ® and Post-Exposure Recovery tests were conducted on 2.5% MWF formulations, containing varying amounts of biocide, before and after the coagulation process. The results of these tests are presented in Figure 4-9. It can be seen that a concentration of 0.5 g/L of Na-OPP was insufficient to cause any significant inhibition to the microbial respiration activity ($p=0.73$) or to the growth of the micro-organisms ($p=0.58$) used for the test. However, concentrations at 1g/L

and higher significantly reduced the both the respiration activity ($p<0.01$) and the number of detectable CFUs within the formulation ($p<0.01$).

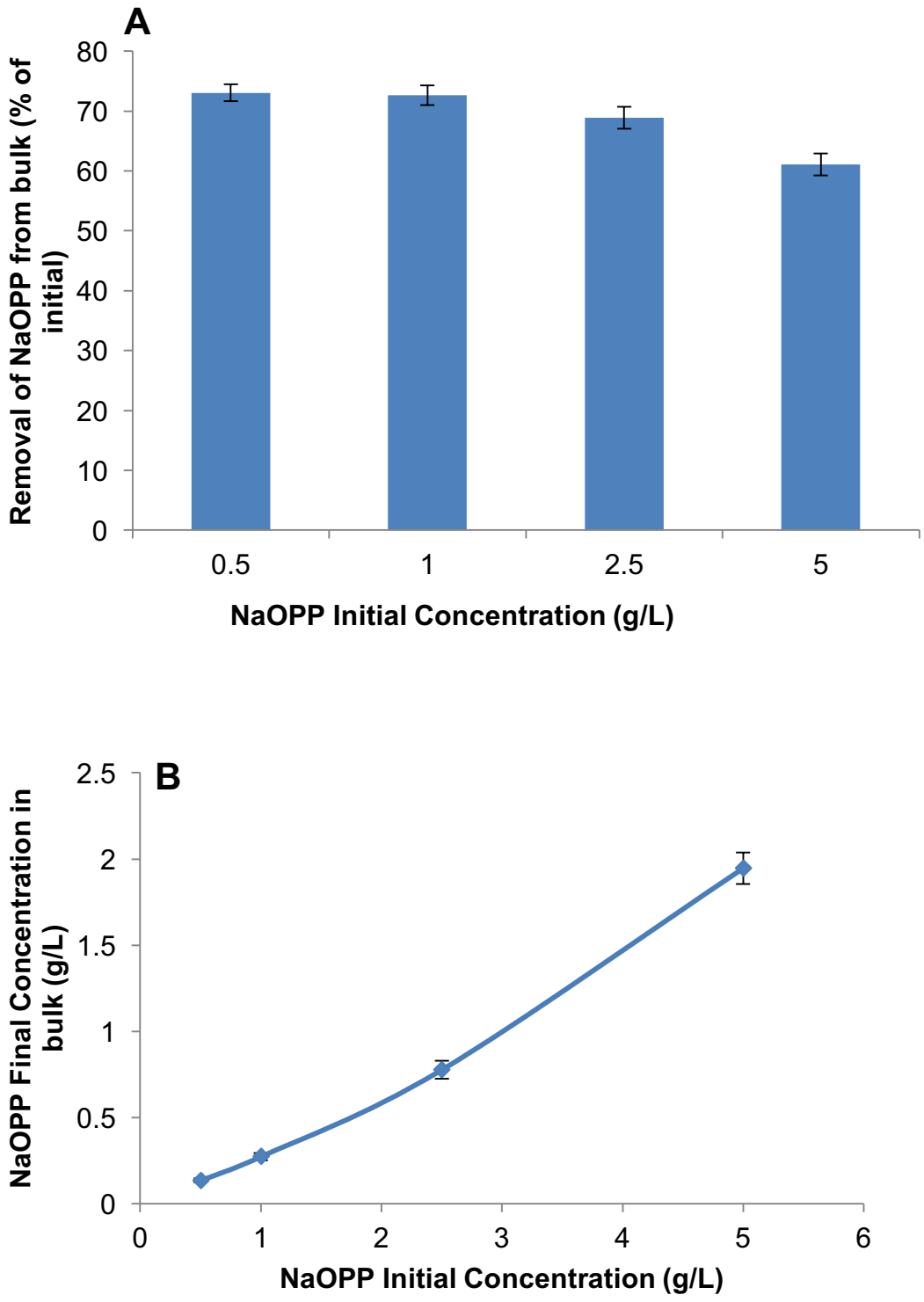

Figure 4-7: A) Removal of different concentrations of Sodium ortho-phenyl phenate from 2.5% metalworking fluid via coagulation/coalescence using 15mM $MgCl_2$. B) Residual concentration in bulk as a function of initial NaOPP concentration. Error bars represent standard deviation of triplicate measurements.

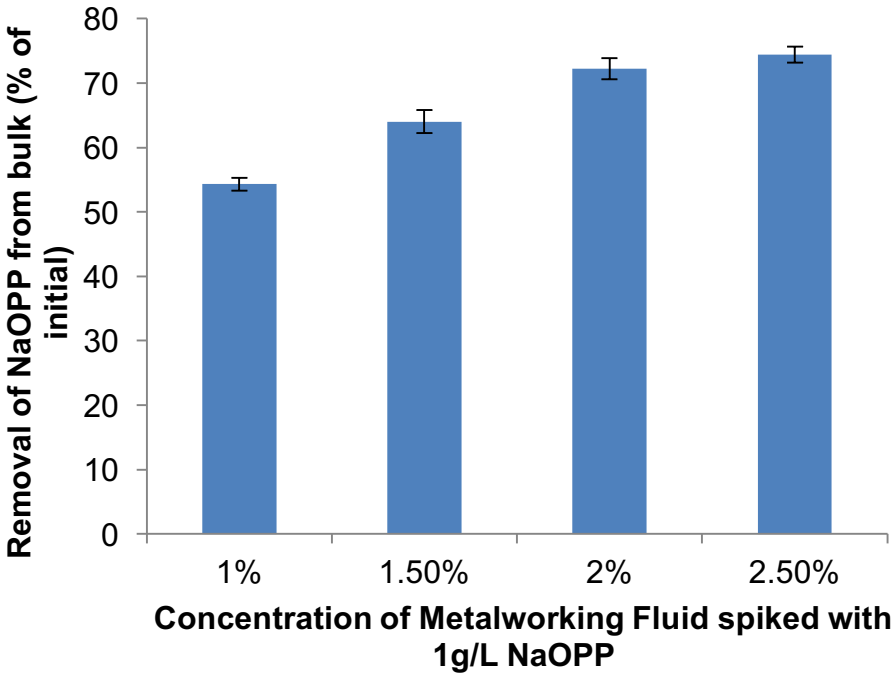

Figure 4-8: Removal percentage of 1g/L NaOPP as a function of metalworking fluid concentration. Error bars represent standard deviation of triplicate measurement.

Figure 4-9 also shows the effect that coagulation/coalescence had on improving the respiration activity and post-exposure growth of the MWF micro-organisms. There was a significant improvement in respiration activity (from a relative respiration activity value of 10.4% ± 11.9% to 110% ± 6.1%, $p < 0.01$) and the post-exposure growth (from a log[CFU] count of 4.9 ± 0.9 to 8.2 ± 0.2, $p<0.01$)) of the micro-organisms exposed to 1g/L Na-OPP. No significant effects were observed for all other concentrations tested. At 0.5 g/L, there were no significant inhibitory effects, and at 2.5g/L and 5.0 g/L, the residual inhibitory effects were still large enough to inhibit both respiration activity and post exposure growth. However, since the process removed more than 60% of the biocide, the extent of dilution that would be required to biologically treat this stream would be much less than that required

without the coagulation/coalescence process. Furthermore, the biocide and the organic constituent load to any subsequent treatment process would be reduced.

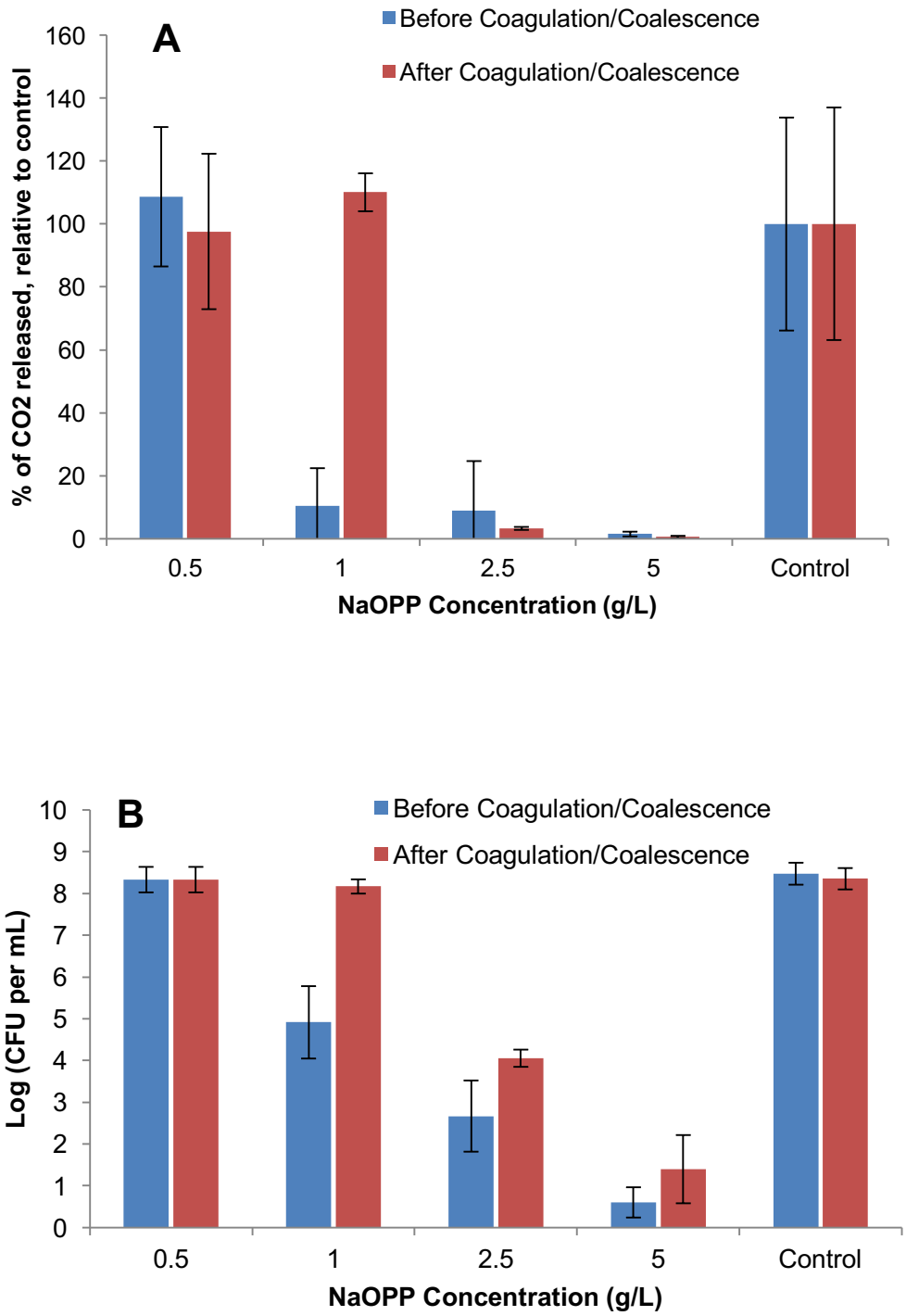

Figure 4-9: A) MicroResp ® results and B) Post-Exposure Recovery Tests on 2.5% MWF with varying concentrations of Na-OPP. Results are given for both before and after the coagulation/coalescence process using $MgCl_2$. Controls did not contain any Na-OPP. All wells contained 5g/L Propylene Glycol, 0.2g/L NH_4Cl and 0.1g/L KH_2PO_4. Error bars represent standard deviation of triplicate measurements.

The results from this section of the chapter showed that coagulation/coalescence was able to significantly reduce Na-OPP concentrations in metalworking fluids since the biocide partitions itself into the resultant oil phase. Over the recommended range of dosages tested, coagulation and coalescence improved biological responses for formulations containing 1g/L Na-OPP or less. At concentrations at or greater than 2.5 g/L, the residual concentration of Na-OPP was still inhibitory to the microbes. This suggests that a further treatment strategy would be required in order to make these formulations more amenable to downstream bio-treatments.

4.4.3.2 3-Iodo-2-Propynyl N-Butylcarbamate (IPBC)

IPBC is a fungicide that is commonly used in metalworking fluids and as a wood preservative (The DOW Chemical Company 2015b). It is provided as either a powder or as being dissolved in a non-aqueous solvent and can be either added within the concentrate formulation or on the tankside of metalworking fluid operations (DOW Microbial Control n.d.).

IPBC has a K_{OW} of 2.81 (25˚C) and limited water solubility of 168 mg/L at 20 ˚C (pH 7) (The European Commission-Denmark 2013), and thus the coagulation/coalescence process is expected to be effective at treating metalworking formulations which contain this anti-microbial agent.

Guidelines for dosage and formulations within metalworking fluids recommended that a maximum concentration of 1000 mg/L be maintained within the final diluted product(The DOW Chemical Company 2015b; Juergensen et al. 2000). Figure 4-10A shows the removal of IPBC by coagulation/coalescence as a function of its concentration. In contrast to the trend that was observed for NaOPP in Figure 4-7A, the extent of IPBC removal significantly increased with increasing concentration

(From 53.1% ± 2.7% for 250 mg/L IPBC to 74.6% ± 2.3% for 1000 mg/L IPBC, $p<0.01$).

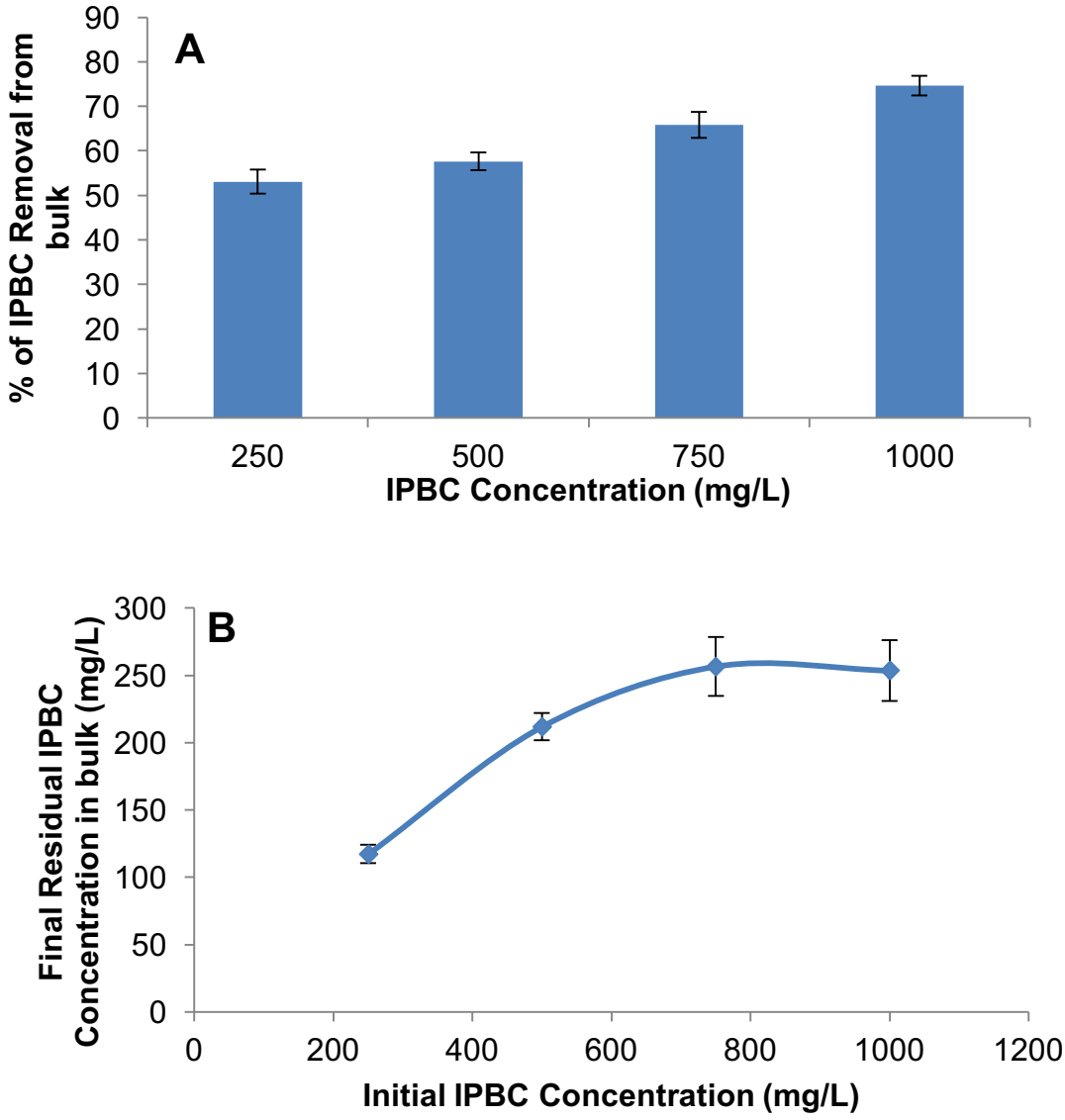

Figure 4-10: Removal of different concentrations of 3-Iodo-2-Propynyl N-Butacarbamate from 2.5% metalworking fluid via coagulation/coalescence using 15mM $MgCl_2$. B) Residual concentration in bulk as a function of initial IPBC concentration. Error bars represent standard deviation of triplicate measurements.

An examination of Figure 4-10B shows that the residual concentration in the bulk solution plateaued to a saturation concentration (it was observed that this was larger than that quoted in literature, possibly due to the experiments being conducted at pH 9). Visual observations of the test tube in which the coagulation/coalescence

process was conducted showed that precipitation had begun to occur. Thus, coagulation/coalescence can be an effective process at removing biocides with limited solubility in the aqueous phase. This is because destabilisation and oil/water separation would either result in the biocides accumulating in the oil phase, or precipitating out of the aqueous phase. Considerations would need to be taken to ensure that the precipitates are removed from the water before further processing. These results imply that dilution should not precede coagulation/coalescence as this will lower the amount of biocide that is removed through the coagulation process.

Figure 4-11 shows the MicroResp® and Post-Exposure Recovery results for formulations containing varying amounts of IPBC. The results for the formulations that have been treated by the coagulation/coalescence process and those that are untreated are given. From an analysis of Figure 4-11, it can be seen that 250 mg/L of IPBC was not able to cause significant inhibition of microbial respiration activity ($p=0.78$) and post-exposure growth ($p = 0.09$) as compared to a biocide-free control. However, increasing concentrations of IPBC began to significantly reduce both the microbial activity and the post-exposure recovery growth, suggesting that higher concentrations of IPBC led to irreversible cell death.

Figure 4-11 shows that the coagulation/coalescence treatment was able to significantly improve the respirational activity and the post-exposure recovery growth for all concentrations tested (with the exception of the 250mg/L concentration, since no significant inhibition was observed).

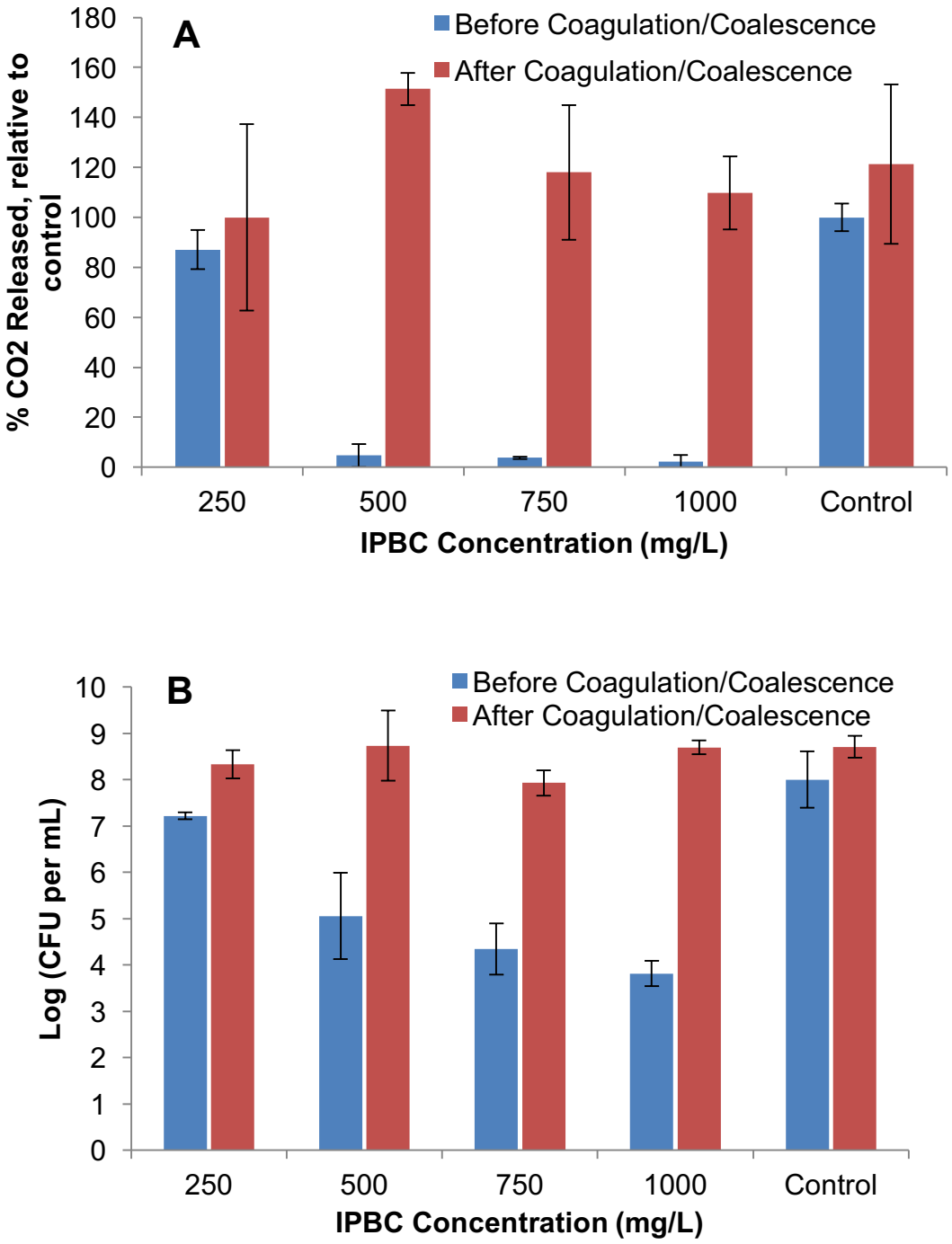

Figure 4-11 A) MicroResp ® results and B) Post-Exposure recovery tests on 2.5% MWF with varying concentrations of IPBC. Results are given for both before and after the coagulation/coalescence process using $MgCl_2$. Controls did not contain any IPBC. All wells contained 5g/L Propylene Glycol, 0.2g/L NH_4Cl and 0.1g/L KH_2PO_4. Error bars represent standard deviation of triplicate measurements.

The improvement was to the extent that there were no longer any significant differences with the biocide free control for both respiration activity (p=0.28) and for post-exposure growth (p=0.14). This is because the concentration of IPBC was

reduced to a saturation concentration within aqueous water, which was not sufficient to cause any microbial inhibition.

The results from this section showed that the coagulation/coalescence process was able to significantly reduce both the concentration of IPBC within the wastewater, and its toxic effect to micro-organisms. This was found to be true for even the maximum recommended dosage concentration. It should be noted however, that IPBC is a fungicide, and its anti-microbial effectiveness is more pronounced for fungal systems as compared to bacterial systems. Thus, the toxicity of the bulk liquid against fungal bio-treatment systems should still be assessed before coagulation/coalescence was adopted as a pre-treatment step. Nevertheless, since IPBC has a limited solubility within water, concentrations of IPBC above the saturation point was easily reduced to the saturation value through coagulation and coalescence. Since IPBC partitions itself more to the oil-phase than the aqueous phase, formulations containing concentrations near or below the saturation value may still be treated with fixed removal efficiency.

4.4.3.3 1,2-Benzisothiazol-3(2H)

Benzisothiazolinone is an anti-microbial agent which is currently used in both domestic and industrial products (Li et al. 2016). It is used within metalworking fluids as bactericide (DOW Microbial Control n.d.) and can be formulated into the concentrate, or can be added to the tank-side since it has a moderate solubility in water (about 1.1 g/L at 20˚C) (Scientific Committee on Consumer Safety 2012). Benzisothiazolinone has a low log octanol-water partition coefficient (0.4 at 20˚C), suggesting that upon oil/water separation, only a relatively small amount would partition within the oil phase. Thus, it was expected that coagulation/coalescence would not be an effective treatment for this biocide. While recommended dosages

can be as high as 3.6 g/L, previous work done by the research group at Begbroke Science Park suggests that concentrations as low as 0.5g/L was capable of causing microbial inhibition (Adapa 2016). Thus, in this study, a range close to 0.5g/L was utilised. Figure 4-12 shows the BIT removal efficiency of the coagulation/coalescence process, as well as the residual concentration of BIT remaining in the bulk after the process had been completed. It can be seen, that unlike the efficiencies obtained for Na-OPP (which ranged from 61.1% ± 1.8% to 73% ± 0.1%) and for IPBC (which ranged from 53.1% ± 2.7% to 74.6% ± 2.3%), the efficiencies obtained for BIT were substantially lower and range between 11.5% ± 0.9% and 12.3% ± 0.9%.

Moreover, there was no significant change in the removal efficiency as the concentration within the bulk was increased from 200 mg/L to 800 mg/L ($p=0.51$) This was likely due to the fact that the range that was tested was below the solubility limit for BIT in water. Since the efficiencies did not drop with increasing concentration, it was likely that the oil phase did not become saturated at the highest concentration tested. The linearity of the residual concentration in the bulk suggests that the removal mechanism was mainly due to partitioning. Since the compound was known to have a low octanol/water coefficient, the low removals obtained using coagulation/coalescence was not surprising.

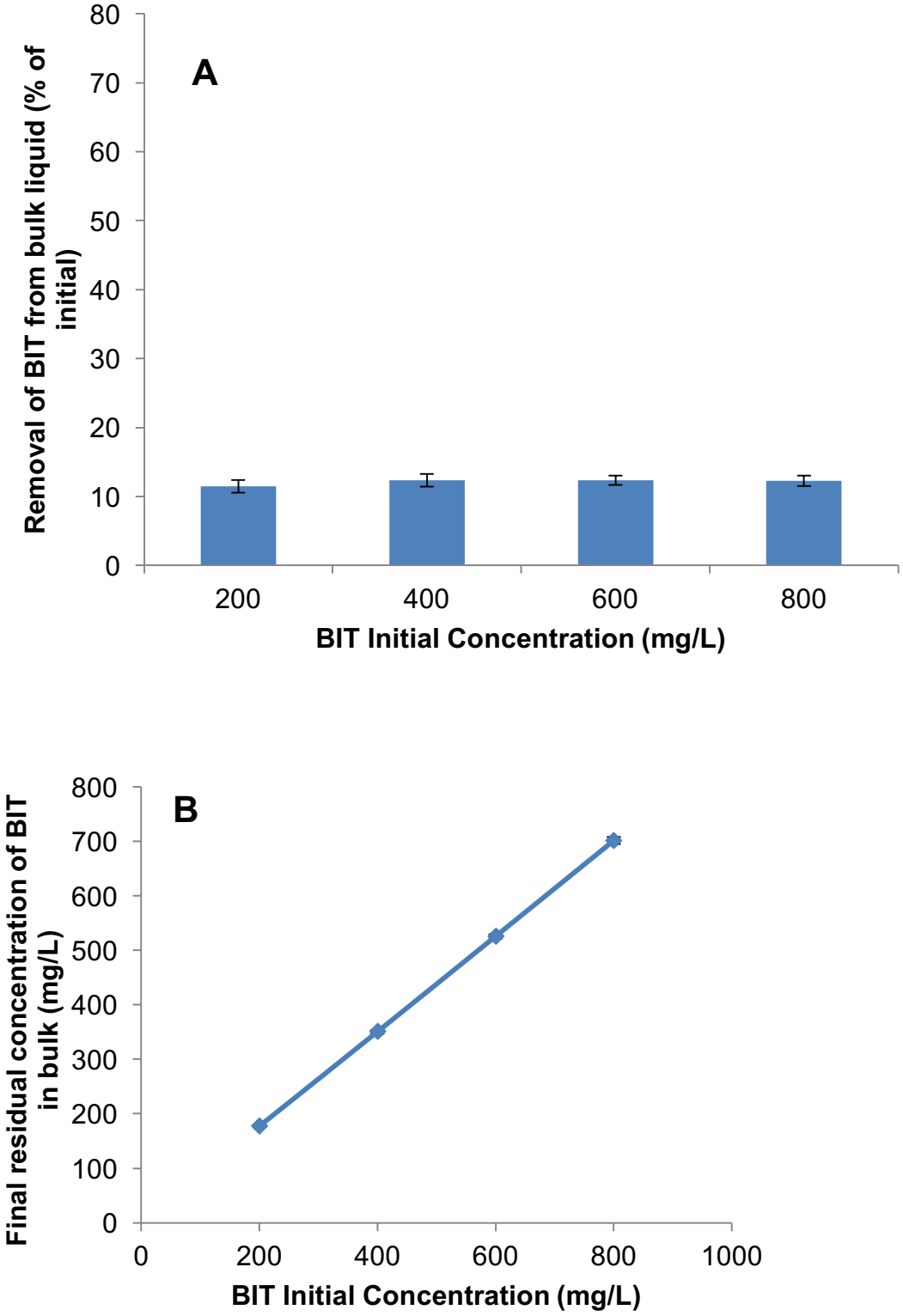

Figure 4-12: Removal of different concentrations of 1,2-Benzisothiazol-3(2H)-one (BIT) from 2.5% metalworking fluid via coagulation/coalescence using 15mM $MgCl_2$. B) Residual concentration in bulk as a function of initial BIT concentration. Error bars represent standard deviation of triplicate measurements.

Since the removal efficiencies obtained were relatively low, it was expected that the coagulation/coalescence process would have a minimal effect on toxicity reduction of metalworking fluids containing BIT in their formulation.

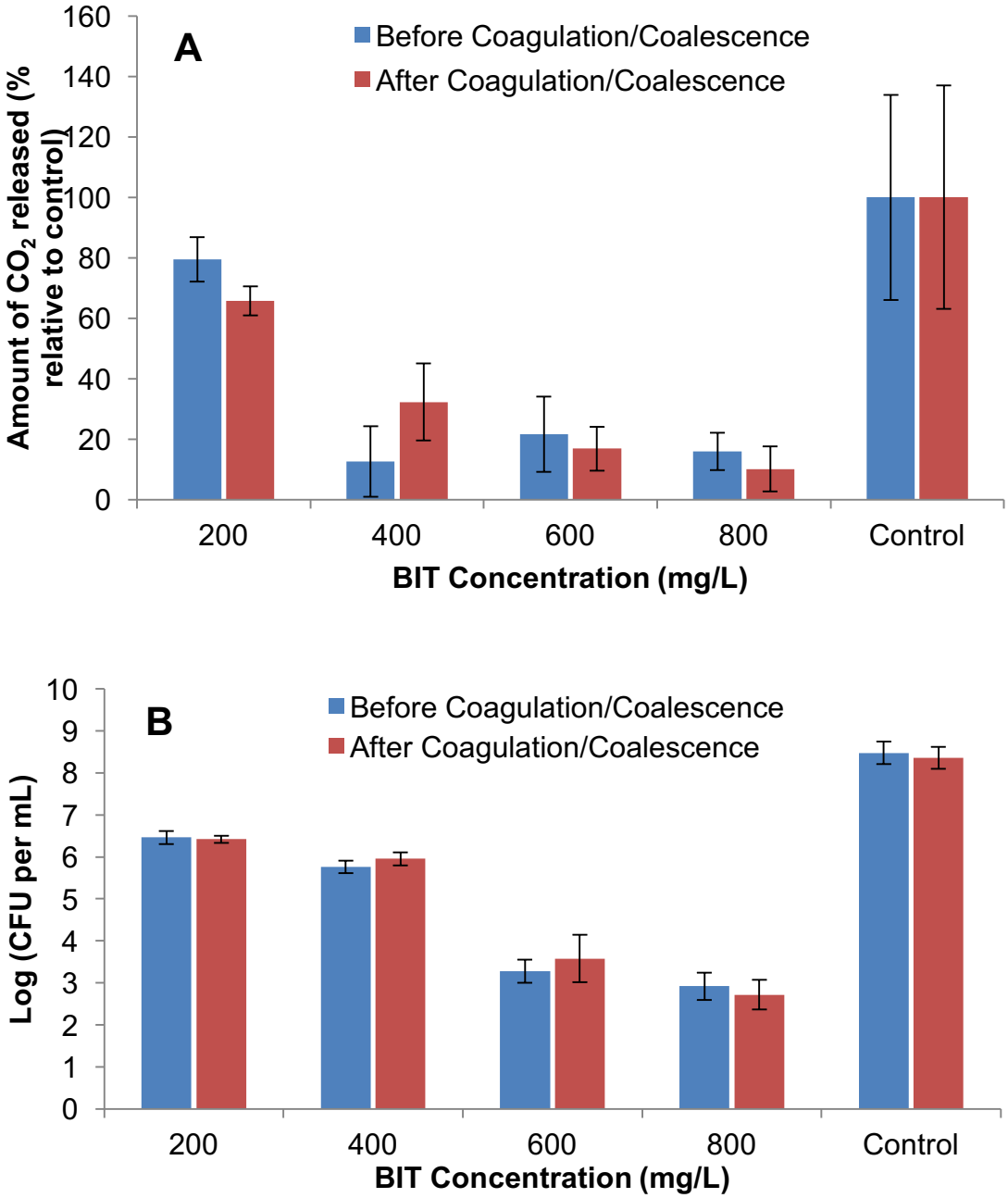

Figure 4-13: A) MicroResp ® results and B) Post-Exposure Recovery Tests on 2.5% MWF with varying concentrations of 1,2-Benzisothiazol-3(2H)-one (BIT) . Results are given for both before and after the coagulation/coalescence process using $MgCl_2$. Controls did not contain any BIT. All wells contained 5g/L Propylene Glycol, 0.2g/L NH_4Cl and 0.1g/L KH_2PO_4. Error bars represent standard deviation of triplicate measurements.

Figure 4-13 shows that BIT had a significant toxic effect on both the microbial respiration activity and the post-exposure recovery growth at concentrations as little as 400 mg/L ($p<0.05$). Treatment via coagulation/coalescence process was ineffective at inducing a significant change to relative respiration activity or to the post-exposure growth as expected.

The results in this section suggested that coagulation/coalescence was ineffective at removing the biocide BIT. This was likely due to the tendency of BIT to partition into the water phase. It should be noted that if formulations contained concentrations above the solubility of BIT in water, then higher removal efficiencies would be obtained. However, since the saturation concentration would most likely inhibit microbial processes, a further treatment step would need to be implemented.

4.5 Discussion

In this chapter, it is shown that coagulation and coalescence is an effective treatment for removing large amounts of organic carbon and organic constituents within the metalworking fluid. While the coagulation/coalescence process removed oil-partitioning components such as fatty amides, water partitioning components such as propylene glycol and diisopropanolamine were not removed. It would thus be advantageous to couple coagulation/coalescence with a downstream biological treatment step to remove biodegradable water soluble components such as these.

Furthermore, it is shown that the presence of $CaCl_2$ and $MgCl_2$ at concentrations equal to the critical coagulation concentration did not inhibit microbes that could be used for the treatment of metalworking fluids. The water soluble coagulants can thus be used for a hybrid process involving coagulation and coalescence, and a subsequent bio-treatment process without the generation of inorganic sludge. If the

effluent from the microbial process is filtered to remove micro-organisms, then this effluent, which would be rich in dissolved coagulant, may be recycled to save chemical costs.

It is shown that the coagulation/coalescence process is effective at removing oil partitioning biocides, as well as those with limited water solubility. NaOPP is a biocide which exhibited high water solubility as well as a high oil partitioning tendency, and thus a removal efficiency that is relatively independent of concentration was achieved. IPBC is a biocide that had a limited solubility in the aqueous phase, and thus the removal efficiency increased in formulations that contained concentrations higher than the saturation concentration in water. Benzisothiazolinone is a biocide which had a low octanol/water partition coefficient, and thus was not adequately removed through the coagulation/coalescence process.

Since the coagulation/coalescence process is found to be able to remove biocides, it is thus able to reduce the toxicity of metalworking fluids. Toxicity within formulations containing IPBC was reduced to the extent that the effluent behaved similarly to that of formulations containing no biocides. For NaOPP, this is only true for biocide concentrations equal to or lower than 1g/L. No toxicity reduction is observed for benzisothiazolinone. This suggests that the effectiveness of coagulation/coalescence is highly dependent on the chemical nature of the biocides that are present within the formulation. From a wastewater treatment perspective, practitioners should assess whether the effluent from a coagulation/coalescence process would be inhibitory to the downstream reactors before processing it. Knowing about the biocide that is present in the wastewater is thus critical for treatment. These sentiments are shared by Cheng et al.(Cheng et al. 2005). Should

the stream prove to be inhibitory, then the down-stream process should be made more resistant to toxicity, or a further treatment process should be employed to remove these components before the biotreatment process. In Chapter 5 and Chapter 6, the potential to develop biofilms within wastewater bioreactors is explored since this is a well-established means of improving resistance.

From a regulatory perspective, formulations that contain oil-partitioning biocides are easily treated, and thus oil-partitioning biocides should be used within formulations so as to mitigate release into the environment. Since coagulation/coalescence is a common means of treating both soluble and semi-synthetic formulations, a preference to include oil partitioning or water insoluble biocides could ensure that the waste from the formulation is easily treated, which may save costs to the end-user.

There is a need to conduct research onto the potential of reclaiming biocides either from the oil phase, or from the solid residue of the coagulation/coalescence process. Reclamation may allow for recycling of biocides which would increase the sustainability of metalworking fluid usage.

4.6 Conclusions

From coagulation and coalescence experiments, the following conclusions were drawn:

- Coagulation and coalescence was able to remove 80% of the total carbon from the metalworking fluid formulations treated. The process was also able to remove oil-partitioning components such as saturated and unsaturated fatty amides. It was not effective at removing water partitioning components such as diisopropanolamine and propylene glycol

- The critical coagulation concentration for different coagulants was found to follow the Schulze-Hardy rule. However, a larger CCC value for $FeCl_3$ and $Al_2(SO_4)_3$ was obtained, possibly due to the reduction in the pH that accompanied the addition of these chemicals
- Microbes exposed to $CaCl_2$ and $MgCl_2$ at concentrations equal to their CCC values did not have their respirational activity or post-exposure growth inhibited. This suggests that the microbial activity of the bioprocess treating the water partitioning components of the metalworking fluid would be unaffected by the presence of these coagulants.
- Coagulation/Coalescence was found to be effective at OPP, NaOPP (oil-partitioning biocides) and IPBC (a low aqueous solubility biocide). For NaOPP, the removal efficiency remained fairly constant up to a concentration of 2.5 g/L suggesting that no saturation in either phase had been approached. A slight decline in efficiency was observed for 5g/L formulations, possibly due to oil/phase saturation.
- Removal efficiencies increased with IPBC concentrations that were above the saturation concentration in water. For concentrations in which the oil-phase had also become saturated, solid precipitate of the biocide was formed.
- The coagulation/coalescence process was found to be ineffective at removing benzisothiazolinone (a water partitioning biocide). Removal efficiencies were found to be constant in the range that was tested.
- Coagulation and coalescence was able to effectively reduce the bacterial toxicity of formulations containing concentrations of IPBC up to the

maximum recommended dosages, and thus this pre-treatment process is highly effective for bacterial biotreatment processes

- The coagulation and coalescence process could only effectively reduce the toxicity of formulations containing NaOPP up to a concentration of 1g/L. Formulations containing higher concentrations would still require an extent of dilution or would require further treatment before being subjected to a vulnerable bioprocess
- No reduction in toxicity was observed for formulations containing the tested concentration range of Benzisothiazolinone. Alternative toxicity mitigation strategies would need to be imposed on such formulations.

Chapter 5- Physico-chemical Factors Influencing the Development and Performance of Fixed-film Reactors Treating Semi-Synthetic Metalworking Fluids

5.1 Introduction

5.1.1 Application of Fixed-film Reactors for Treating Chemical Wastes

When considering the design of microbial reactors, the mode of growth is an important parameter that must be taken into account (Leslie et al. 2011). Microbes such as bacteria are able to grow in both a sessile, planktonic form, or in a fixed-form that is associated with a solid surface through the formation of biofilms (Leslie et al. 2011; Dunne 2002; O 'toole et al. 2000; Donlan 2002). A biofilm is defined as being a community of microbes encased in an exopolymeric matrix which is associated with a substratum (Hall-Stoodley et al. 2004). From an engineering perspective, physically fixed modes of growth within bioreactors offer the advantage of increased biomass concentration in a reactor, protection against hazardous waste, and inherent biomass retention (Nicolella et al. 2000; Ødegaard 1999; Das et al. 2012).

The biological treatment of waste metalworking fluids is a cost-effective means of remediation (Cheng et al. 2005). However, both the presence of inhibitory components, and the slow degradation kinetics associated with complex organic components hinders the effectiveness of the treatment process (Thill et al. 2016; Jagadevan et al. 2013). The application of biofilms to the treatment of hazardous wastes is a means of compensating for these disadvantages. While studies have utilised biofilms for the treatment of metalworking fluids in the past (Dong et al. 2011;

Borghei & Hosseini 2004; Li et al. 2011; Tziotzios et al. 2005), none have looked into the conditions that are required to develop biofilms using the metalworking fluid waste itself. Insights into the optimum conditions for the physico-chemical parameters, the nutritional factors, and the mechanisms for removal of fixed-film reactors will help practitioners to quickly develop microbial biofilm reactors within metalworking fluid wastes. This could lead to reduced start-up times and reduced recovery times in the event of an inhibitory shock load. In this Chapter, and in Chapter 6, both physico-chemical and nutritional factors influencing the growth of metalworking fluid acclimated biofilms will be provided.

5.1.2 Known Physiological Influences on Biofilm Development

Within this chapter, the effects of physiological parameters such as temperature, pH and air flow-rate on biofilm development and reactor are investigated. Physiological parameters are able to influence nutrient uptake rates, attachment rates, as well EPS production rates of micro-organisms and can thus influence biofilm formation(Garrett et al. 2008). Temperature can influence the rate at which micro-organisms are able to utilise carbonaceous matter within wastewaters (Deepak et al. 1994; Cheng et al. 2006), and hence can have a direct impact on the rate at which biofilms are cultivated. Temperature can also alter the hydrophobicity of cells, and can thus have an impact on attachment rates (Di Bonaventura et al. 2008; Donlan 2002). Temperature can also have significant impacts on the number of flagella that the micro-organisms possess and the physical properties of the EPS, both of which lead to altered attachment rates (Garrett et al. 2008). Both temperature and pH have a direct impact on enzyme activity, which means there exists optimum values for bioreactor growth and performance (Hoštacká et al. 2010; Di Bonaventura et al. 2007). Finally, continuous airflow serves to provide oxygen

and mixing needed for reactor functioning. Airflow may have both positive and negative effects on biofilm formation and is thus a parameter that would need to be optimized in order to facilitate biofilm growth (Liu & Tay 2002; Choi & Morgenroth 2003). Increasing the airflow reduces the hydrodynamic boundary layer at the surface of the substratum, making it easier for the cells to attach to the surface. However, increasing airflow by too much will result in substantial shear stress, which may lead to detachment (Donlan 2002).

One of the aims of this chapter is to investigate the optimum physico-chemical parameters that are required for biofilm growth. A secondary aim of the chapter is to investigate the impact that these parameters would have on carbon and organic component removal within the bioreactors.

5.2 Aims and Objectives

This chapter addresses the following research aim:

- To use a micro-cosmic system to study physico-chemical factors influencing the development and performance of metalworking fluid biofilm reactors

The objectives to achieve this aim are as follows:

1- To determine the influence of temperature, pH and airflow on the development of biofilms within metalworking fluid fixed-film reactors
2- To determine the effects of temperature, pH and airflow on the carbon removal performance of the fixed-film reactors

5.3 Materials and Methods

5.3.1 Micro-organisms

The micro-organisms used for the experiments in this chapter were the indigenous consortium described in section 3.3.3. The inoculum procedure used was the same as that descried in section 3.3.3, with the exception of using an artificial metalworking fluid formulation in place of CoolEdge BI.

5.3.2 Metalworking Fluid

An artificial, semi-synthetic concentrate was developed and used for all of the experimental work in this chapter. The formulation used was modified from that given by Childers (Childers 2006). In previous chapters, soluble oil was used. The decision to use a semi-synthetic formulation for growth stemmed from the fact that it was mainly the additives (i.e emulsifiers, couplers etc), and not mineral oil, that was utilised by the micro-organisms for growth. Semi-synthetic formulations contain greater concentrations of additives as compared to soluble oils.

An artificial formulation was developed since metalworking fluid wastewaters and commercial concentrates usually contain a mixture of organics which are difficult to identify and characterise. By developing an artificial proxy, observed trends can be investigated in further detail. To make the semi-synthetic concentrate, 13.5 g of Naphthenic Mineral Oil (base oil), 4.5 g of Sodium Sulfonate (emulsifier), 10g of Tall Oil Fatty Amide (emulsifier), and 1.8g of Diethylene Glycol Butyl Ether (coupler) was mixed together. 28g of this mixture was added to 75g of water to create the final semi-synthetic concentrate. All experiments described in this chapter use a 2.5% w/w metalworking fluid concentration made by diluting this metalworking fluid using

an artificial tap water (the recipe for which is described in section 3.3.2). Upon dilution, a transparent emulsion was formed. The chosen components are common ingredients that are found within metalworking fluid formulations (Childers 2006).

5.3.3 Reagents

Analytical grade inorganic salts ($CaSO_4.2H_2O$, $MgSO_4.7H_2O$, $NaNO_3$, NaCl, $FeSO_4.7H_2O$, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$) Triton X-100, acetonitrile (99.9% purity), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and acetic acid were purchased from Sigma Aldrich.

Samples of naphthenic mineral oil were provided by Nynas Base Oils. Samples of sodium sulfonate were provided by Sonneborn. Tall oil fatty amides were provided by Colonial Chemical.

5.3.4 Bioreactor System

A schematic for the bioreactor system is provided in Figure 5-1 and a photo of the set-up is provided in Figure 5-2 B. Briefly, air was passed through a 0.22 micron syringe filter to ensure sterility and through a humidifier made from a 50 mL centrifuge tube, before being bubbled into a 100 mL bioreactor (100 mL Duran bottles). The air was humidified so that evaporation was negligible in the course of the experiment. The air was pumped at 0.5 L/min. The reactor was submerged in a water batch kept at 27 ˚C. Biofilms were grown on a 3cm x 5cm polypropylene matrix which is commercially sold as BioBlok 300. A picture of the matrix is given in Figure 5-2A.

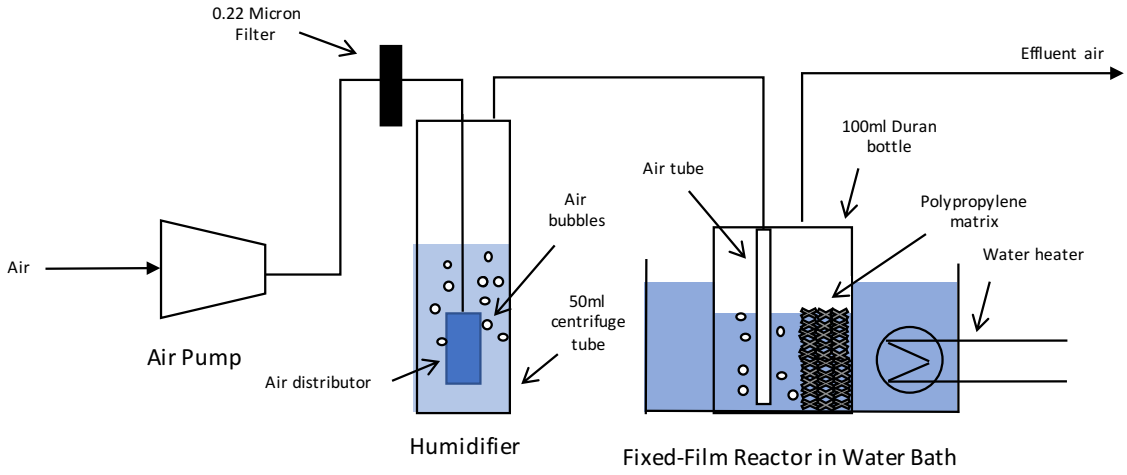

Figure 5-1: Schematic of bioreactor set-up.

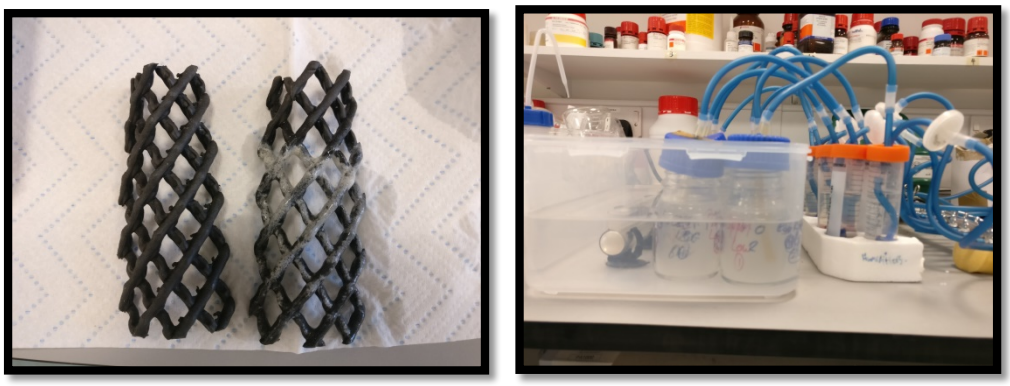

Figure 5-2: Left- Polypropylene Matrix before and after growth. Right- Photo of experimental set-up.

5.3.5 Bioreactor Operation

In order to achieve a significantly measurable amount of biomass, bioreactors were operated as sequential batch reactors. Biofilm growth was encouraged by limiting the amount of ammonium chloride (which is the nitrogen source) to 10 mg/L within this metalworking fluid growth medium. The medium was stimulated with phosphates, by adding 100mg/L KH_2PO_4.The sequential batch biofilm reactors were operated for 2 cycles. The first cycle lasted for 4 days, and the second cycle lasted for 3 days (since nutrients are consumed faster in the second cycle). After the first cycle, the matrix which held the biofilms was immediately transferred to a

reactor containing new media (containing the same composition and physico-chemical settings as the first cycle). This simulated the act of drawing and refilling a sequential batch biofilm reactor, but ensured that residual fluid from the first cycle was not transferred to the second one.

At the end of each cycle, the total carbon was measured, and the total carbon removal efficiency of the bioreactor was calculated using the following equation:

$$TC\ removal\ efficiency\ (\%) = \left(1 - \frac{TC_{cyc1} + TC_{cyc2}}{2 * TC_{initial}}\right) * 100 \quad \text{Equation 5-1}$$

TC_{cyc1} and TC_{cyc2} are the total carbon concentrations at the end of the first and second batch cycles respectively. TC_{in} is the initial total carbon concentration of the artificial metalworking fluid used for the experiments (4600 mg/L).

Unless otherwise specified, the conditions for both cycles in operation involved 2.5 wt% of semi-synthetic metalworking fluid diluted in artificial tap water. The temperature was kept constant at 27℃, the starting pH was 8 and the airflow was 0.5 L/min.

5.3.6 Biofilm Dry Weight and Biofilm Yield

Biofilm dry weight measurements were taken at the end of the second cycle of bioreactor operation. To measure the weight of the biofilm attached to the substratum, the matrix was taken out of the reactor, and placed within a 50 mL centrifuge tube. The matrix was gently rinsed with deionised (DI) water twice, and then the biofilm was dislodged into 20 mL of PBS solution through a combination of vigorous shaking and vortexing using a Vortex Genie 2. 0.2mL of a 10% Triton X-100 solution was added to the centrifuge tube to re-emulsify the oils that had dislodged from the biofilm and the matrix. The tube containing the dislodged biofilm was centrifuged at 4100 RPM for 15 minutes, which resulted in a distinct biomass

layer floating on the top of the tube, and a cell pellet at the bottom of the tube. The cell pellet was found to be of a negligible mass as compared to the floating biomass, and thus only the floating biomass was considered in dry-weight determinations. To determine the mass of the floating layer, the supernatant of the tube, together with the floating biomass, were passed through a piece of Whatman filter paper (Qualitative Number 1). The filter paper was dried overnight at 60 ˚C and the difference in its weight before and after the biomass addition was taken as the dry-weight of the biofilm.

The biofilm yield can be calculated using the total carbon (TC) removed from the bioreactor over both cycles of operation, and the biofilm dry weight obtained:

$$Biofilm\ Yield\ \left(\frac{mg}{mg\ TOC\ removed}\right) = \frac{Dry\ weight}{(2*TC_{in}-TC_{cyc1}-TC_{cyc2})*0.1} \quad \text{.....Equation 5-2}$$

TC_{cyc1} and TC_{cyc2} are the total carbon concentrations at the end of the first and second batch cycles respectively. $TC_{initial}$ is the initial total carbon concentration of the artificial metalworking fluid used for the experiments (4600 mg/L).

5.3.7 TOC Analyses

TOC analyses are as described in section 3.3.5.

5.3.8 Statistical Analysis

Single-factor ANOVA was used to test for significance of a response as a function of a varied parameter. The 2-tail student t-test with equal variance was used to test for significance between two individual responses. Tests were deemed to be significant for results were $p<0.05$.

5.4 Results

5.4.1 Effects of Temperature

Figure 5-3 shows the effect of temperature on the development of biofilms and on the total carbon removal performance within the biofilm reactors. It can be seen that the increases in temperature from 20℃ to 30℃ constantly resulted in increases in carbon removal performance, from 45.5% ± 0.7% to 80.6% ± 1.0%. The increase in the extent of carbon removal may have been due to the increase in metabolic activity as a result of achieving more optimum conditions for enzyme functions (Richard & Walker 2006). Similar results were obtained by Deepak et al. who investigated the kinetics of chemical oxygen demand removal as a function of temperature using a soluble oil metalworking fluid and found that increasing the temperature increased the rate of removal (Deepak et al. 1994). Cheng et al. also showed that temperature had a significant effect on the treatment of synthetic metalworking fluids (Cheng et al. 2006).

It can be seen that there were no significant differences in the dry-weight of biofilm achieved in culture conditions ranging between 20 to 27 ℃ ($p = 0.528$) but a significantly higher biofilm dry weight was achieved at 30℃. This could be due to a number of reasons, such as the alteration of the cell surface (Di Bonaventura et al. 2008) or the alteration of the EPS structure and binding properties(Garrett et al. 2008). Results of temperature having a significant impact on biofilm development have been reported in previous studies (Hoštacká et al. 2010; Piao et al. 2006; Stepanović et al. 2003; Donlan 2002). As can be seen from Figure 5-4, there was a sudden and significant increase in the yield of biofilm obtained at 30℃. This suggests that biofilm formation within reactors was controlled using temperature. If

the objective was to maintain suspended growth and limit biofilm formation, then lower temperatures with higher hydraulic retention times would be favoured. If the objective, as was in this work, was to produce large biofilm yields, then higher temperatures are preferred.

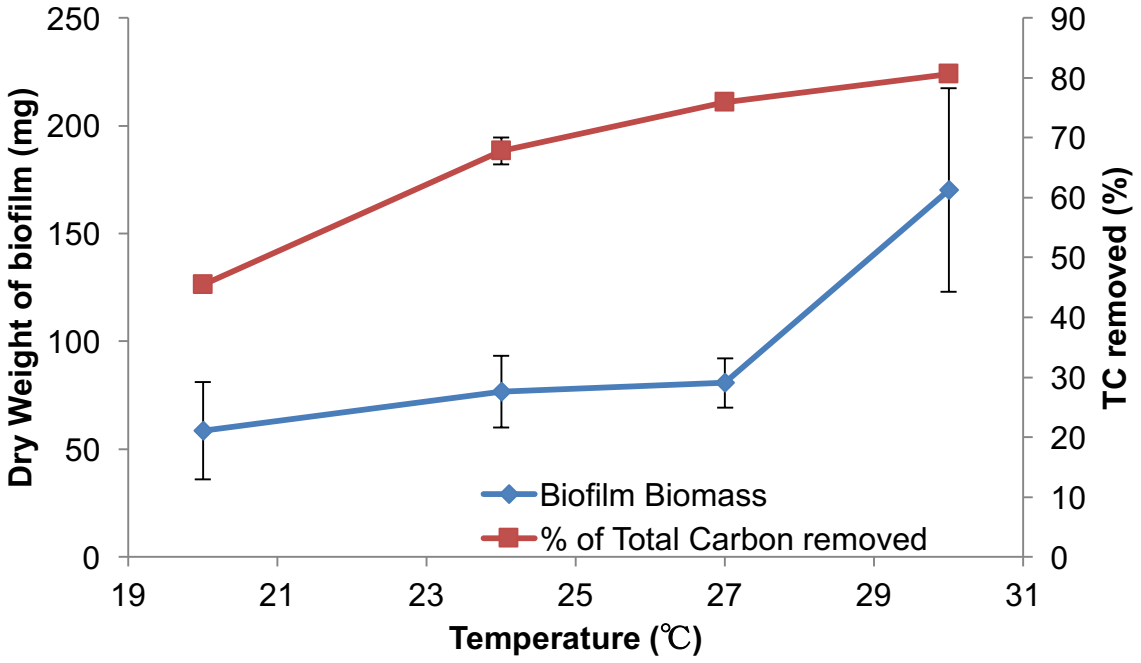

Figure 5-3: Biofilm development and total carbon removal performance in biofilm reactor operating over two 4 day cycles. Error bars represent standard deviations of triplicate measurements.

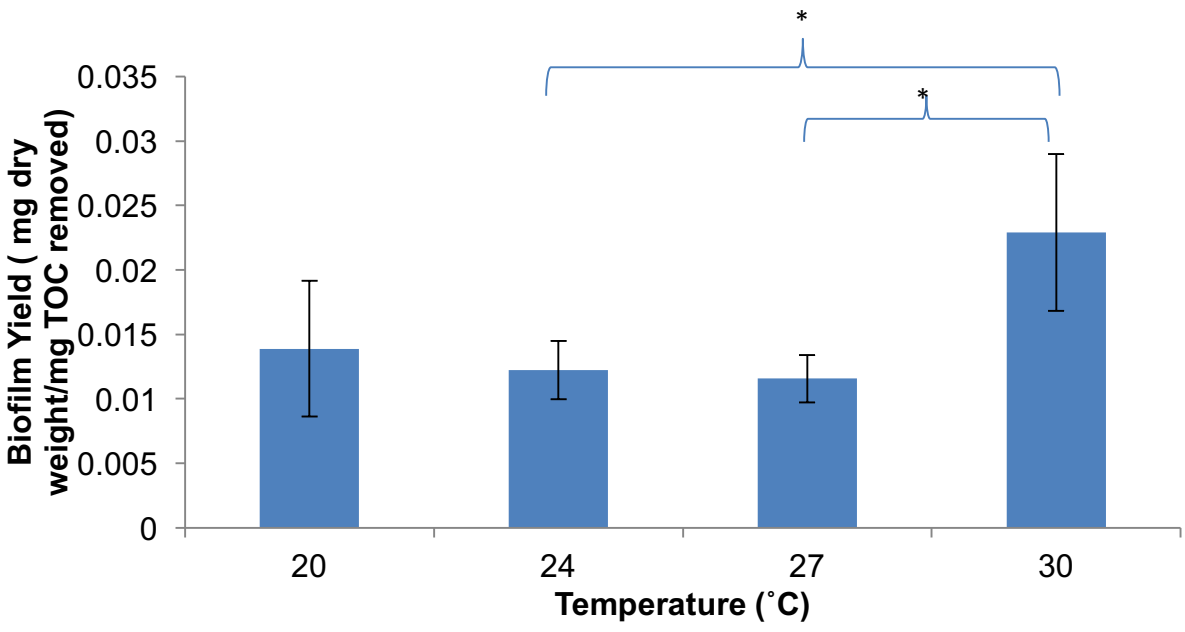

Figure 5-4: Biofilm yield as a function of temperature. Error bars are represented as standard deviations of triplicate measurements. * indications represent significant differences where p < 0.05.

5.4.2 Effects of pH

Another physicochemical parameter that may influence biofilm formation is the pH of the bulk fluid.

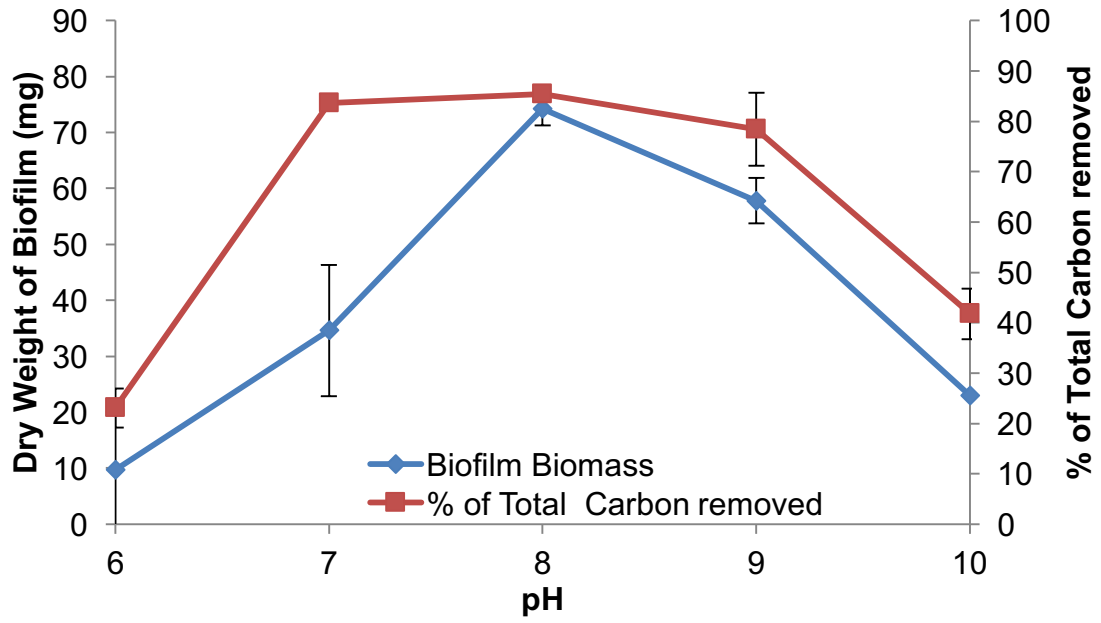

Figure 5-5: Biofilm biomass and reactor performance as a function of the starting pH of the bulk liquid. Error bars represent standard deviations of triplicate measurements.

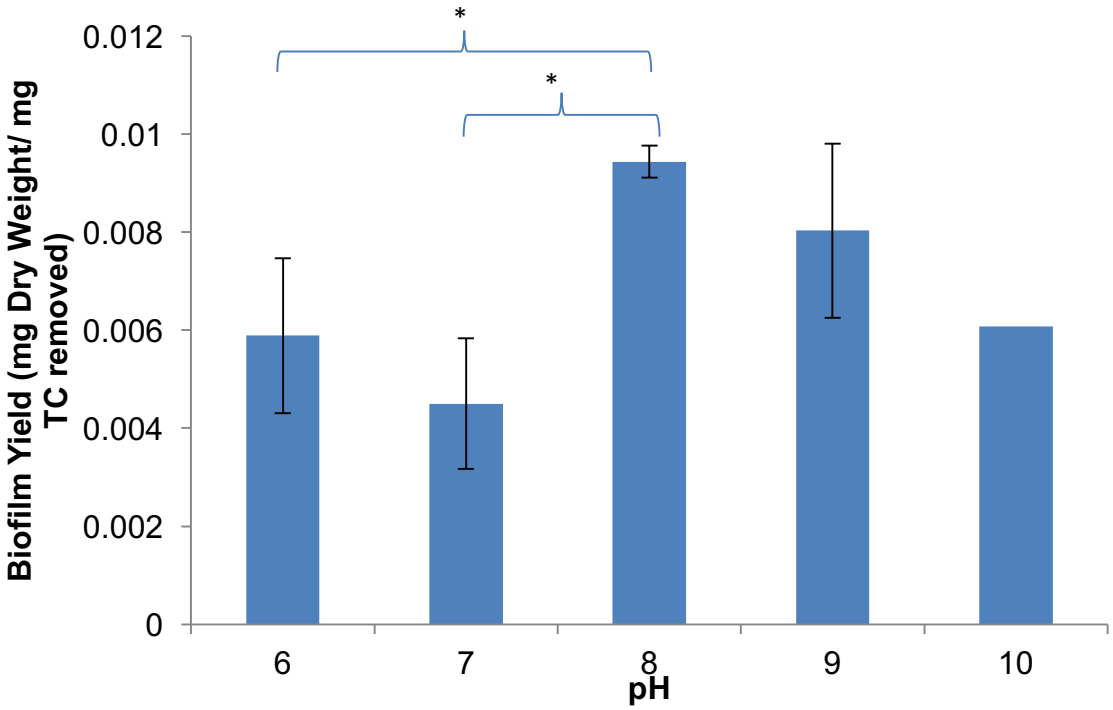

Figure 5-6: Biofilm yield as a function of pH. Error bars represent standard deviations to triplicate measurements. * indicates significant differences where p<0.05

Figure 5-5 shows the effect of pH and biofilm formation and reactor performance during the treatment of the artificial metalworking fluid waste. Total carbon removal performance was found to peak at 85.4% at a pH value of 8. This removal efficiency did not significantly alter within the pH range of 7 to 9 ($p=0.07$), but significantly declined at pH 6 ($p<0.01$) and 10 ($p<0.01$). Thus, the results suggest that the microbes within the reactor were more proficient in slightly alkaline conditions, which was representative of the natural pH of metalworking fluid wastes. Deepak et al. found that activated sludge had an optimum pH range of 6-7.5 (Deepak et al. 1994)., which suggests that the indigenous community used in this study had become more adapted to the natural pH of the metalworking fluid (ranging from 8-9).

van der Gast and Thompson investigated the effect of pH on the treatment of a semi-synthetic metalworking fluid formulation (van der Gast & Thompson 2004) and found the optimum range for their process to be 6 to 7. Moreover, they observed that the pH of the reactor fluid tended towards a value of 8 as biodegradation progressed. In this study, the pH declined towards a value of 6 as biodegradation progressed (data not shown). It is important to note that van der Gast and Thompson used a defined microbial consortium as a starting community, while this work made use of an indigenous community. Thus, different responses to pH may be anticipated for different starting communities.

Biofilm formation was found to be optimum at pH 8. In comparison to 74.2 ± 11.7 mg achieved at the optimum pH, biofilm growth was significantly reduced to 34.6 ± 12.0 mg at pH 7 ($p<0.01$), even though the removal efficiency had not significantly changed. This suggested that pH may be used as a physicochemical parameter that can be used to trigger biofilm growth. It can be seen in Figure 5-6 that the yield of biofilms obtained under alkaline conditions was higher than that achieved under

neutral to slightly acidic conditions. Similar results had been found by Hoštacká et al. who found that increasing the pH of a media solution from 5.5 to 8.5 led to significant increases in the biomass of *Pseudomonas aeruginosa, Klebsiella pneumoniae and Vibrio cholerae* biofilms (Hoštacká et al. 2010), although their results were not for metalworking fluids. Results suggesting increased biofilm formation versus increasing pH were also reported in other studies (Di Bonaventura et al. 2007; Wulff et al. 2008).

The increase in biofilm production and yield at higher pH may be due to change in the physiology of the cells or due to a change in the composition of the microbial community. As an example, Harjai et al. found that the alginate production in *Pseudomonas aeruginosa* was significantly higher at pH 8 as compared to that produced at pH 6 (Harjai et al. 2005). van der Gast demonstrated that a consortium community may change when exposed to different pH values (van der Gast & Thompson 2004), and thus it may also be possible that microbes more suitable for growth in a biofilm were preferentially cultivated under slightly alkaline conditions.

pH may also affect the detachment rate of the biofilms. It was observed that as the biological process progressed, the pH of the fluid in the reactor approached a slightly acidic value of 6. It was observed that once the bioreactor pH had dropped below 6.5, large amounts of biofilm had begun to dislodge (data not shown). This suggested that maintaining the solution at an alkaline pH may be beneficial for biofilm formation. In order to test this hypothesis, biofilms were cultivated in the presence of 20mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), which is a common physiological buffer used in cell culture experiments. The results in Figure 5-7 show that the buffered system maintained the pH above 7.5, and achieved a biofilm dry weight of 120.6 ± 9.1 mg, which was significantly higher

($p<0.01$) than the 48.7 ± 10.3 mg achieved in the un-buffered system. HEPES concentration was measured before and after the bio-treatment process using the same method for the detection of fatty amides outlined in section 6.3.6. HEPES was not utilised as a carbon source during degradation as evident by the observation that its HPLC peak did not decline during the bio-treatment process (data not shown).

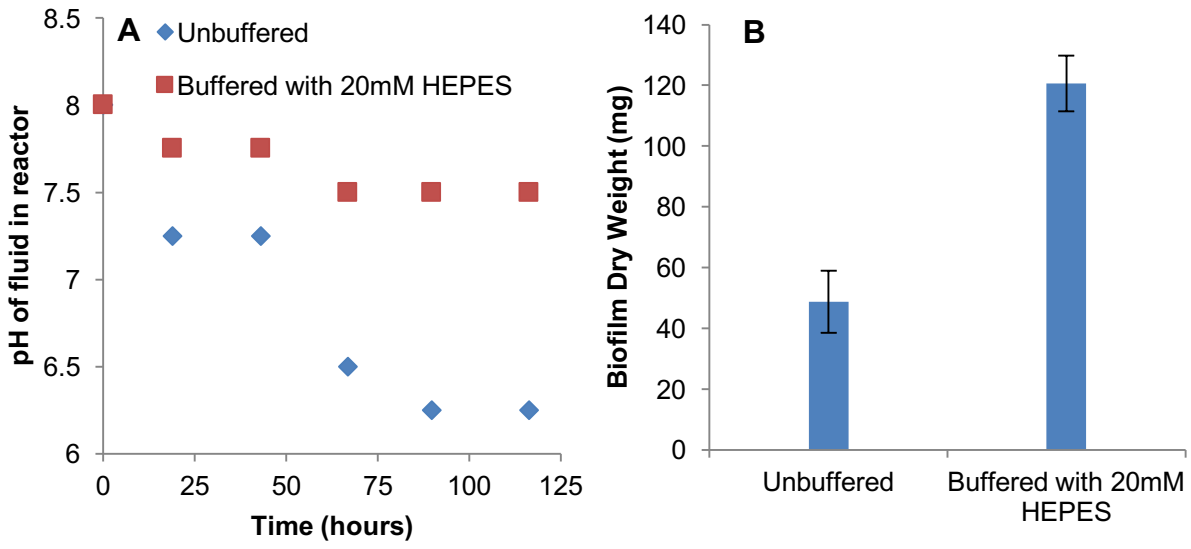

Figure 5-7: A) pH changes in the first cycle of operation in the bioreactors treating the artificial metalworking fluid (MWF) with and without 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. B) Biofilm dry-weight achieved at the end of the second cycle in the reactor treating the artificial MWF with and without HEPES buffer. Error bars represent standard deviation of triplicate measurements.

The results suggested that maintaining a slightly alkaline pH was beneficial to biofilm growth, and thus mechanisms for pH control should be considered when cultivating a fixed-film reactor.

5.4.3 The effects of air flow-rate

The continuous bubbling of air within the bioreactors serves two purposes. The first is to deliver oxygen to the micro-organisms to provide an aerobic environment to

promote degradation. The second is to provide shear and mixing to allow for effective reactor functioning. Mixing ensures that there is bulk fluid motion within the reactor and thus ensures that micro-organisms are in intimate contact with the pollutants which they are removing. In terms of biofilm growth, mixing provides a shear stress on the micro-organisms that are attached to the substratum. There are reports that shear stress can have both beneficial and detrimental effects on biofilm growth, and thus it is necessary to optimize a biofilm growth system with regards to air flow-rate (Liu & Tay 2002; Donlan 2002).

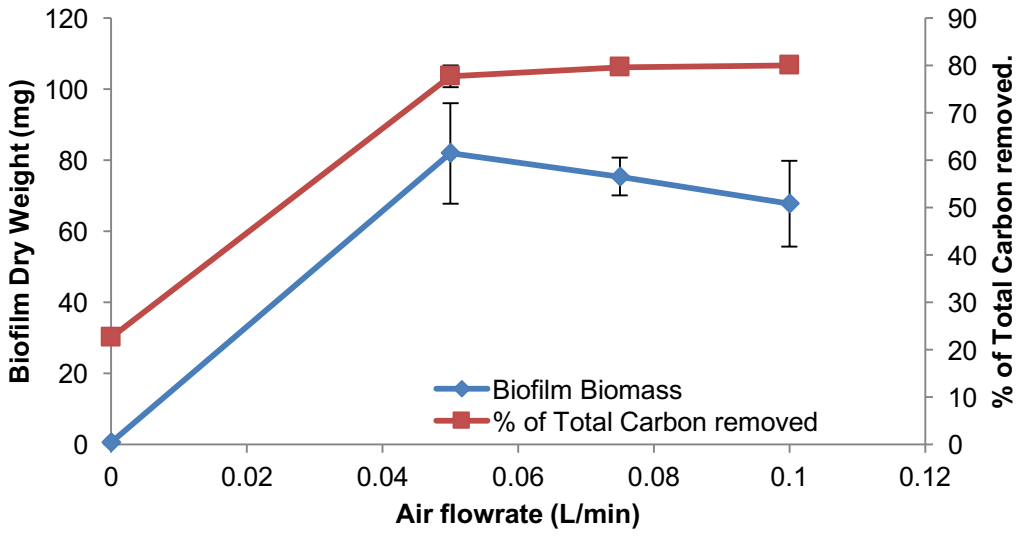

Figure 5-8: Effect of air flow-rate on biofilm development and reactor performance in bioreactors treating metalworking fluid. Error bars represent standard deviations of triplicate measurements.

Figure 5-8 shows the effect of air flow-rate on biofilm development and reactor performance. In the absence of air-flow, the reactor achieved only 22.6% ± 1.3% total carbon removal as compared to the 77.8% ± 2.3% removal when the airflow was 0.05 L/min. A declining trend for biofilm biomass as a function of air flow-rate was observed, but the change in biomass was not significant ($p=0.296$). It was likely that at higher airflow rates, the shear forces would have resulted in significant

detachment of biofilm biomass through sloughing. The results show that shear due to mixing was beneficial both to biofilm formation and reactor performance.

5.5 Discussion

In this chapter, it was shown that fixed-film reactor development and performance is significantly influenced by both the temperature and the pH of the metalworking fluid. In this work, the carbon removal efficiency within the reactors monotonically increased with increases in temperature. However, continuously increasing the temperature would eventually result in a plateau in the carbon removal efficiency. Practitioners would thus need to balance the cost of maintaining the reactors at elevated temperatures with the benefit of having an increased reaction rate. Furthermore, although not observed here, increasing the temperature of the reactor could result in significant microbial inhibition since the enzymes that are typically involved in the biodegradation of metalworking fluids may become denatured (Cheng et al. 2006). Practitioners should thus determine the maximum temperature at which the reactor performance peaks in order to run their operation.

With regards to pH, it is found that there exists an optimum range of starting pH values to achieve optimal carbon removal efficiencies within the reactors. Deviation from this range results in reduced performance, possibly due to microbial inhibition caused by the denaturing of enzymes. Waste practitioners should thus be wary that there is an optimum pH range for metalworking fluid treatment.

The results in this chapter also show that both temperature and pH have a significant effect on the biofilm yield that is obtained within the fixed-film reactor. In particular, it is shown that the biofilm yield can be significantly changed by adjusting the starting pH of the metalworking fluid, without having a significant effect on the

reactor performance. This means that the starting pH of the metalworking fluid can act as a 'switch' to control the amount of biofilm that is obtained within the reactor system. During the start-up of the system, or after the event of an inhibitory shock, practitioners may wish to switch to a pH to promote biofilm growth so as to re-commission the reactor in the shortest time possible. However, for a mature reactor, practitioners may from time to time switch to a pH value which does not promote biofilm growth so as to prevent the problems that could be associated with overgrowth (such as clogging of air-diffusers or down-stream pipelines) (Nicolella et al. 2000). Further work would need to be done to determine the fitness of the biofilm community when it is exposed to a pH value that is sub-optimum for growth.

Airflow is found to have significant impacts on biofilm formation and reactor performance. Having no airflow did not result in good reactor performance. This is expected since good performance requires good degrees of mixing and since aerobic reactors usually outperform anoxic/anaerobic ones for metalworking fluid treatment. However, practitioners should be conscious that high airflows increases the energy input of the treatment operation, and could result in detachment. Airflow should thus be controlled at a moderate value that is suitable to provide mixing.

5.6 Conclusions

In this chapter, the effects of physico-chemical effects on biofilm formation and bioreactor performance were explored. The following conclusions were made:

- Biofilm growth and reactor performance increased with increasing temperature, but there was a significant increase in the yield of biofilms at higher temperatures than at lower temperatures.

- Both biofilm yield and reactor performance is sensitive to the starting pH of the metalworking fluid. High reactor performances could be attained for a range of pH values from 7-9, biofilm growth and yields were significantly optimized at pH 8. This suggests that pH may act as a switch for changing between suspended and biofilm modes of growth without compromising reactor efficiency. Furthermore, as the pH declined in the bioreactors, biofilm detachment was observed.
- Maintaining an alkaline pH lowered the extent of detachment and thus increased yields attained.
- Increases in airflow were found to increase reactor performance and biofilm growth. A decreasing trend was observed at relatively higher airflows suggesting that physical detachment may become significant with continuous increases.

Chapter 6- Nutritional Factors Influencing the Development and Performance of Fixed-film Reactors, and Reactor Application to Biocide-containing Metalworking Fluids

6.1 Introduction

6.1.1 Nutritional Factors Influencing Biofilm Formation

The amount and the availability of essential nutrients in the environment in which micro-organisms are contained can have several types of influences on the formation of biofilms. This is because micro-organisms are able to sense the amount of nutrients that are present within the environment, and make physiological adjustments that are necessary to ensure their continued survival(O 'toole et al. 2000). As an example, research has shown that the carbon catabolite induced gene regulation can play a strong role in the formation of biofilms (Jefferson 2004). Micro-organisms such as pseudomonads, *V.chloerai* and *E.coli* express a marked increase in exopolysaccharide production in the presence of high glucose concentrations (O 'toole et al. 2000; Jefferson 2004). The hypothesis behind this is that it is done to ensure that the micro-organisms remain in a high nutrient setting, which is a rarity in the natural environment.

An alternative hypothesis is that the EPS functions as a storage component which may be utilised during periods of nutrient unavailability (Wang et al. 2006; Flemming & Wingender 2010). Since exopolymers can serve as a reserve source of carbon, it is plausible to think that the deficiency of an essential nutrient can lead to enhanced EPS production, and hence, enhanced biofilm formation. Dewanti and

Wong observed that *E.Coli* O157:H7 formed greater amounts of biofilm in low nutrient conditions than in those with higher concentrations (Dewanti & Wong , 1995). Zhang et al. demonstrated that nutrient depletion led to an increased production of EPS in *B.Subtilis* biofilms (Zhang et al. 2014). Recently, Liu et al. found that biofilms may be cultivated under nitrogen limiting conditions (Liu et al. 2015). Furthermore, nutrient concentrations may also influence the composition of the EPS formed (Hoa et al. 2003).

The carbon source that is available to micro-organisms may also influence biofilm development. Allan et al. found that lactose limitation resulted in large biofilm biomass production in their studies, but glucose limitation did not (Allan et al. 2002). Other studies have shown that the carbon source may influence the structure of the biofilms formed by micro-organisms by either influencing the shape or the swarming motility of the biofilm (Shrout et al. 2006; Stoodley et al. 1998; Klausen et al. 2003)

While there is an abundance of data on factors influencing biofilm development in water distribution systems and in domestic water treatment processes, there are no studies looking at the development of biofilms in metalworking fluid treatment systems. Since different nutrient conditions may trigger different biofilm responses, a study looking into the effects of stimulation on the development of an indigenous consortium biofilm in metalworking fluid reactors is warranted. In this study, the effects of phosphate and ammonium stimulation and limitation are presented. These compounds were chosen since they are commonly added to wastewater systems to promote growth and development of biomass, and to promote reactor performance.

Moreover, biofilm growth as a function of carbon sources commonly occurring in metalworking fluids are investigated to promote understanding as to which constituents are likely to result in biofilm proliferation.

Finally, the effects of nutrient stimulation on the reactor performance are also presented. This is because the primary function of a fixed-film reactor is the removal of carbon and organics from water, and thus it is necessary to know how the performance of the reactor is influenced with biofilm formation.

6.1.2 Application of Fixed-film Reactors for the Treatment of Metalworking Fluid with Biocides

It is shown in Chapter 4 that while the coagulation/coalescence pre-treatment was able to remove oil partitioning biocides, as well as those with limited solubility, it was found to be ineffective for water partitioning biocides. Results also showed that while Na-OPP had a substantial amount of biocide removed, the residual biocide was still in sufficient quantity to cause microbial inhibition. Having determined the influences of physico-chemical and nutritional parameters on the formation of biofilms, the next step is to show that the developed biofilm reactors are capable of treating metalworking fluids with hazardous components. In this chapter, the application of a well-developed fixed-film reactor to metalworking fluid formulations containing the varying concentrations of Na-OPP is provided. Effects of Na-OPP on the removal of the organic components within the metalworking fluids, and the microbes own ability to remove Na-OPP itself is also provided. Within literature, information regarding the biodegradation of OPP and its sodium salt are fairly limited. Within the few studies which examine biotreatment techniques, OPP is only removed when it is diluted to relatively low concentrations (approx. 150mg/L) (Perruchon et al. 2016; Wick & Gschwend 1998; Gonsior et al. 1984). Since OPP and Na-OPP may

be present in metalworking fluids at concentrations up to 5g/L (The DOW Chemical Company 2015c), vast amounts of inhibition may occur if the bioreactor is not made to be resilient. The aim of this is to show that the optimization strategies for developing a biofilm results in a reactor that is able to operate in the presence of Na-OPP.

6.2 Aims and Objectives

The research aim addressed in this chapter is:

- To study the effects of bio-stimulation on the development and performance of metalworking fluid biofilm bioreactors. Furthermore, to understand how carbon sources within the metalworking fluid may influence biofilm development.

The objectives of this chapter to address this aim are as follows:

1- To determine the effects of phosphate and ammonium stimulation on both biofilm development in metalworking fluid bioreactors, and on the ability of the bioreactors to remove carbon and organic constituents
2- To determine the carbon source that is required for biofilm formation in order to increase understandings of which components in metalworking fluids promote biofilm formation
3- To determine the mechanisms for removal of carbon and individual organic components in the bioreactors.
4- To apply the developed fixed-film reactors to the treatment of metalworking fluids containing the biocide Na-OPP within their formulation.

6.3 Materials and Methods

6.3.1 Micro-organisms, Bioreactor System, Biofilm Dry Weight and Biofilm Yield, TOC Analyses

Details of these materials and methods are provided in section 5.3.

6.3.2 Metalworking Fluid

Details of the artificial metalworking fluid used in this chapter are provided in section 5.3.2. In order to add the biocide Na-OPP into the system, a stock solution containing 100g/L of sodium ortho-phenyl phenate was prepared in DI water. This stock, together with the metalworking fluid concentrate, was used to make formulations containing the indicated concentrations of biocide used in the experiments of this chapter.

6.3.3 Reagents

Analytical grade inorganic salts ($CaSO_4.2H_2O$, $MgSO_4.7H_2O$, $NaNO_3$, NaCl, $FeSO_4.7H_2O$, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$) Triton X-100, Acetonitrile (99.9% purity), Acetic Acid and 2-Phenylphenol sodium salt were purchased from Sigma Aldrich.

Samples of Naphthenic Mineral Oil were provided by Nynas Base Oils. Samples of Sodium Sulfonate were provided by Sonneborn. Tall Oil Fatty Amides were provided by Colonial Chemical.

6.3.4 Bioreactor Operation

For experiments involving nutritional factors, the bioreactor operation was as described in section 5.3.5. The difference now being that the reactors were held at a fixed temperature of 27 °C and a pH of 8.

For experiments applying the fixed-film reactors to the treatment of biocide-containing metalworking fluids, the fixed-film reactors were developed using the artificial metalworking fluid described in section 4.3.2 with no biocide. Bioreactors were operated as sequential batch reactors to grow the biofilms. When the growth medium was exhausted, the matrix onto which the biofilm was growing on was removed and quickly re-immersed into a new reactor containing fresh growth medium. This simulated the act of drawing and refilling a batch reactor with growth media, and ensured that no residual fluid was present within the new medium. Three batches of artificial metalworking fluid, at a concentration of 2.5% (v/v) made in an artificial tap water, were used to develop the biofilms. Biofilm growth was encouraged by limiting the amount of ammonium chloride (which is the nitrogen source) to 10 mg/L within this medium. The medium was stimulated with phosphates, by adding 100mg/L KH_2PO_4. The temperature of the reactors was held at 27 ± 1℃. The pH of the metalworking fluid was adjusted to pH 8, and a 1M stock solution of HEPES was added to the formulation to was added to prevent rapid pH change. The final concentration of HEPES was 20mM.

The first cycle of growth lasted for 4 days, while the subsequent cycles lasted for 3 days each. After the three growth cycles, the biofilms were immersed in metalworking fluids now containing varying amounts of Na-OPP biocide. The treatment of this metalworking fluid was set to 6 days.

6.3.5 Re-emulsification Protocols

For suspended systems, the re-emulsification protocols are as those previously described in section 3.3.6.

For the re-emulsification of bulk fluid in the biofilm reactors, the biofilm matrices were first removed, and then the re-emulsification procedure for the suspended reactors was followed.

For the re-emulsification of adsorbed oil on the biofilm, the biofilm matrices were removed from the reactor and placed within 50 mL centrifuge tubes. The biofilms were rinsed three times with phosphate buffered saline solution and then were dislodged (by vigourous shaking) into 15 mL of phosphate buffered saline solution. The matrix, which now did not contain any biofilm was placed into a second centrifuge tube containing 25 mL of 0.5% Triton X-100 solution. The tube together with the matrix was sonicated using a Branson 3510 sonnicator set at 41.5 kHz for 5 mins. This was done to ensure that the majority of adsorbed carbon was recovered into the Triton X-100 solution. The Triton-X100 solution containing re-dispersed oils was then mixed with the PBS containing the biofilm. The total contents were mixed on a rocking incubator for 10 minutes, before being centrifuged at 4100 RPM for 20 mins. Samples of this mixture were extracted and diluted 10 times before TOC analysis and LC-Analysis. The total amount of a component in adsorbed onto the biofilm can be determined with the following equation:

$$TC_{adsorbed} = TC_{measured} * DF * V_{sample} - \frac{V_{triton}}{V_{sample}} * TC_{triton}$$

Where $TC_{adsored}$ is the total carbon adsorbed on the biofilm, $TC_{measured}$ is the total carbon of the sample (prepared above), V_{sample} is the volume of the sample (40 mL), $V_{trition}$ is the volume of 0.5% Triton X-100 added (25 mL), $TC_{trition}$ is the total carbon of 0.5% Triton X-100 (3000 mg/L) and DF is the sample dilution factor (10).

6.3.6 HPLC

HPLC analyses were used for the determination of the concentrations of amides, DGBE, DIPA and Na-OPP within the reactors. All analyses were done on an Agilent 1120 compact HPLC system equipped with an Agilent C18 Eclipse Plus Column and a UV-Vis detector. All analyses were done at 210 nm using an isocratic elution made up of a ratio of Acetonitrile acidified with 0.2% acetic acid and DI water (for amides and Na-OPP, the ratio was 90:10, for DGBE and DIPA, the ratio was 70:30). 2.5 µL of amide samples were directly injected into the system for analysis. DGBE and DIPA samples were derivitized before 5µL was injected into the system.

A method for HPLC sample derivitization developed by Sinjewel et al. (Sinjewel et al. 2007) was modified and applied to samples with DGBE and DIPA.

Briefly, 100 µL of sample was added to an Eppendorf tube, together with 100 µL of Propylene Glycol (which served as the internal standard). To this tube, 250 µL of a 300g/L NaOH solution was added with 15 µL Benzoyl Chloride. The tube was then vigourously shaken for 15 mins using a TOC X-5 shaker. After this, 100 µL of 100g/L Glycerol solution was added to terminate the derivitization reaction. The sample tube was then shaken for another 5 mins. After this, 400 uL of Hexane was added to the tube, which was then shaken for another 5 mins. After shaking, the tube was centrifuged for 5 mins at 14 500 RPM. The hexane layer was extracted and dried under a stream of air. To the residue, 300 uL of Acetonitrile was added. This acetonitrile sample was injected into the HPLC for analysis.

All calibration curves made were linear with an R^2 value of greater than 0.98.

6.3.7 TOC Analyses

TOC analyses are as described in section 3.3.5.

6.3.8 MicroResp™ and Post-Exposure Recovery Analysis

MicroResp™ and Post-Exposure Recovery Analyses were conducted as described in section 3.3.10, except that the 2.5% (v/v) artificial metalworking fluid was used instead of glucose.

6.3.9 Statistical Analysis

Single-factor ANOVA was used to test for significance of a response as a function of a varied parameter. The 2-tail student t-test with equal variance was used to test for significance between two individual responses. Tests were deemed to be significant for results were $p<0.05$.

6.4 Results

Within this section of the chapter, the effects of NH_4Cl and KH_2PO_4 addition on the development and the performance of fixed-film reactors are presented. These nutrients were chosen since they are a source of ammonium and phosphates, which are common stimulants added to treatment processes which are deficient in nitrogen or phosphorus (Jefferson et al. 2001; Byung R Kim et al. 1994; Wang et al. 1984). Furthermore, ammonium and phosphates are included in many recipes which are used for the promotion of bacterial growth (Neidhardt et al. 1974).

Thereafter, an investigation into which carbon source in the metalworking fluid is most utilised during biofilm growth is explored.

Finally, having established the influence of nutrient addition and carbon sources on biofilm growth, the fixed-film reactors are applied to the treatment of metalworking fluids containing biocides in order to determine if reactor inhibition still occurs.

6.4.1 Ammonium Influences

For the experiments in this section, the artificial metalworking fluids were stimulated with 100ppm of KH_2PO_4 to ensure that there was an ample phosphorus source. Figure 6-1 shows the effect of ammonium addition on both the extent of biofilm development and the total carbon removal performance in the bioreactors treating the defined metalworking fluid formulation. The biofilms were cultivated in the artificial metalworking fluid over two cycles of operation (the first being 4 days, and the second being 3 days). It can be seen that the addition of ammonium chloride significantly reduced biofilm formation within the reactor. An increase of ammonium stimulation from 10 ppm to 200 ppm resulted in a significant biomass decline from 79.7 ± 2.0 mg to 19.2 ± 2.1 mg ($p< 0.001$). Further to the absolute biomass decline, there was also a significant decline in the yield of the biofilm. Figure 6-2 shows that the increase of the addition of NH_4Cl from 10 ppm to 200 ppm resulted in a significant decline in biofilm yield from 0.012 ± 0.0006 mg/mg TC removed to 0.0047 ± 0.0004 mg/mg TC removed ($p < 0.001$). This suggests that there was an anabolic mechanism which favours biofilm growth under nitrogen limiting conditions. Similar results were obtained by Thompson et al. who showed that C:N:P ratios with smaller amounts of nitrogen resulted in more biofilm growth of *Enterobacter cloacae* and *Citrobacter freundii* (Thompson et al. 2006). Punal et al. also showed that nitrogen limitation led to increased cell attachment rates in an anaerobic upflow sludge blanket reactor (Puñal et al. 2000).

Another possible reason for increased biomass may be due to increased production of EPS, or due to the synthesis of carbon storage components under nitrogen limiting conditions. Zhang et al. found that EPS production increased with nitrogen

limitation (Zhang et al. 2014). Miqueleto et al. showed that EPS production was maximized at high C/N ratios (Miqueleto et al. 2009).

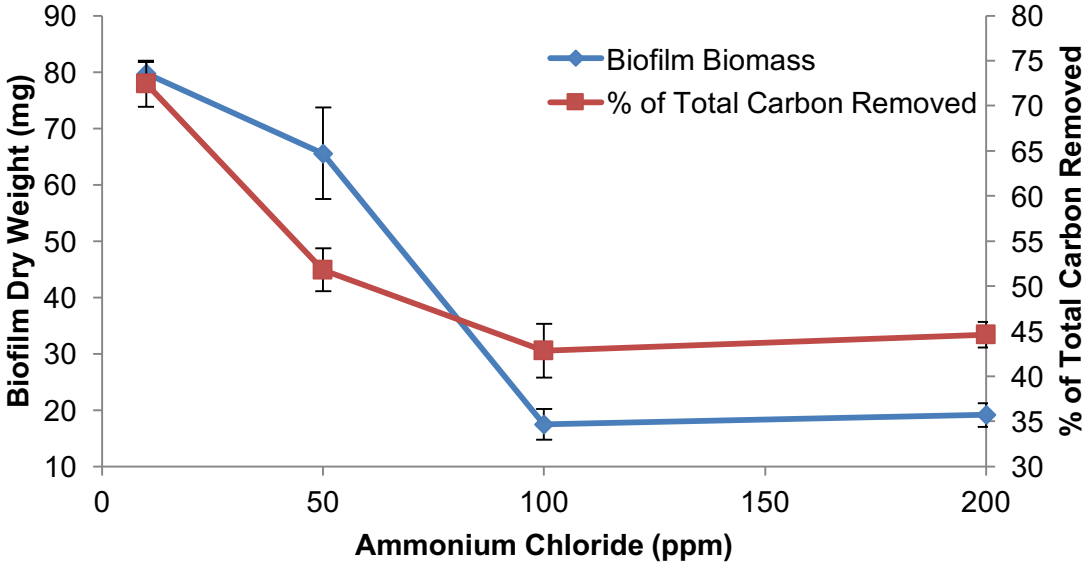

Figure 6-1: Effects of ammonium chloride stimulation on biofilm development and total carbon removal performance of reactors treating the defined artificial metalworking fluid. Reactors were stimulated with 100ppm KH_2PO_4. Error bars represent standard deviation of triplicate measurements.

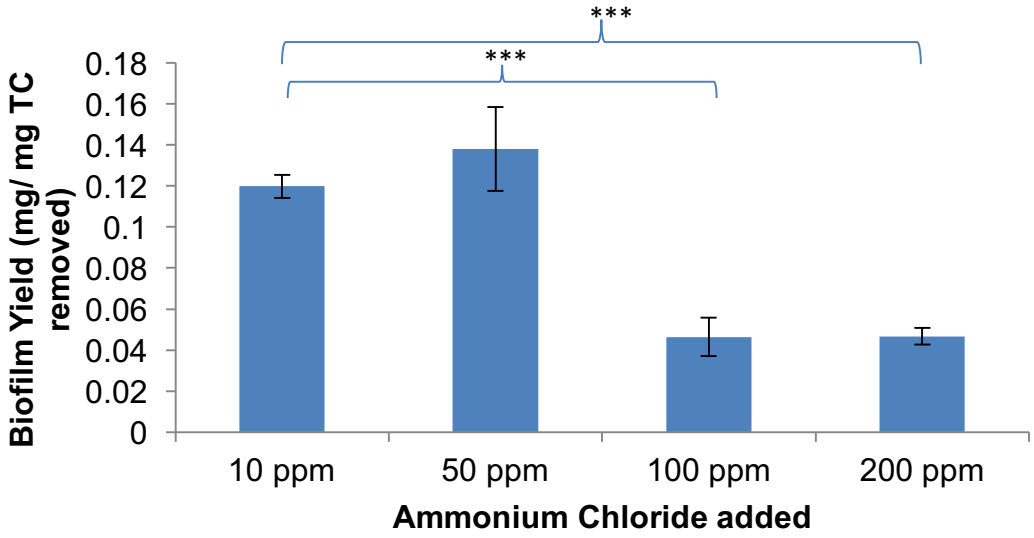

Figure 6-2: Effect of ammonium chloride addition on biofilm yield in reactors treating the defined artificial metalworking fluid. Reactors were also stimulated with 100ppm KH_2PO_4. Error bars represent standard deviation of triplicate measurements. * indicates significance with $p < 0.001$**

In addition to biofilm decline, NH_4Cl stimulation also resulted in a significant decline in total carbon removal performance. Surprisingly, the total carbon removal efficiency declined from 72.4% ± 2.6% to 44.6% ± 1.4% ($p < 0.01$) when the NH_4Cl concentration increased from 10 ppm to 200 ppm. This was not due to a toxic effect (in fact, the number of colony forming units suspended in the bulk liquid was much greater in the 200 ppm condition than the 10 ppm condition- data not shown. Furthermore, it will be shown later in the chapter that ammonium stimulation accelerated the removal of some of the constituents of the metalworking fluid, and resulted in a similar mineralisation extent as the 10 ppm condition).

Figure 6-3 shows the kinetics of carbon removal. While the addition of NH_4Cl resulted in a significant decline over the long term in overall performance, it resulted in an increase in the rate of carbon removal during the early stages of treatment. This suggests that NH_4Cl may have been stimulating the degradation of some components of the metalworking fluid, but may have had no effect or even an inhibitory effect on others. The dynamics of component degradation were further investigated in section 6.4.4 and a final discussion on why the removal efficiency was lowered is presented in section 6.4.5.1. In brief, these sections will attempt to demonstrate that ammonium addition results in a decrease in diisopropanolamine degradation, and results in a more stable final emulsion possibly due to the production of biosurfactants.

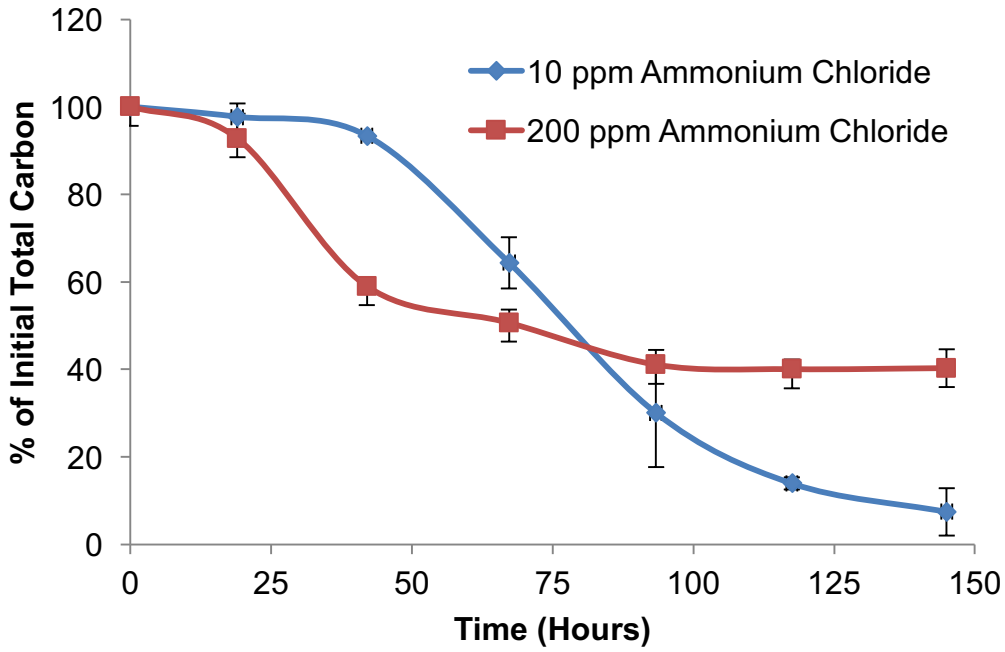

Figure 6-3: Kinetics of total carbon removal as a function of ammonium stimulation. Data is presented for cycle 1 of the 2 cycle batch process. Error bars represent standard deviation of triplicate measurements.

From the results provided in this section of the chapter, it can be seen that wastewater treatment operators should utilise nitrogen limiting conditions in order to stimulate both biofilm growth and carbon removal performance in their reactors. It is possible that large additions of NH_4Cl can lead to reduced reactor performance.

6.4.2 Phosphate Requirements

Having investigated the effects of varying the concentration of the easily utilisable nitrogen source to the reactor, this section proceeds to investigate the effects of varying the concentration of the easily utilizable phosphorus source. Phosphorus was provided in the form of KH_2PO_4, which is a common stimulant added to wastewaters that are phosphate deficient (Cheng et al. 2005; Jefferson et al. 2001). Since NH_4Cl addition has a significant effect on biofilm growth, the effects of phosphate addition on conditions involving 10 ppm and 200 ppm NH_4Cl addition were investigated, and the results are presented in Figure 6-4.

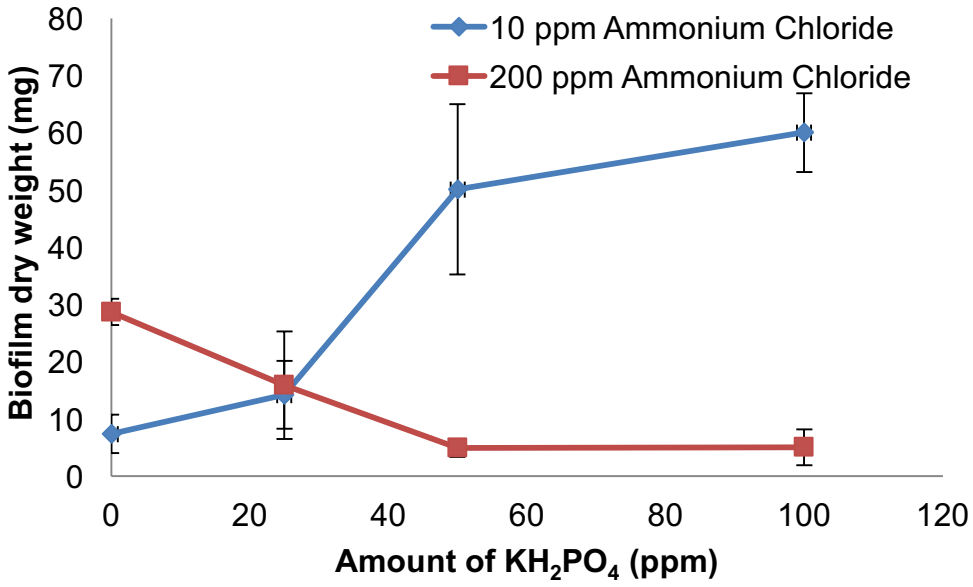

Figure 6-4: Biofilm dry weight as a function of KH_2PO_4 addition to reactors treating artificial metalworking fluid. Reactors were further stimulated with either 10 ppm or 200 ppm NH_4Cl. Error bars represent standard deviation of triplicate measurements.

In Figure 6-4, systems stimulated with 10 ppm NH_4Cl and those stimulated with 200 ppm NH_4Cl show opposite trends. For 10 ppm NH_4Cl systems, addition of KH_2PO_4 resulted in a significant increase in biofilm biomass production ($p<0.01$). Specifically, reactors stimulated with 100 ppm of KH_2PO_4 had a biofilm biomass of 60.0 mg ± 6.9 mg as compared to reactors with no phosphates, which had only 7.4 mg ± 3.4 mg ($p<0.01$). This suggests that at relatively low levels of NH_4Cl stimulation, phosphate addition results in growth stimulation. This was consistent with the findings in section 6.4.1, but shows further that limiting both nutrients does not stimulate biofilm growth.

On the other hand, the results indicate that at relatively high levels of NH_4Cl stimulation, KH_2PO_4 addition results in a significant decline in biofilm growth ($p<0.05$). In reactors stimulated with 200 ppm NH_4Cl, reactors containing no phosphates had 28.3 mg ± 2.3 mg of biofilm biomass, which was significantly more

than the 5.0 mg ± 3.1 mg of biomass found in reactors containing 100 ppm of KH_2PO_4 ($p<0.01$). This suggests that phosphate limitation may be a means to enhance biofilm growth. Previous research has shown that phosphate limitation may enhance specific types of EPS production in bacteria (Mendrygal & Gonzalez 2000; Fang et al. 2009) or may enhance attachment of bacteria on surfaces (Xu et al. 2012). Both of these mechanisms would result in a relative in biofilm production, which may be observed here as well. However, it important to note that having both nutrients present in relatively large amounts quenched biofilm formation in the reactors.

The results of the effect on reactor performance as a function of phosphate stimulation in both low and high NH_4Cl reactors are presented in Figure 6-5. It can be seen that there was a significant increase in the total carbon removal performance with KH_2PO_4 for both the low ($p<0.01$) and high ($p<0.01$) NH_4Cl conditions. An addition of 25 ppm of KH_2PO_4 to the 10 ppm NH_4Cl reactor enhanced total carbon removal from 38.7% ± 0.9% to 80.9% ± 0.8%. An addition of 50 ppm KH_2PO_4 to the 200 ppm NH_4Cl reactor increased the total carbon removal from 24.4% ± 2.7% to 50.8% ± 2.9%.

This behaviour was anticipated since the artificial metalworking fluid wastewater was phosphate deficient, which was representative of real metalworking fluid wastewaters (Cheng et al. 2005).

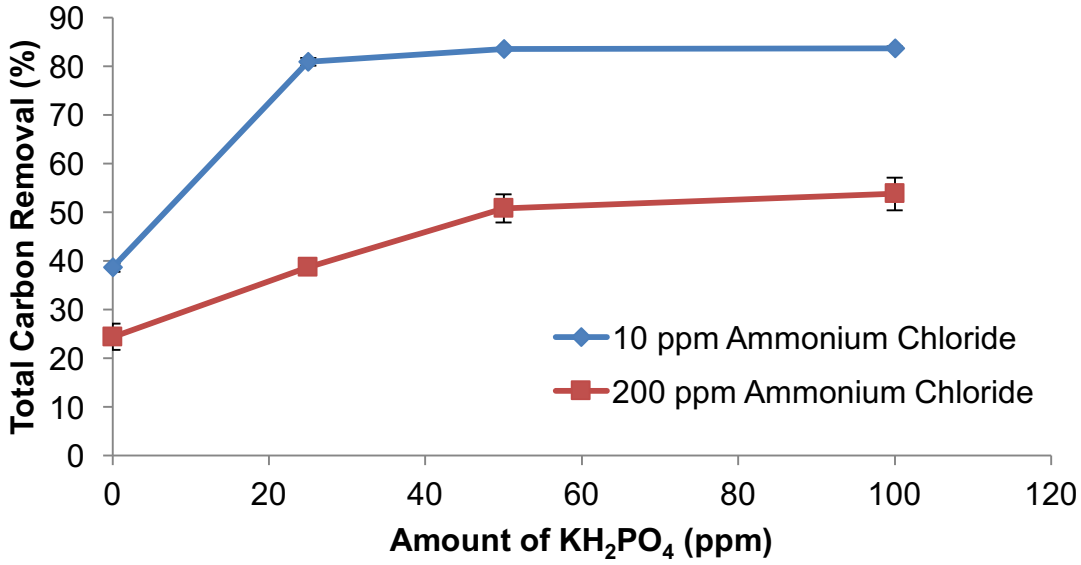

Figure 6-5: Total carbon removal performance as a function of KH_2PO_4 addition. Data is presented as the cumulative total carbon removed over 2 cycles of operation. Error bars represent standard deviation of triplicate means.

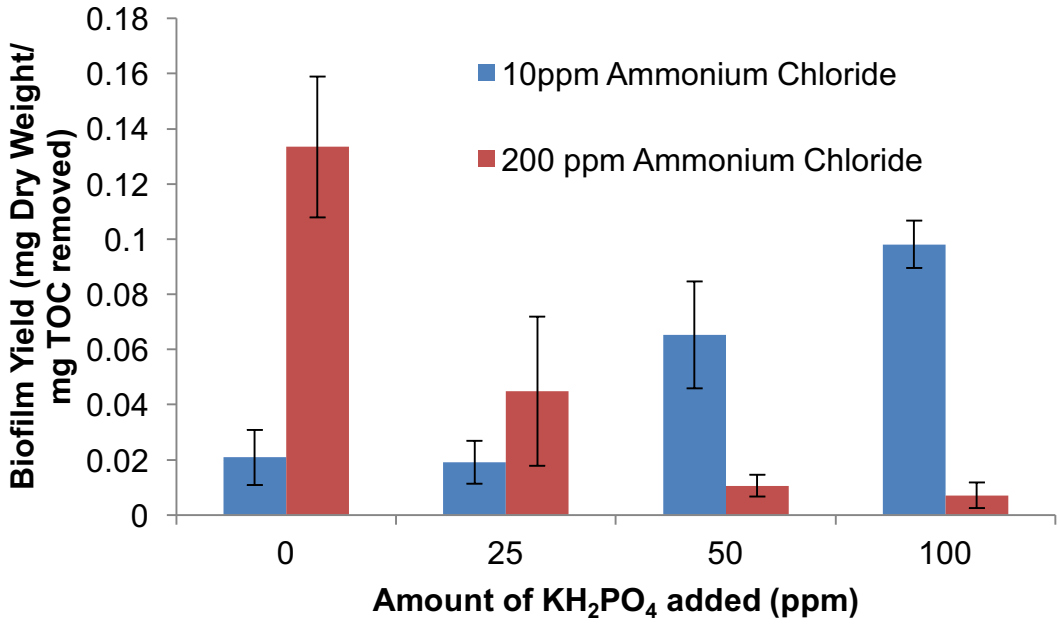

Figure 6-6: Biofilm yield obtained in MWF reactors as a function of KH_2PO_4 addition. Results are given for 10 ppm and 200 ppm NH_4Cl stimulation conditions. Error bars represent standard deviation of triplicate measurements.

Similar results of phosphates improving the treatment efficiency of bioreactors have been reported (Schreyer & Coughlin 1999; Cheng et al. 2005). The trend of increasing NH_4Cl concentration reducing total carbon removal performance was

consistent with the findings of section 6.4.1. Increasing phosphates increases carbon removal efficiency since providing a phosphorus source allows for greater extents of microbial growth (Sperling 2007). It was observed that the number of colony forming units present in both the 10ppm and 200ppm NH_4Cl reactors at the end of each cycle increased with increasing KH_2PO_4 concentration (data not shown). This suggested that while KH_2PO_4 addition quenched biofilm growth in the 200ppm NH_4Cl condition, it stimulated the production of cells in the suspended state. This could explain why there was a carbon removal increase in the 200ppm NH_4Cl reactor, even though biofilm biomass was reduced.

Figure 6-6 shows that the yield in the reactor was maximized when either NH_4Cl or KH_2PO_4 was limited. Limiting both of the nutrients or having both of the nutrients present in an excess amount significantly reduced the yield ($p< 0.01$ for both stimulatory conditions). Thus, nutrient limitation may be an effective strategy that can be utilised for the development of fixed-film reactors treating metalworking fluids. Several studies have shown how nitrogen limitation and phosphate limitation may be used to stimulate biofilm growth (Puñal et al. 2000; Thompson et al. 2006; Liu et al. 2015; Xu et al. 2012). Nutrient limitation may have created a stress response that led to more biofilm development (Jefferson 2004), or may have stimulated EPS production as a means of storing the nutrients are present in excess in anticipation for favourable conditions to return (Flemming & Wingender 2010).

The results from this section of the chapter indicated that nutrient limitation was an effective strategy for developing biofilm reactors. However, even though phosphate limitation resulted in a similar yield to that of ammonium limitation, limiting the phosphates in general severely reduced total carbon removal efficiency. In section 6.4.4, it is shown that the microbes were able to obtain a source of nitrogen from

the alkanolamines in the artificial metalworking fluid. However, there was no other sufficient source of phosphorus aside from what was added through stimulation. Without phosphates, microbes were not able to replicate to form new cells, which could be an explanation as to why the carbon removal performance was reduced.

The results thus indicate that fixed-film reactors for the treatment of metalworking fluids are best developed with both phosphate stimulation, and with ammonium limitation. Practitioners who wish to employ the fixed-film bio-treatment process may use this information to simultaneously maximize the treatment performance and the yield of biofilm obtained in the reactor.

6.4.3 Carbon Sources

In this section, insights into the carbon sources that are utilised for biofilm growth are provided. The artificial metalworking fluid has numerous carbon sources, and so it is possible that only specific ones are utilised for biofilm growth. Being able to understand which carbon source is required for biofilm growth will greatly assist practitioners in establishing biofilm reactors for treating wastewaters. Figure 6-7 shows the dry weights, the TC removal percentages, and the yields of biofilms grown on the individual components that are used in the artificial metalworking fluid formulation. Note that the concentration of the components used in each experiment is the same as the concentration as they would appear in the artificial metalworking fluid mixture. It can be seen that growth on Tall Oil Amides produced the significantly higher biofilm yields than that achieved by using the other components ($p<0.01$). This suggests that the fatty amides in the reactor are specifically required for the formation of biofilms. Furthermore, it can be seen that in addition to producing the largest yield of biofilm, Utilisation of the fatty amides produces the largest dry weight of biofilm in the reactor. Furthermore, when the biofilm dry-weights obtained from

growth using the other components are added together (4.1mg), it total value does not exceed that produced from growth using Tall Oil Amides (27.9mg).

Interestingly, the % of carbon removal increased when all components are added together, and the biomass obtained was much greater than the sum of the individual amounts achieved with each component. During the dry weight measurement procedure (see section 5.3.6), it was noted that biofilms that were grown on Tall Oil only accumulated at the bottom of the PBS-containing tube into which they were dislodged, while biofilms that were grown with all components together floated on the top of the tube. This suggests that the density of the biofilms grown in the mixture containing all of the components is much less dense than that grown in tall oil only. A possible explanation for this was that some components, in particular, naphthenic mineral oil, were adsorbed onto the biofilm (Adapa et al. 2016).

In section 6.4.5.1, it is shown that a large fraction of the carbon removed within the system was recovered by re-suspending the adsorbed oil with Triton X-100. However, this does not explain the large increase in biomass when all components are together since the biomass was washed with Triton X-100 before the dry-weight is measured. Furthermore, the biomass still floats in DI water, suggesting that there may be some actual incorporation of the oily components into the biofilm structure.

Furthermore, the total carbon removal percentage was also much larger when all of the components where combined. While this may be due co-metabolic activities, another possible explanation for this may be that sorption of other components onto the biofilm during its formation.

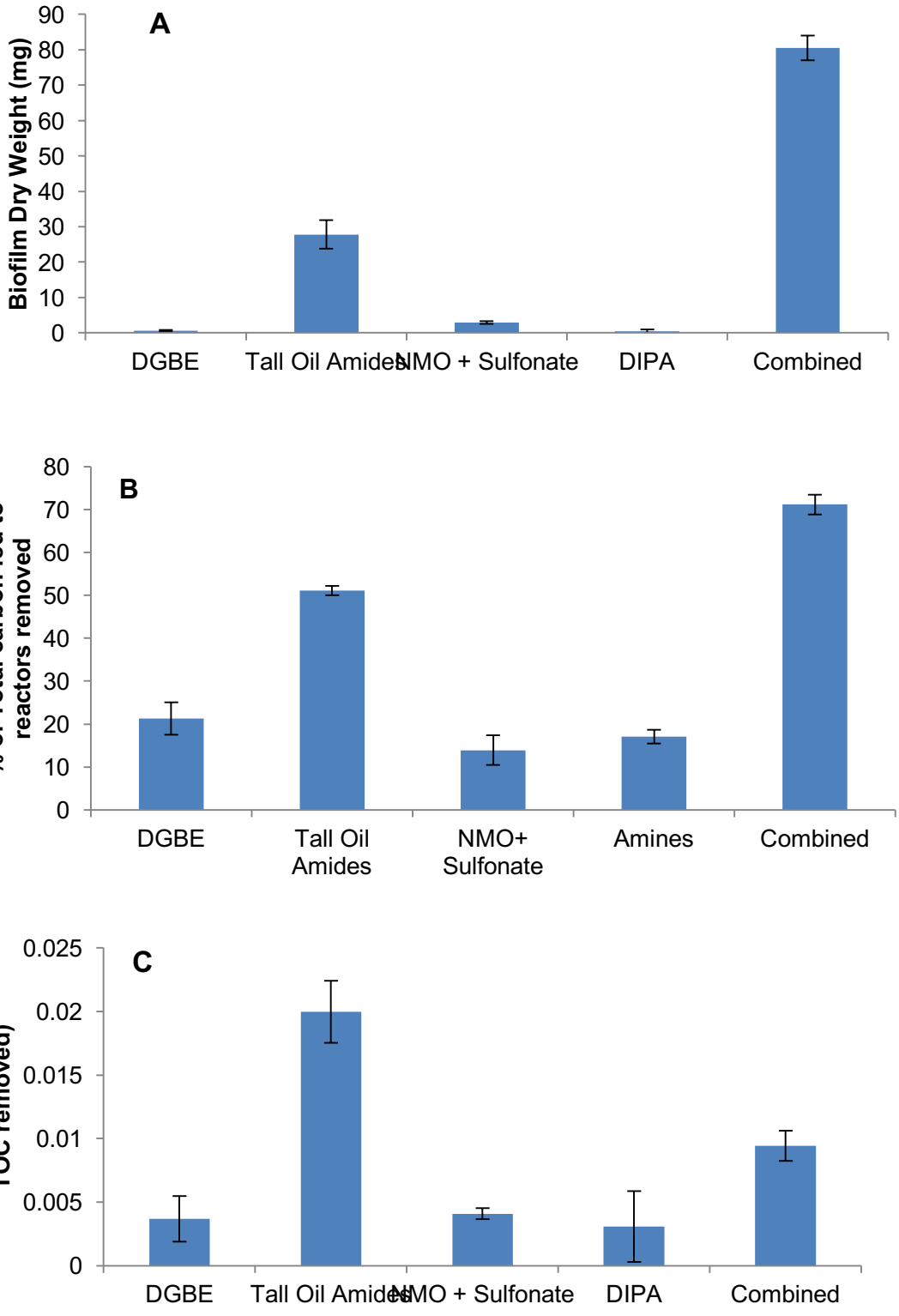

Figure 6-7: A) Biofilm Dry Weight B) Total carbon removal percentages and C) Biofilm yields obtained from growing biofilms on carbon sources used in the artificial metalworking fluid . DGBE is Diethylene Glycol Butyl Ether, NMO+ Sulfonate is Naphthenic Mineral Oil and Sodium Sulfonate, DIPA is Diisopropanolamine, and Combined is all of these components together in the artificial metalworking fluid. Concentrations of the individual components are the same as the concentration in which they appear in the artificial metalworking fluid formulation. Error bars represent standard deviation of triplicate measurement.

Furthermore, as shown in Chapter 3, and as will be shown in section 6.4.5.1, the removal mechanisms for carbon are not completely metabolic, but a large percentage of removal was due to oil/water separation. Thus, in the combined system, oil/water separation may have been the dominant removal mechanism.

Figure 6-7 B shows that in addition to producing the greatest yield, bioreactors developed on tall oil amides also had the greatest total carbon removal efficiency. Similar results showing that fatty amides are readily biodegradable components within metalworking fluids have been reported by Rabenstein et al. (Rabenstein et al. 2009) and by Kim et al. (Byung R. Kim et al. 1994). The ease of biodegradability of fatty amides may be the reason as to why they led to the highest biofilm yield. It is possible that microbes were able to readily develop the enzymes that were necessary for the EPS production leading to the development of biofilms through the use of fatty amides, while synthesis of such enzymes could not be accomplished for other components due to their lack of biodegradability.

It should be noted that although DGBE and DIPA had relatively low removal rates when fed to the reactors in isolation, when fed with the other components in the metalworking fluid they were almost completely removed (as demonstrated in section 6.4.4). This was likely due to the microbes first utilising more biodegradable components for growth before utilising the more bio-resistant molecules. This would lead to higher removal rates of the bio-resistant molecules since soft-component utilisation would lead to more biomass, which would lead in turn to higher removal rates and extents.

Attempts were made to dissolve the biofilm biomass that was found floating at the top of the PBS-containing tube during the dry-weight measurement protocol (section

5.3.6). A number of solvents (Hexane, Ethyl Acetate, Dichloromethane, Acetonitrile, Acetone and Ethanol) were used, but none of these were successful in dissolving the biomass obtained. However, upon the addition of Diethylene Glycol Butyl Ether, a large fraction of the biofilm was found to dissolve, leaving only a small residue which had a density less than that of water. DGBE was used as a coupling agent that was mutually soluble in both the oil and water phases of the semi-synthetic metalworking fluid (The DOW Chemical Company 2004). Since the biofilm was produced from the components in the artificial metalworking fluid, it dissolving in a solvent which was able to accommodate such components is not surprising.

HPLC analysis of the DGBE, together with the dissolved components, revealed that it had contained the unsaturated fatty amides that were initially present within the metalworking fluid wastewater. No saturated amides were detected. Figure 6-8 shows the percentage of each of the fatty amides that are recovered from the biofilm biomass using the DGBE dissolution method. This suggests that one of the mechanisms involved in biofilm formation could the accumulation of fatty amide material within the bacterial cells. This is consistent with the component storage functionality that is associated with biofilm growth. Another explanation could be that these amides are physically adsorbed onto the biofilm and could only be released upon the dissolution of biofilm components

This may explain why the fatty amides are not chemically altered in the bio-treatment process. Nevertheless the dissolution method and the results from Figure 6-8 suggest that unsaturated amides are incorporated into the biofilm structure, and that these may be recovered.

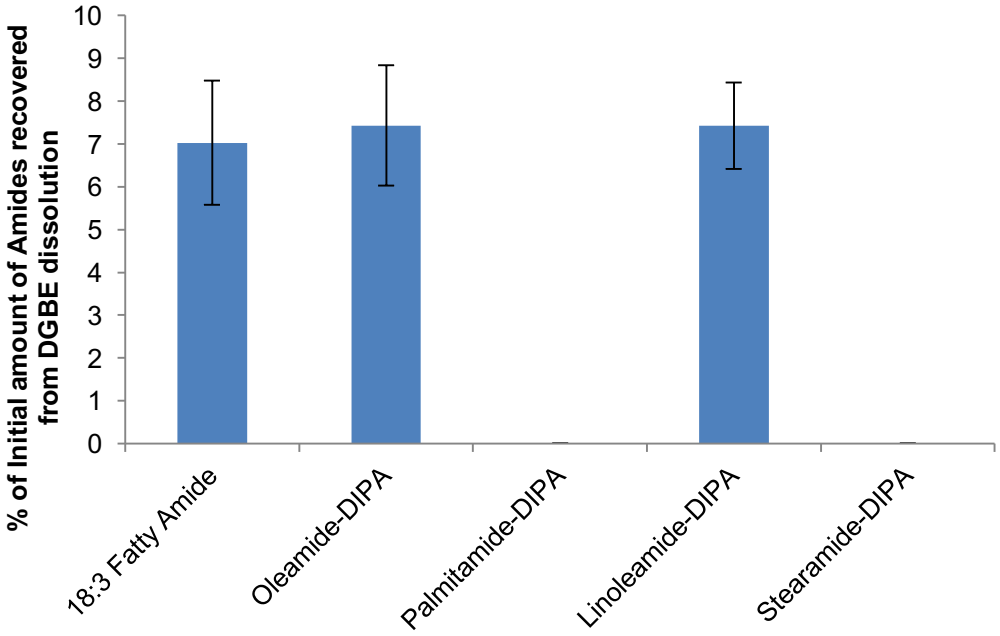

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Figure 6-8: Fatty amides recovered from the dissolution of biofilm biomass in Diethylene Glycol Butyl Ether. Recovery is expressed as the percent of the total amount that is put into the bioreactors over the 2 cycles of treatment. Error bars represent standard deviation of duplicate measurements.

Roughly only 7-8% of the total amount of amides that were used for the production of the biofilm was recovered, but considering that the biofilm will be used to treat a number of waste cycles over its commissioning life, this absolute amount may make the process of recovery a feasible one in industry.

In this section of the chapter, it was shown that fatty amides are the main carbon source for biofilm growth under the nitrogen limiting conditions that were utilised. When comparing the removal extents for reactors treating individual components of the artificial metalworking fluid, it was shown that the reactor treating Tall Oil amides demonstrated the highest total carbon removal efficiency, indicating that it tall oil amides are the most biodegradable substance in the artificial formulation. The ease of biodegradability could be the reason as to why tall oil amides led to the highest biofilm yields.

6.4.4 Dynamics of metalworking fluid component removal as a function of NH_4Cl concentration

In section 6.4.1, and specifically in Figure 6-3, it was shown that NH_4Cl stimulation resulted in a significant decline in the total carbon removal efficiency of the metalworking fluid. Ammonium chloride is a common stimulant that is added to wastewater treatment processes in order to enhance microbial growth and reactor performance, and thus the effect of reducing total carbon removal was an unexpected one. This section attempts to explain this anomaly. Specifically, the kinetics of utilisation of some of the organic constituents within the metalworking fluid is presented. The artificial metalworking fluid contains diethylene glycol butyl ether (DGBE), saturated and unsaturated fatty amides (from the tall oil that is used in the formulation), diisopropanolamine (DIPA), naphthenic mineral oil (NMO) and sodium sulfonate. Kinetics for DGBE, the amides, and DIPA are presented since the degradability of NMO and Sodium Sulfonate was known to be low in comparison (see section 3.5).

Figure 6-9 shows the removal pattern of DGBE and the detected fatty amides (Palmitamide-DIPA and Stearamide-DIPA) in the reactor. It can be seen that for these compounds, NH_4Cl addition increased the rate of removal from the wastewater. An interesting observation from this data was that in both stimulatory conditions, the fatty amides were utilised before the glycol. This suggests that the fatty amide was more biodegradable than the glycol and that the microbes preferentially utilised these softer components before harder ones. Furthermore, it can be seen that Palmitamide-DIPA was utilised faster than Stearamide-DIPA. This was most likely due to Palmitamide-DIPA being a smaller molecule than Stearamide-DIPA. The results from Figure 6-9 show the expected trend of

ammonium chloride being a stimulant for biodegradation. Thus, this data does not explain the anomalous result of reduced carbon removal efficiency.

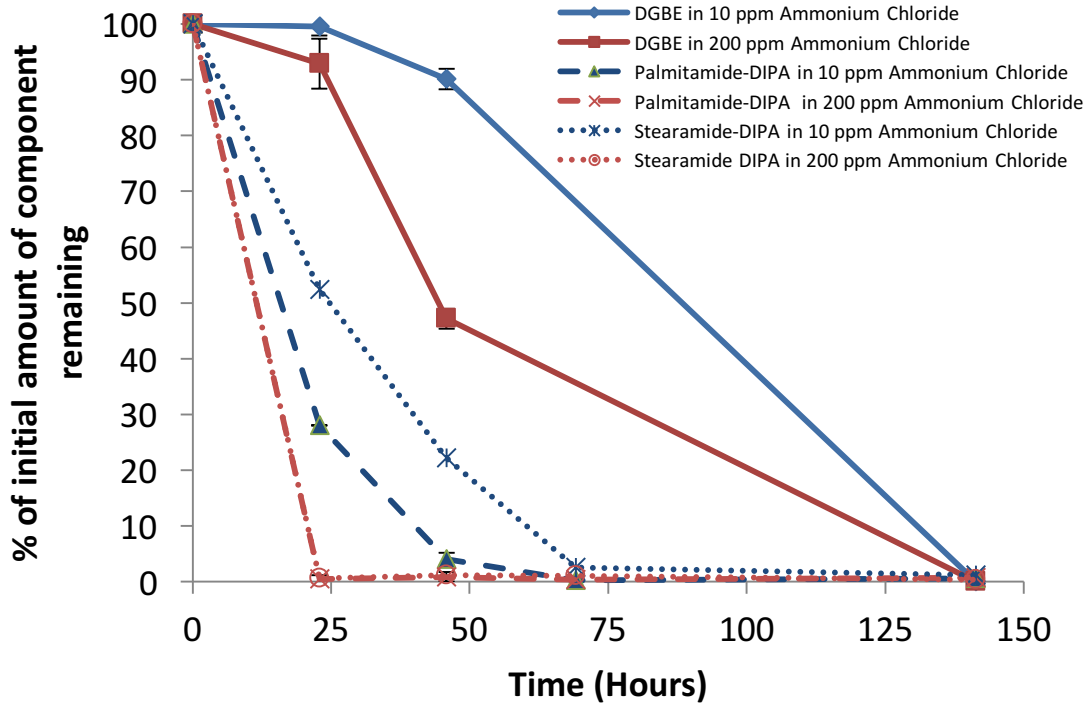

Figure 6-9: Dynamics of diethylene glycol butyl ether, palmitamide-DIPA and stearamide-DIPA removal in bioreactors treating the artificial metalworking fluid. Data is presented for the first cycle of operation only. Error bars represent standard deviation of triplicate measurements.

Figure 6-10 shows the dynamics of removal of the detected unsaturated fatty amides present within the artificial metalworking fluid (oleamide-DIPA, linoleamide-DIPA and an amide with an 18:3 fatty acid component, which could either be α-linolenic acid or γ-linolenic acid). Unlike the clear difference in removal rates observed for the saturated fatty amides, the removal rates for the unsaturated fatty amides were extremely similar ($p>0.05$). This was the first piece of evidence that suggests that removal of unsaturated fatty amides within the bioreactor does not proceed via microbial utilisation. As observed in Chapter 3, saturated amides were removed to a greater extent than unsaturated fatty amides. This was consistent with observations by Berg et al. who showed that unsaturated fatty amides were more

difficult to biodegrade than saturated amides since they required an .additional step in the metabolic mechanism (Berg et al. 2002)

If microbial utilisation were indeed the mechanism responsible for removal, then the utilisation rates of these fatty amides would be expected to be different considering that they have different degrees of saturation, and have different molecular weights. In section 6.4.5.2, it is shown that the mechanism for removal of the unsaturated fatty amides was likely due to demuslfication induced by the biodegradation of the emulsifiers (saturated fatty amides) and due to both oil/water separation and adsorption of the oil droplets onto the biofilm. As oil/water separation occurred, the unsaturated fatty amides, which have a greater affinity for the oil phase than the water phase (see Chapter 4), were removed from the reactor.

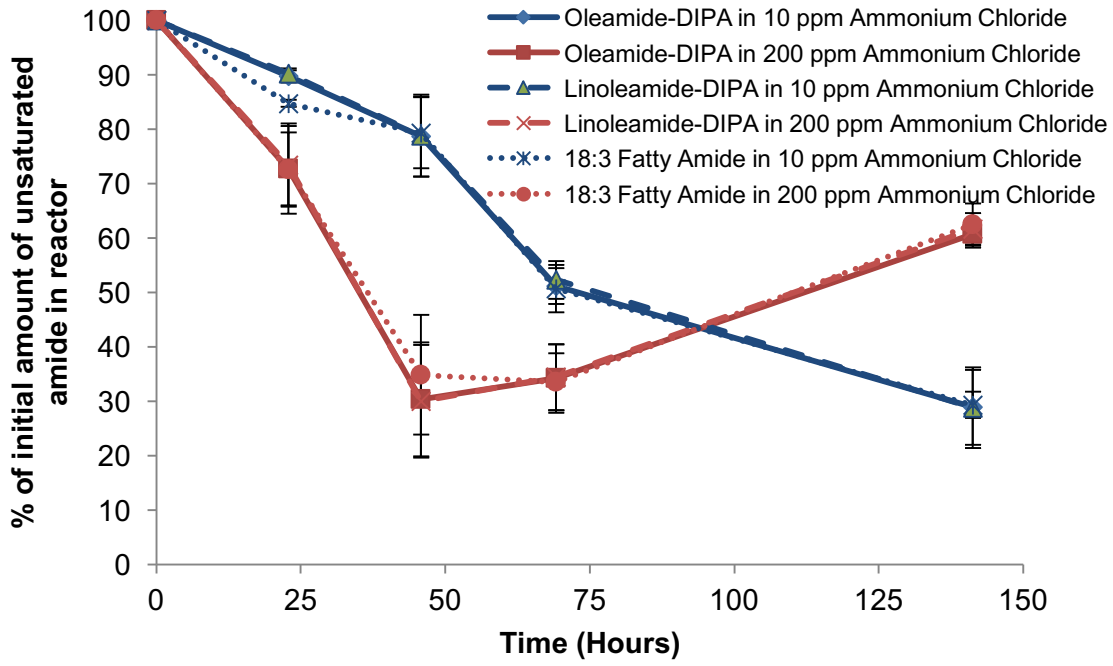

Figure 6-10: Dynamics of unsaturated fatty amide removal in bioreactors treating the artificial metalworking fluid. Data is presented for the first cycle of operation only. Error bars represent standard deviation of triplicate measurements.

When comparing the removal trends of the unsaturated fatty amides between reactors stimulated with 10ppm NH_4Cl and those stimulated with 200ppm NH_4Cl, vast differences can be observed. For the reactors containing 10 ppm NH_4Cl, there was a constant decline of fatty amides, whereas in the 200 ppm reactors, there was a sharp decline followed by a re-emergence of unsaturated fatty amides within the reactor. A possible explanation for this could be due to the production of biosurfactants within the reactor which results in a re-dispersion of the oils that have been removed through demulsification, leading to a re-dispersion of the amides that have been partitioned into the oil phase. This is discussed further in section 6.4.5.2.

The overall removal of the unsaturated fatty amides in the 200 ppm NH_4Cl reactor ranged between 37-39% while the removal achieved in the 10ppm reactors ranged between 71-72%. The removal extents for each component were found to be significantly different in the 10 ppm NH_4Cl reactor and the 200 ppm NH_4Cl reactor ($p< 0.01$ for all unsaturated fatty amides). Keeping in consideration that the overall removal mechanism for unsaturated amides in the reactor was due to oil/water separation induced by biodegradation of emulsifiers, the difference in extents can be explained by the difference in the biodegradation of saturated amides. In Figure 6-9, it was shown that the saturated amides are removed at greater rates with 200 ppm NH_4Cl stimulation as compared to 10 ppm NH_4Cl stimulation. In section 6.4.5.2, it will be shown that this removal was most likely due to biodegradation. Since the saturated amides, which are emulsifiers in the metalworking fluid, degrade much quicker in reactors stimulated with 200ppm NH_4Cl, oil soluble components, such as the unsaturated amides, were also removed at greater rates

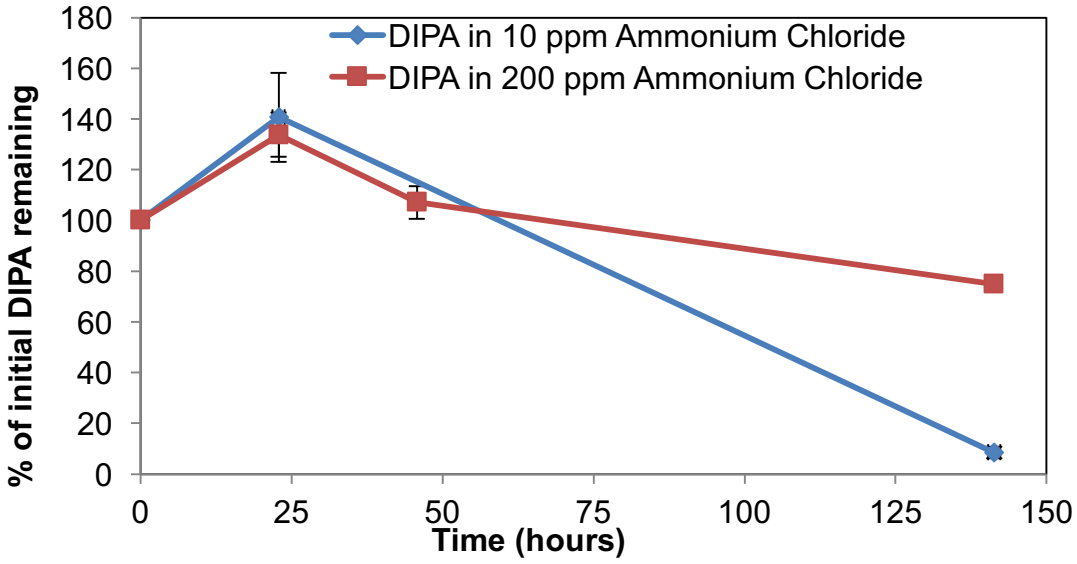

Figure 6-11: Dynamics of Diisopropanolamine removal in bioreactors treating artificial metalworking fluid. Data is presented for the first cycle of operation of the SBBR only. Error bars represent standard deviation of triplicate measurements.

Figure 6-11 shows the removal dynamics of the alkanolamine component in the metalworking fluid, namely Diisopropanolamine (DIPA), for both relatively low and high stimulatory conditions of NH_4Cl. It can be seen that in both instances, the DIPA concentration first increased to a concentration that was higher than that initially present within each of the reactors, and then began to decline as the treatment process progressed. The increase in DIPA concentration was likely due to the biodegradation of the saturated fatty amides within the system. The saturated amides were made up of C16-C18 fatty acid chains that have undergone an amidation reaction with diisopropanolamine to produce the fatty amide surfactants. It was likely that the biodegradation of these amides occurs via a mechanism that involves cleaving the molecule at the carbonyl group, which led to the production of amines in the process. This was consistent with the findings of Geerts et al.(Geerts et al. 2014) who investigated the biodegradation mechanisms of a number of fatty amides, including tall oil amides. They found that the biodegradation of fatty amides released amines, while the fatty acid component was metabolized immediately. In

the bioreactor system, saturated fatty acids Palmitamide-DIPA and Stearamide-DIPA where biodegraded to produce DIPA, while the fatty acid component was completely removed (as shown by Figure 6-9).

As can be seen in Figure 6-11, the extents of removal of diisopropanolamine were significantly different for different amounts of NH_4Cl stimulation. Reactors stimulated with 10 ppm NH_4Cl removed 91.4% ± 2.3% of the initial amount of DIPA in the reactors, while those stimulated with 200 ppm NH_4Cl only removed 25.1 ± 1.8% of the initial amount ($p<0.01$). Thus, overstimulating the reactors with NH_4Cl inhibited the removal of diisopropanolamine within the system. A likely reason for this was that the degradation of amines can lead to the production of NH_4Cl (Greene et al. 1999; Kim et al. 2010; Ndegwa et al. 2004; Gieg et al. 1999). Microbes utilise NH_4Cl as a readily accessible source of nitrogen. It was likely that, in the presence of excess NH_4Cl, microbes did not metabolize DIPA since a readily available alternative nitrogen source was available. The implication of this is that DIPA was not readily used as a carbon source, but was rather utilised as a nitrogen source. Some researchers have shown that amines can readily be degraded in the presence of ammonium chloride (Mrklas et al. 2004; Greene et al. 1999). It is important to note that in these cases, there were no other carbon sources and hence the microbes would have utilised DIPA irrespective of the NH_4Cl concentration.

In this section, it has been shown that NH_4Cl concentration can have significant effects on both the removal rates and removal extents of the organic constituents within the artificial metalworking fluid. While stimulating the bioreactors with 200ppm NH_4Cl resulted in more rapid removal of certain compounds, the extents of degradation of others were significantly reduced. Specifically, overstimulation of NH_4Cl led to re-dispersion of unsaturated fatty amides (due to re-emulsification of

the oil phase in which it is dissolved), and removal inhibition of DIPA. This suggests that NH_4Cl stimulation of metalworking fluid bioreactors was not a good strategy for treatment, and that it was more preferable to allow for microbes to utilise the nitrogen-containing organics, such as alkanolamines, to obtain their nitrogen requirements for growth.

6.4.5 The Mechanisms for the Removal of Total Carbon and Organic Constituents from Metalworking Fluids

In this section, insights into the mechanisms of removal for the individual organic constituents as well as the total carbon of the artificial MWF waste are provided. In Chapter 3, it was demonstrated that the bulk of the carbon removal in the bio-treatment of metalworking fluids was accomplished through oil/water separation induced by emulsifier degradation. While those findings were presented for a soluble oil metalworking fluid, it may be equally valid for semi-synthetic metalworking fluids. Over and above looking at the carbon removal, specific insights into the removal mechanisms for the organic constituents were also provided. In Chapter 4, it was observed that oil/water separation can be used as a strategy for the removal of oil soluble organics present in metalworking fluids. If oil/water separation occurs through a biological means, then this action will also lead to physical removal of organics that partition themselves into the oil phase rather than the water phase. Furthermore, the mechanisms for removal investigated in Chapter 3 were for suspended systems as opposed to the fixed-film systems investigated in this chapter. Therefore, a comparative study on the performance and removal mechanisms for fixed and suspended systems is also provided in this section.

6.4.5.1 Carbon Removal Mechanisms

Figure 6-12 shows the total carbon removal performance of the suspended system and the fixed system in treating the artificial metalworking fluid supplemented with 10ppm NH_4Cl and 100 ppm KH_2PO_4. Included are also the results of the abiotic controls, one without the polyethylene matrix on which the biofilm forms, and one in which it was present. It can be seen that the total carbon removal performance of both the fixed and suspended systems (before re-emulsification) are statistically similar ($p=0.543$) suggesting that there was no difference in removal extents between suspended and biofilm modes of growth at this stage.

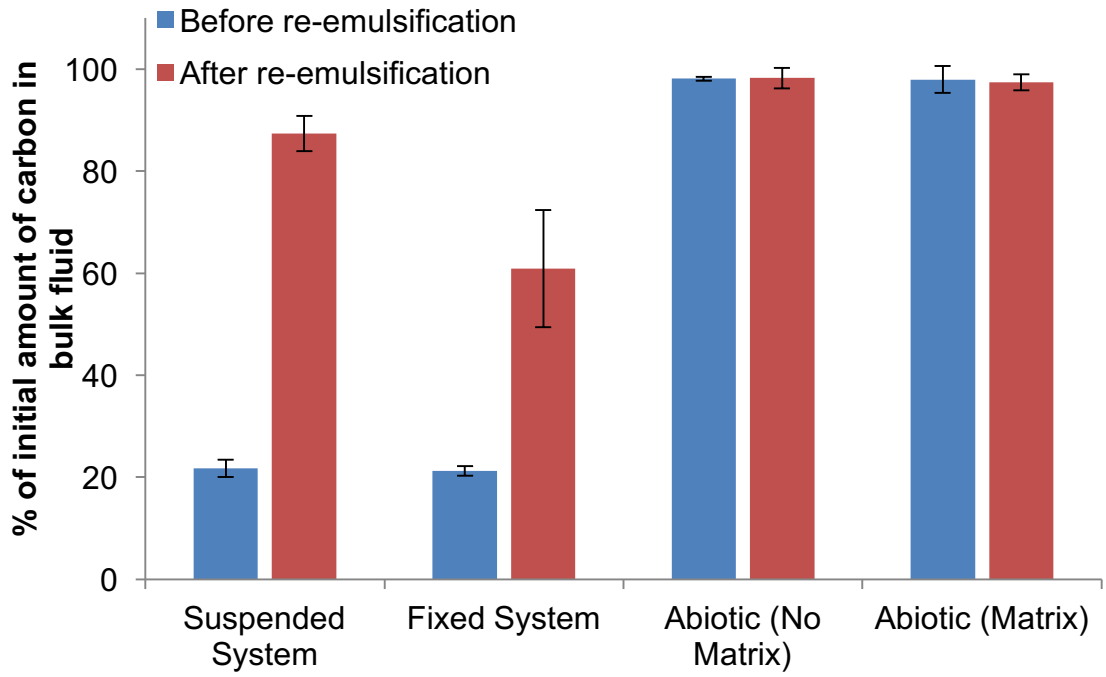

Figure 6-12: % of initial bulk carbon in reactors before and after re-emulsification using 0.1% Triton X-100. Matrices were removed before remulsification was carried out in the fixed systems. Data is presented for the end of the first cycle of treatment. Error bars represent standard deviation of triplicate measurement.

Similar to the results obtained for the soluble oil metalworking fluid, as the bio-treatment process proceeded, an oily-sludge began to appear at the top of the

reactor. Upon re-emulsification of the sludge using the oil-emulsifying reagent Triton X-100, a large proportion of the total carbon was re-dispersed into the bulk liquid. This suggests that, similar to the results obtained in Chapter 3, the predominant mechanism for removal was oil/water demulsification induced by microbial activity (such removal does not occur in the abiotic system). However, the percentage of the initial carbon that was present in the reactor after re-emulsification of the biofilm system is only 60.9% ± 11.5%, which was significantly lower than the 87.4% ± 3.4% in the suspended system ($p<0.01$). This suggests that, over and above oil/water separation, there was an additional mechanism for removal within the fixed systems.

Table 6-1: Carbon partitioning in suspended and fixed systems treating the artificial metalworking fluid. Standard deviations are given for triplicate measurements.

	Total Carbon Removed from Bulk (mg)	% of carbon Removed due to Oil/Water separation (%)	% of carbon removed through adsorption on biofilm	% of unaccounted Carbon removed (including that used for microbe growth and energy)
Suspended System	359.8 ± 7.7	83.9 ± 4.0	N/A	16.1 ± 4.0
Fixed System	362.3 ± 4.2	31.9 ± 13.8	42.7 ± 18.8	25.4 ± 5.0

Upon applying a re-emusification protocol (see 6.3.5) to the biofilm, 42.7% ± 18.8% of the total carbon that had been removed was recovered, suggesting that the de-emulsified oils had been adsorbed onto the biofilm and the polyethylene matrix. Such re-emergence of carbon did not occur in the abiotic system (data not shown). Previous work has shown that Triton X-100 does not cause any structural damage to biofilms grown in metalworking fluid (Adapa et al. 2016); thus, the carbon increase cannot be attributed to lysis or biofilm dissolution. Since Triton X-100 is an oil re-emulsifying agent, it is plausible that the naphthenic mineral oil, together with oil soluble constituents was adsorbed on the biofilm. Table 6-1 presents a comparative

study of the removal contributions of oil/water separation, or adsorption and of other mechanisms (which include growth and energy usage). Unlike the complete characterization that was done for suspended systems in Chapter 3, it was difficult to measure the amount of carbon utilised for biofilm growth and energy usage due to the difficulty in solubilizing the biofilm, and due to the uncertainty associated with the re-emulsification protocol in recovering carbon (no inhibited biofilm control was utilised to determine the efficiency of recovering carbon). Nevertheless, the results in Table 6-1 show that adsorption plays a significant role in the carbon removal mechanism.

6.4.5.2 Organic Constituent Removal Mechanisms

In section 6.4.5.1, it was found that both oil/water separation and adsorption (in the case of fixed-film systems) play a substantial role in the carbon removal mechanisms for metalworking fluid bioreactors. In this section, the same analysis was applied to some of constituents of the artificial metalworking fluid (the fatty amides, DGBE and DIPA). An analysis of this nature will help researchers to understand which metalworking fluid components are removed through metabolic activities, and which are removed through physical processes such as oil/water separation and adsorption. For components that are removed through physical processes, additional treatment steps may need to be applied either to complete the disposal process or to recycle the chemicals since they are not mineralised to

carbon dioxide.

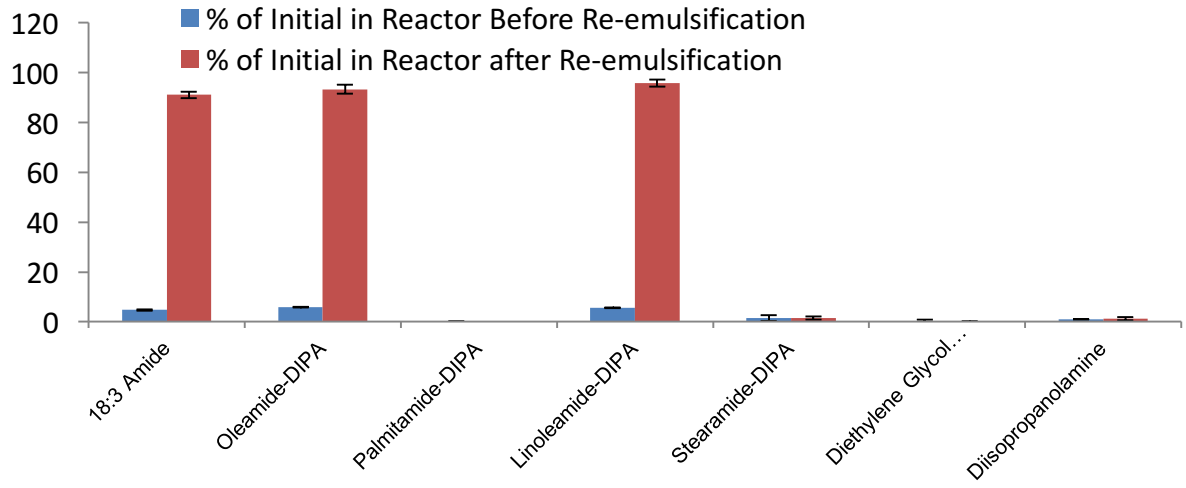

Figure 6-13: % of Fatty Amides, DGBE and DIPA in a suspended bioreactor after one cycle of bio-treatment (5 days). Re-emulsification is done after 5 days using 0.1% Triton X-100. Error bars represent standard deviation of triplicate measurements.

Figure 6-13 shows the percentages of the initial amounts of fatty amides, DGBE and DIPA in a suspended reactor that has treated the artificial metalworking fluid for five days. The reactor was stimulated using 10ppm NH_4Cl and 100 ppm KH_2PO_4. It can be seen that the removal extents for all of the components shown was greater than 94%. However, upon the addition of the oil-emulsifying agent, Triton X-100, there was a significant re-emergence of unsaturated fatty amides which suggests that these compounds were removed from the reactor by the oil phase when the emulsion broke during the biodegradation process. As the reactor fluid contents split into an oil and water phase, the predominantly hydrophobic unsaturated fatty amides partitioned themselves into the oil phase. Addition of Triton X-100 re-dispersed the oil phase, leading to the re-dispersion of the dissolved fatty amides. The percentage of the initial amount present in the reactor increased from 4.8± 0.3% to 91.1% ± 1.3% for the 18:3 amide ($p<0.005$), from 5.8% ± 0.1% to 93.3% ± 1.8% for oleamide-DIPA ($p<0.005$) and from 5.7% ± 0.1% to 95.8% ± 1.4% ($p<0.005$) for linoleamide-DIPA. There were no significant increases for the saturated fatty

amides, for diethylene glycol butyl ether and for diisoproponolamine. This was consistent with the results from Chapter 4, which showed that glycols and amines were not significantly removed through coagulation processes. For these compounds, demulsification was not a mechanism for removal. Inhibited controls for the suspended reactors did not provide substantial removals for any compound (removal was found to be < 1% of the initial amount -data not shown) so it can be concluded that the removal for saturated fatty amides, for DGBE and DIPA was due to metabolic usage for microbial growth and energy. The suspended reactor was able to remove 100% of Palmitamide-DIPA, 99.6% ± 0.5% of DGBE and 99.0% ± 0.2% of DIPA during the bio-treatment process.

The results from Figure 6-13 show that the unsaturated fatty amides within the metalworking fluids were not easily utilised as a carbon source by the microbes, and that removal was due to oil/water separation due to the biodegradation of the soft emulsifiers (i.e the saturated fatty amides). Similar results showing that unsaturated fatty acids were not as biodegradable as saturated fatty acids was shown by Berg et al. (Berg et al. 2002). They showed that unsaturated fatty amides require additional steps in the biodegradation process in order to be oxidised. Saturated fatty amides, DGBE and DIPA were almost completely utilised by the microbes for growth and energy. This was consistent with research which has shown that DGBE and DIPA were readily utilised by microbes in biological processes (Gieg et al. 1999; Greene et al. 1999; Kawai 1995).

In 6.4.5.1, it was shown that in fixed systems, there was a large amount of carbon that partitions itself onto the biofilm during the biodegradation process. This was hypothesized to be due to the adsorption of naphthenic mineral oil onto the biofilm. Since it has been shown that the major removal mechanism for unsaturated fatty

amides was due to partitioning into the oil phase after oil/water separation, it was likely that there will be a partitioning of amides onto the surface of the biofilm of fixed-film reactors. The application of the re-emulsification protocol for adsorbed oil on the biofilm (see section 6.3) led to a re-emergence of unsaturated fatty amides, but did not lead to a re-emergence of saturated fatty amides, DGBE or DIPA. This is shown in Table 6-2. The percentage of removal attributed to adsorption for the unsaturated amides ranged between 26.7% ± 1.5% (for Oleamide-DIPA) and 29.0% ± 0.9% (for Linoleamide-DIPA). This suggests that, along with the adsorption of mineral oil, the biofilms are also able to retain the components that partition into the oil phase. Further analysis shows that, similar to the case with suspended bioreactors, there were large proportions of unsaturated fatty amides that are removed through demulsification (ranging from 59.5% ± 3.0% for Linoleamide-DIPA and 60.6% ± 2.5% for the 18:3 amide).

Table 6-2: Removal extents and removal mechanisms of Fatty amides (with Diisopropanolamine groups), Diethylene Glycol Butyl Ether and Diisopropanolamine in fixed film reactors treating artificial metalworking fluids. Data is given for the summation of two cycles of treatment (5 days each). Standard deviations are given for triplicate measurements.

	% of initial removed from reactor	% of removal attributed to adsorption	% of removal due to demulsification	% of removal unaccounted for (includes microbial usage for growth and energy)
18:3 Amide	83.9% ± 1.3%	28.3% ± 2.8%	60.6% ± 2.5%	11.2% ± 5.3%
Oleamide-DIPA	84.9% ± 0.3%	26.7% ± 1.5%	60.3% ± 0.6%	13.0% ± 2.1%
Palmitamide-DIPA	100% ± 0%	0% ± 0%	0% ± 0%	100% ± 0%
Linoleamide-DIPA	79.6% ± 2.2%	29.0% ± 0.9%	59.5% ± 3.0%	11.6% ± 3.8%
Stearamide-DIPA	99.2% ± 0.02%	2.9% ± 0.3%	0.4% ± 0.2%	96.7% ± 0.5%
DGBE	99.6% ± 0.01%	0% ± 0%	0.1% ± 0.1%	99.9% ± 0.1%
DIPA	98.8% ± 0.2%	3.1% ± 1.3%	0.9% ± 0.2%	96.1 ± 1.1 %

Thus, like suspended reactors, the main mechanism for removal of unsaturated amides was due to physical processes induced by microbial metabolic activities, rather than by actual microbial activities themselves. This was not found to be the case for the saturated amides, for DGBE and for DIPA, for which the majority of removal was attributed to un-accounted for mechanisms (which include microbial activity or direct adsorption of the components on the biofilm). While the relative importance of the mechanisms of direct adsorption onto the biofilm and metabolic activities were not determined, there was evidence which suggests that most of the unaccounted for mechanisms were due to microbial utilisation. Firstly, this is because it was shown microbial activities were more important than adsorption for the case of the suspended reactors (by using an inhibited control), and because it was unlikely that microbes would stop utilizing these carbon sources when switching to fixed-film modes of growth. This, in turn, was because the microbes would not switch to a mode of growth which would inhibit their utilisation of carbon sources. Secondly, upon dissolution of the biofilm in DGBE, there was no recovery of saturated fatty amides, suggesting that there were no saturated fatty amides on the biofilm. Thirdly, since the saturated and unsaturated amides have very similar chemical structures, it was unlikely that the extents of direct adsorption would vary considerably between these compounds. Finally, Figure 6-11 shows that DIPA removal by biofilms was dependent on the ammonium chloride concentration of the reactor, suggesting that there was a metabolic mechanism, rather than a physical mechanism associated to removal. The conclusion that can be made with absolute certainty was that demulsification is not responsible for the removal of saturated amides, DGBE and DIPA, and that the re-emulsification protocol does not recover substantial amounts of these components.

The results in the section showed that de-emulsification was a major mechanism of removal for unsaturated fatty amides in suspended reactors, and that both demulsification and adsorption of demulsified oils was the mechanism of removal in fixed-film reactors. Since these components are removed but not degraded in the reactors, there exists potential to recycling of these components as re-usable surfactants. Feasibility studies into separating oil from unsaturated fatty amides and into formulating metalworking fluids with recycled surfactants should be researched to see if there is value in recovering these products. The alternative would be that of ensuring that an alternative disposal process is put into place for the treatment of demulsified oils and the chemicals contained within.

6.4.6 Application to Metalworking Fluids with Biocides

In order to determine if the fixed-film reactors that were developed in both this Chapter and in Chapter 5 were effective at treating metalworking fluids which contain biocides in their formulation, developed reactors were exposed to varying concentrations of the biocide sodium ortho-phenyl phenate. This biocide was chosen as a core part of the investigation since it was shown (in Chapter 4) that this biocide was not completely removed through the pre-treatment process of coagulation/coalescence (for certain concentrations). Thus, it was imperative to understand if the fixed-film reactors were able to remove organic constituents in the presence of this biocide.

6.4.6.1 Toxicity of Na-OPP

In Chapter 4, toxicity levels for Na-OPP as a function of concentration was given. The toxicity tests were conducted using propylene glycol, a compound for which degradation data was readily available (Bausmith & Neufeld 1999; Toscano et al. 2013). In order to understand if Na-OPP was able to have a significant impact on

the degradation activity of the artificial metalworking fluid used in this chapter, the MicroResp™ and Post-Exposure Recovery tests were repeated using the metalworking fluid as the sole carbon source.

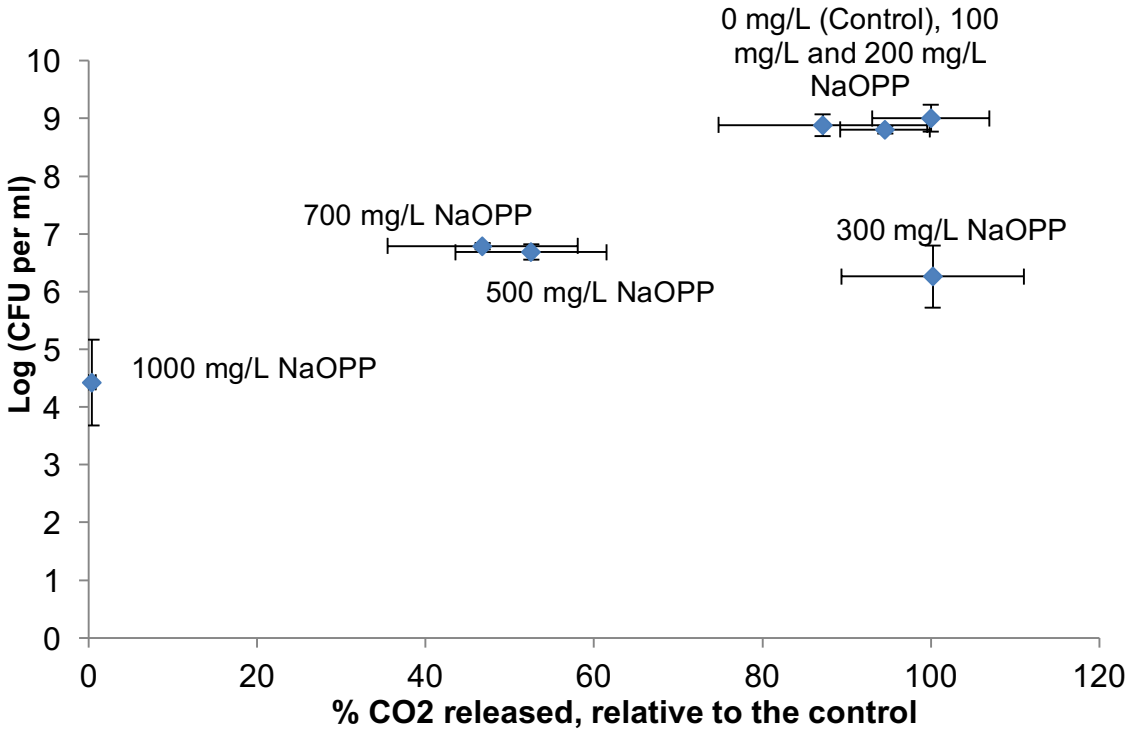

Figure 6-14: MicroResp™ and Post-Exposure Recovery results as a function of biocide (sodium Orthophenyl phenate) concentration in 2.5% MWF. Each well contained 200 mg/L NH_4Cl, 100mg/L KH_2PO_4, 2.5% MWF, and the stated concentration of biocide. Controls did not contain biocide. Error bars are given as standard deviations of triplicate measurements.

Figure 6-14 shows that increasing the concentration of Na-OPP in the metalworking fluid had significant inhibitory effects on both the microbial respirational activity ($p<0.01$) as well as the growth and post-exposure recovery ($p<0.01$). At 1000 mg/L, all microbial activity during the test ceased, but a portion of the micro-organisms were able to survive the test indicating that there was not a significant dosage to kill them. It was expected that dosages above 1000 mg/L would result in more killing, so 1000mg/L was chosen as the minimum dosage to apply to the fixed-film reactors.

6.4.6.2 Organic Removal in the Presence of Na-OPP

Metalworking fluids containing 1000 mg/L , 2500 mg/L and the maximum recommended dosage of 5000 mg/L of biocide Na-OPP were fed into fixed-film reactors that had developed over 3 cycles of growth (see methods). The removal of oil-partitioning components (tall oil fatty amides), water-partitioning components (DGBE and DIPA) and the biocide itself was measured over 6 days of treatment.

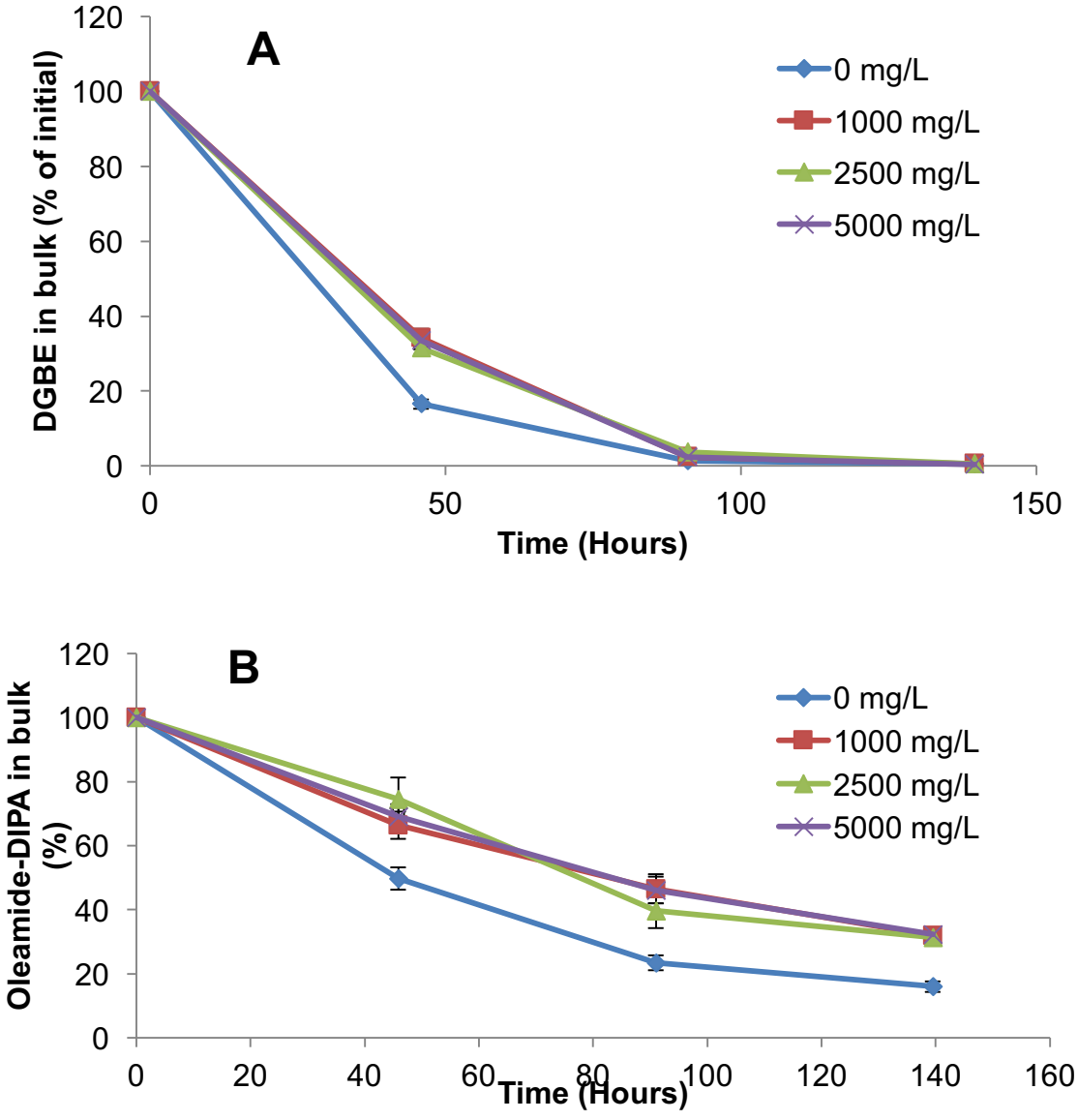

Figure 6-15: A) Diethylene Glycol Butyl Ether and B) Oleamide-DIPA removal by developed fixed-film reactors as a function of Sodium Orthophenyl phenate concentration in 2.5% MWF. Error bars represent standard deviation of triplicate measurements.

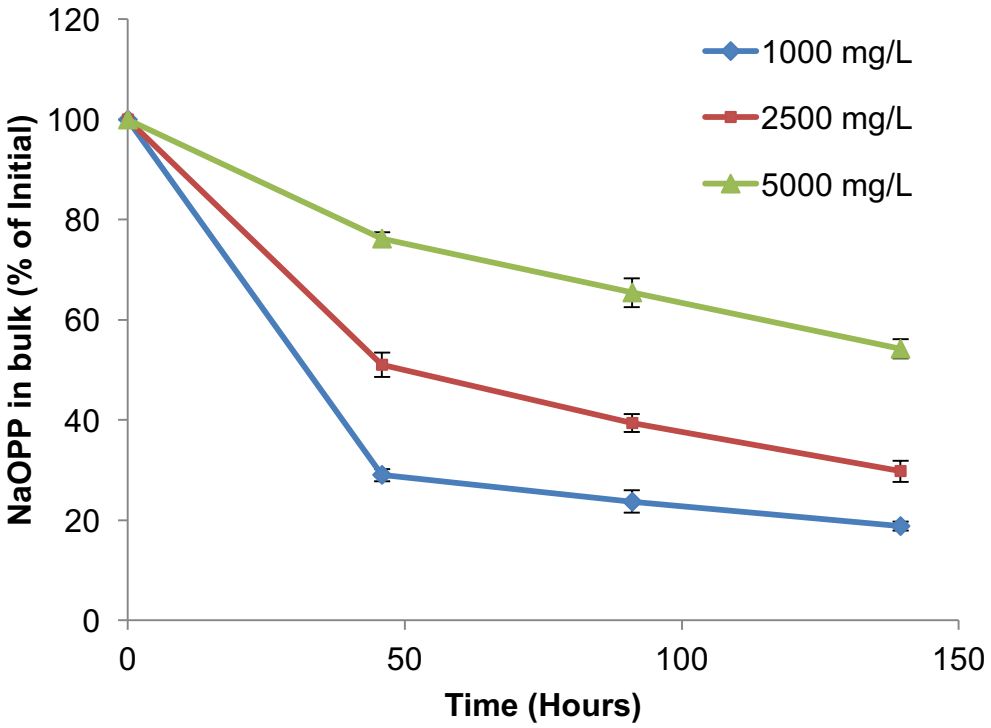

Figure 6-16: Sodium Orthophenyl phenate removal by developed fixed-film reactors as a function of its concentration in 2.5% MWF. Error bars represent standard deviation of triplicate measurement.

Results given in Figure 6-15 indicated that the rate of removal of DGBE and Oleamide-DIPA in systems containing Na-OPP was smaller than that of systems with no Na-OPP. It can be seen from Figure 6-15A, after 2 days of treatment, the amount of DGBE remaining in the bulk liquid of the reactor containing no Na-OPP was 16.5% ± 1.2%, which was significantly lower than the amounts present in the reactor systems containing Na-OPP (which ranged from 31.5% ± 1.6% to 34.2% ± 2.4%, $p<0.01$). Similar results were shown for Oleamide-DIPA, DIPA and the other tall oil fatty amides (data not shown). However, the presence of the biocide had no significant effect on the extent of DGBE removal obtained at the end of the biotreatment process (percentages left in the bulk liquid ranged from 0.41% ± 0.13% to 0.58% ± 0.11%, $p = 0.467$). Similar removal extents were achieved even at the maximum dosage of 5000 mg/L, even though MicroResp™ results of Figure 6-14 suggested that only 1000 mg/L was required for the inhibition of the microbes. Thus, the biocide only hindered the rate of the biodegradation process within the fixed-film

reactors, but did not inhibit the reactor functioning. This may have been due to the enhanced resistance mechanisms that fixed-film reactors have towards hazardous chemicals (Tziotzios et al. 2005; Nicolella et al. 2000).

However, for the time-scale investigated, the extent of removal of Oleamide-DIPA was significantly higher in the reactors without Na-OPP as compared to those with Na-OPP (the amount of oleamide remaining in the reactor with no Na-OPP was 16.0% ± 1.7%, while those with Na-OPP had between 31.4% ± 1.5% and 32.3% ± 2.0% remaining, $p<0.01$). This difference may be due to the fact that unsaturated fatty amides were more difficult to degrade in this system as compared to DGBE (see Figure 6-13 and Table 6-2). Thus, the presence of biocides impacted the removal rates and extents of bio-resistant compounds as compared to bio-supporting compounds (similar trends were observed for DIPA, saturated fatty amides, and other unsaturated amides, data not shown). Even though there was a significant reduction in the removal extents as compared to the control, there were no significant differences in increasing the concentration of the biocide above 1000 mg/L ($p=0.8$). This once again may have been due to the enhanced resistance mechanisms present within the fixed-film reactor.

Figure 6-16 shows the removal of the Na-OPP as a function of its starting concentration in the fixed-film reactor. It can be seen that as the concentration increased from 1000 mg/L to 5000 mg/L, the amount of biocide remaining within the reactor significantly increased from 18.8% ± 0.9% to 54.2% ± 1.9% ($p<0.01$). Thus, the removal extent significantly declined with increasing biocide concentration. Possible explanations for this could be that the biocide was self-inhibiting, or that the removal mechanism was predominantly due to oil/water separation and adsorption (which would decline with increasing concentration biocide)

Nevertheless, the results from this section of the chapter indicated that, while the performance of the fixed-film reactors may be affected by the presence of biocides, they were still capable of removing organic constituents, albeit at a lower rate.

6.5 Discussion

In this chapter, it is shown that nutrient stimulation and limitation has a significant effect on biofilm formation and reactor performance. In order to stimulate performance of the reactor, phosphates should be supplied and ammonium should be limited. This is because microbes would be able to utilise the amines as a carbon source within the metalworking fluid, negating the need for ammonium stimulation. An oversupply of ammonium resulted in a decline in the carbon removal performance since amine degradation was inhibited, and since the emulsion in the metalworking fluid was not completely destabilised. Evidence provided in this chapter suggested that the emulsion was not destabilised due to the possibility of biosurfactants being present in the system. Another disadvantage of ammonium stimulation would be the need to remove excess ammonium from the reactor via a downstream process. Thus, it is recommended that ammonium stimulation be minimized for metalworking fluids which contain amines as a possible source of nitrogen.

A further benefit of ammonium limitation is that it resulted in the formation of biofilms at greater yields than reactors having ammonium in excess. Thus, the dual benefit of higher reactor performance and the development of biofilms may be exploited through ammonium limitation.

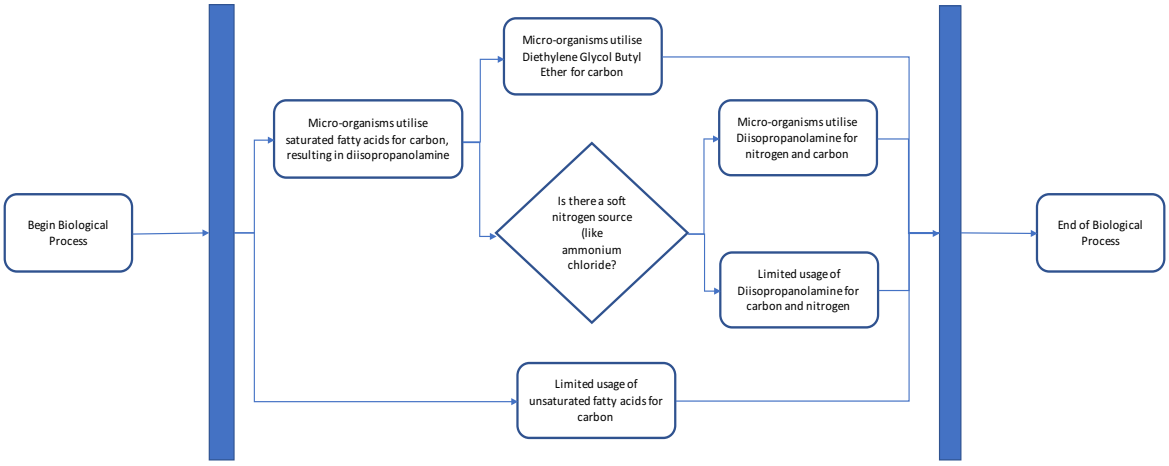

Figure 6-17: Summary of hypothesized sequence for the biological removal of contaminants within the artificial metalworking fluids.

Within this chapter, it was shown that components within metalworking fluids are utilized within a specific sequence. Figure 6-17 shows the summary of this sequence. Saturated fatty amides, being the most biodegradable component within the artificial waste created, was the first to be utilised by the micro-organisms. This resulted in the production of free amines. Next to be utilised was Diethylene Glycol Butyl Ether, and if there was no alternate nitrogen source (provided via ammonium chloride), then Diispropanolamine was utilised in the same timescale. When ammonium was present, DIPA was not effectively utilised. Furthermore, it was found that unsaturated amides were not effectively utilised in the process.

Research in this chapter also showed that it is primarily the utilisation of tall oil fatty amides that were responsible for the formation of biofilms. Practitioners who wish to develop a biofilm reactor for the treatment of metalworking fluid waste should thus use a waste stream that has a high concentration of fatty amides to promote biofilm formation during start-up or after a shock load. Furthermore, since unsaturated fatty amides appear to accumulate in the biofilm, there is a possibility for extraction, recovery and recycling of this component.

Finally, it was also shown in this chapter that the fixed-film reactors developed under ammonium limiting conditions were unaffected by the biocide present within the system. While the mechanism for resistance was not studied, evidence in this chapter shows that fixed-film reactors are capable of treating formulations that contain biocides at inhibitory concentrations. Practitioners may thus use the information provided in this chapter to cultivate biofilms in order to improve the resilience of their metalworking fluid reactor systems.

The biocide Na-OPP was not effectively removed by the biotreatment process at the maximum dosage concentration. An alternative treatment technique may need to be applied in order to ensure that the effluent water is able to meet discharge regulations.

6.6 Conclusions

The role of ammonium chloride and potassium phosphate associated with biofilm development and reactor performance were investigated.

- Biofilm yields were sensitive to both ammonium chloride and potassium phosphate concentrations. Biofilm yields were found to be significantly higher when one of the components was present in relatively low quantities. When both components were deficient in the metalworking fluid, significantly lower biofilm yields were obtained. Maximum biofilm yields were obtained in ammonium chloride deficient conditions.
- While ammonium chloride increased the rate of removal of saturated amides and DGBE, it was shown to inhibit the removal of diisopropanolamine in the reactor. This suggested that

diisopropanolamine was utilised as a nitrogen source under conditions of ammonium limitation.

- Reactor total carbon removal performance was shown to increase with increases in potassium phosphate concentration and to decrease with increases in ammonium chloride concentrations. While the removal of carbon in the bulk was shown to decline with increases in ammonium chloride concentration, the absolute removal of carbon through mineralization was shown to be similar. This suggests that addition of ammonium chloride led to more stable emulsions in the reactor, possibly due to inducing the production of biosurfactants. This would explain the decline and re-emergence of unsaturated fatty amides that are observed for high ammonium chloride concentrations.
- Upon dissolution of the biofilm in DGBE, unsaturated fatty amides were recovered, suggesting that these components are incorporated into the biofilm structure.

Furthermore, the mechanisms of removal for both carbon and the organic constituents of the metalworking fluids were investigated.

- For suspended systems, a large proportion of carbon is removed due to demulsification and oil water separation. This suggests that the emulsifiers are degraded within the biotreatment process
- For fixed-film systems, the removed carbon was found to be partitioned onto the biofilm and in the oil phase on the top of the reactor. This suggests that demulsified oils are adsorbed onto the biofilm during the biotreatment process.

- For suspended systems, unsaturated amides were found to be mainly removed through demulsification and oil/water separation. Saturated amides, DGBE and DIPA were found to be removed through microbial Utilisation.
- For biofilm systems, removed unsaturated amides were found to be partitioned onto the biofilm and in the oil phase on the top of the reactor. Like the suspended system, DGBE, DIPA and the saturated amides were likely removed due to microbial activity.

Finally, an application of the fixed-film reactors to formulations containing biocides was made:

- The presence of the biocide Na-OPP at concentrations deemed to be inhibitory by the MicroResp™ and Post-Exposure Recovery test significantly slowed down the biotreatment process. Removal of unsaturated fatty amides was more affected than removal of DGBE and DIPA.
- The reactors were not inhibited by concentrations deemed to be inhibitory by the MicroResp™ and Post-Exposure Recovery Tests.
- Biocide removal efficiencies declined with increasing biocide

Chapter 7- Conclusions and Future Work

7.1 Conclusions

Due to tightening regulations by environmental protection agencies, traditional means of disposing of spent metalworking fluids are no longer environmentally acceptable. The biological treatment of waste metalworking fluids is a cost-effective alternative to traditional disposal techniques. The biological treatment process is capable of removing vast amounts of carbon (and hence COD) from large volumes of wastewater. However, there are uncertainties in the mechanism by which carbon is removed from the wastewater, and the bioreactors are susceptible to anti-microbial agents.

This project firstly aimed to determine the mechanisms by which carbon was removed from bioreactors treating oil-containing metalworking fluids. It then proceeded to determine strategies which could be used to mitigate the effects of toxicity caused by biocides within metalworking fluid formulations. One of the strategies that were investigated was to remove the biocides via a pre-treatment process which involved coagulation/coalescence. The second strategy was to make use of fixed-film bioreactors, instead of suspended reactors, in order to increase resistance to biocides. Physico-chemical and nutritional influences were investigated in order to optimize bioreactor development and pollutant-removal performance.

7.1.1 Mechanisms for Carbon Removal in Bioreactors Treating Soluble Oil Metalworking Fluids

In order for the biological treatment process to be an effective option for treating waste metalworking fluids, the waste streams needed to be diluted in order to reduce the effects of anti-microbial agents. It was found that dilution of metalworking fluids with relatively hard waters resulted in emulsion destabilisation. It was shown in Chapter 3 that dilution of Castrol CoolEdge BI with a water containing 240 ppm of $CaCO_3$ hardness resulted in 69.4% ± 3.6% of carbon removal in 3 days. Thus, physico-chemically induced oil/water separation may occur within bioreactors and may be a contributory mechanism for carbon removal.

The main mechanism for carbon removal within live bioreactors was found to be oil/water separation, even in instances where physico-chemical destabilisation did not occur. Within bioreactors treating Castrol CoolEdge BI, oil/water separation induced by microbial activity accounted for 67.9% of the total amount of carbon removed from the system while an estimation of carbon removed through metabolic activity is found to be 30.7%.

Since the main mechanism for removal was found to be oil/water separation, carbon removal rates and efficiencies were found to be dependent on the biodegradability of surfactant packages. Metalworking fluid formulations containing only sodium sulfonate (a biostable surfactant) were not amenable to treatment. Formulations using a surfactant package that consisted of biosupportive tall oil fatty amides were found to treatable using the bioprocess. This suggests that a key determinant on whether an oil-containing metalworking fluid may be treated using the bioprocess was the biodegradability of the surfactant.

Another implication of oil/water separation being the main mechanism for removal was that rate of carbon removal was dependent on the water hardness of the wastewater, even when physico-chemical removal does not occur. For an artificial metalworking fluid waste, bioreactor treatment efficiencies were found to be 84.4% ± 2.3% for water hardness values of 180 ppm, and only 38.4% ± 4.1% water hardness values of 30ppm. This suggests that wastewater practitioners may artificially harden metalworking fluid wastewaters in order to improve reactor performance.

A final implication of oil/water separation being the main mechanism of removal of carbon was that an oily sludge containing recalcitrant hydrocarbons and microbes was produced as a by-product of the process. This sludge will continue to accumulate within sequential batch biofilm reactors and will eventually need to be disposed. Thus, the biological treatment of oil-containing metalworking fluids would incur an additional cost for the treatment of this sludge.

7.1.2 Coagulation and Coalescence as a Pre-treatment Process for the Removal of Biocides from Metalworking Fluids

There were two disadvantages to directly utilising the biological treatment process for oil-containing metalworking fluids. Firstly, the treatment resulted in an oily sludge that was contaminated with micro-organisms. Secondly, the treatment could only proceed if the metalworking fluids are diluted enough so that the anti-microbial activity was sufficiently suppressed. Coagulation and coalescence was employed as a pre-treatment process in order to address these challenges.

The critical coagulation concentration of conventional inorganic coagulants was determined, and applied in order to separate artificial metalworking fluids

(containing no biocides) into distinct oil and water phases. While this process led to significant removals of oil-partitioning organics such as tall oil fatty amides, it was not effective at removing water-partitioning components such as diisopropanolamine and propylene glycol. This created the need for the biological treatment process. The toxicity of the resulting water phase, which contained residual coagulant, and the toxicity of the coagulants themselves were determined. It was found that neither the coagulants, nor the water phase of the metalworking fluid was toxic, so it could be concluded that the coagulation/coalescence process itself did not result in any toxicity.

The artificial metalworking fluid was then made toxic through the addition of different concentrations of three conventional biocides (namely sodium orthophenyl phenate, iodopropanyl butyl carbamate, and benzisothiazolinone). The ability of the coagulation/coalescence process in removing each one of these biocides was determined.

It was found that the coagulation/coalescence process was more effective at removing oil-partitioning biocides (such as Na-OPP) than water-partitioning biocides (such as benzisothiazolinone). For Na-OPP it was found that the removal efficiency was dependent on the amount of oil that was present in the formulation, and that the removal efficiency declined as the ratio of the amount of oil to biocide decreased. This suggested that the oil phase acted as an extractant during the coagulation/coalescence process, and removed the biocide from the water as phase separation occurred. For the metalworking fluid formulation investigated, a removal efficiency of 61.1% ± 1.8% was achieved at the maximum recommended dosage of 5 g/L. This removal efficiency increased in formulations containing concentrations smaller than the maximum concentration. The

coagulation/coalescence process was able to significantly reduce the toxic effects of formulations containing up to 1 g/L of Na-OPP, but was not effective at concentrations at or greater than 2.5g/L. This means that further treatment may be required to further reduce the toxicity if necessary.

For IPBC, the coagulation process was able to achieve relatively high removal efficiencies in the concentration range investigated. At the maximum recommended dosage of 1g/L, the removal efficiency attained was 74.6% ± 2.3%. Converse to what was observed for Na-OPP, the removal efficiency of IPBC declined as the concentration within the formulation was lowered. This was because IPBC had a poor solubility in the water phase, and at formulations greater than the solubility limit, precipitation had occurred as an additional mechanism of biocide removal. Thus, the coagulation/coalescence process is also highly effective at removing biocides with limited water solubility. The coagulation/coalescence process was able to significantly reduce the toxicity effects of all concentrations investigated (up to the maximum recommended dosage of 1g/L).

Benzisothiazolinone was an example of a biocide which had a moderate water solubility and was water-partitioning, and thus the coagulation/coalescence achieved relatively low removal efficiencies at the concentration range investigated (an efficiency of 12.3% ± 0.9% was achieved for a concentration of 800 mg/L of biocide). The coagulation/coalescence process was not effective at reducing the toxicity of formulations containing these biocides in concentrations ranging from 200mg/L to 800 mg/L (which was well- below the maximum recommended dosage of 3.2g/L).

It can be concluded that coagulation/coalescence as a pre-treatment strategy was effective at removing oil-partitioning biocides, as well as those with limited water solubility. It was not effective at removing water partitioning biocides with moderate water solubility.

7.1.3 Physico-chemical Factors Influencing the Development and Performance of Fixed-film Reactors

In Chapter 4, it was shown that the coagulation/coalescence process was capable of removing oil-partitioning biocides, as well as those with limited water solubility. However, it was not always effective at reducing the toxic effects that the metalworking fluid formulation had on the micro-organisms used for the treatment process. Thus, Chapters 4 and 5 focussed on the development of fixed-film reactors since such bioreactors are known to be more effective at treating hazardous wastes.

Chapter 4 focussed on the physico-chemical influences on fixed-film reactor development and performance. It was found that temperature, pH and airflow had a significant effect on the development of bacterial biofilms on the polyethylene matrices within the bioreactors. Increases in temperature from 20ºC to 30ºC resulted in significant carbon removal efficiencies (from 45.5% ± 0.7% to 80.6% ± 1.0%). While there were no significant increases in the biofilm yield between 20 to 27 ºC, the biofilm yield significantly increased between 27 ºC and 30 ºC. This suggests that temperature can be used as a means to control the amount of fixed biomass that develops in a reactor, but lower temperatures would result in lower carbon removal rates.

A different trend was observed for the pH of the reactor. For the pH investigations, it was found that the starting pH had a significant effect on both the reactor

performance and the biofilm development, but that the optimum reactor performance was achieved over a range of starting values (pH 7 to 9). Starting pH values lower or higher than this range resulted in lower carbon removal efficiencies. Unlike the carbon removal efficiencies, the biofilm yield obtained an optimum at starting pH of 8, and was found to decline at the other starting pH values investigated. These results suggested that pH can be a useful parameter that can control biofilm biomass without significantly influencing reactor performance.

Finally, for the range of airflows investigated, no significant reduction in biomass was observed. However, the a trend of declining biomass with increased airflow was observed, so it may be possible that large air-flow rates could result in significant losses of biofilm biomass due to the induced shear stress.

7.1.4 Nutritional factors influencing the development and performance of fixed-film reactors

Having determined the physico-chemical influences on fixed-film reactor development and performance, Chapter 6 explored the effects of nutrient stimulation and carbon sources on reactor development and performance.

It was shown that both ammonium and phosphate stimulation had significant effects on both biofilm development and reactor performance. Adding one of the nutrients in excess and limiting the other was found to be the best strategy for maximizing the biofilm yield obtained (having both in excess, or both limiting did not result in optimum biofilm yields). However, when phosphates were limited, there was a significant decline in reactor carbon removal performance. Limiting ammonium did not result in such a decline. Thus, waste practitioners should provide phosphates and limit ammonium supply to bioreactors for the promotion of fixed-film reactors.

Furthermore, in Chapter 6, it was shown that ammonium stimulation resulted in a significant decline of carbon removal performance in the bioreactors. Further chemical analysis showed that this occurred even though the stimulation improved the rate of removal of saturated fatty amides, and of diethylene glycol butyl ether. However, the addition of ammonium resulted in the re-dispersal of unsaturated fatty amides, and the inhibition of diisopropanolamine. The inhibition of diisoproponalamine suggested that the amines within the metalworking fluid were utilised as a nitrogen source and its utilisation was suppressed in a nitrogen-rich environment. The re-dispersal of amides, together with the result that the extents of ultimate carbon removal in ammonium-rich and ammonium-deficient reactors were similar, suggested that ammonium stimulation may promote the formation of biosurfactants within the system. This would result in an increase in emulsion stability, leading to reduced carbon removal rates (since the main mechanism for removal is oil/water separation).

Unlike the results obtained for ammonium stimulation, stimulation with phosphates resulted in improved performance of carbon removal. This is likely due to the fact that there were no phosphate sources present within the metalworking fluid formulation.

When the micro-organisms of the bioreactor were fed with the different individual components of the metalworking fluid, it was found that fatty amides resulted in the highest yield of biofilm. This suggests that formulations of metalworking fluids which contain Tall Oil Fatty Amides were likely to result in biofilm growth. Furthermore, it was shown that there was a small fraction of tall oil fatty amides that were accumulated within the biofilm, creating the potential for reagent recovery.

7.1.5 Application of fixed-film reactors to treating metalworking fluids containing biocides

Having determined the optimum conditions that were required for the development of fixed-film reactors, the reactors were developed and applied to the treatment of simulated metalworking fluid waste streams that consisted of varying amounts of inhibiting sodium o-phenyl phenate concentrations.

It was first determined that concentrations greater than 1000 mg/L were inhibitory to the micro-organisms that were used for the treatment process, and formulations containing concentrations greater than 1000 mg/L were used for the treatment process.

It was shown that the presence of biocides reduced the rate of removal of the organic constituents present within the wastewater as compared to a control which did contain biocides. However, even at the maximum recommended concentration of 5000 mg/L, the reactors were able to achieve relatively high removal efficiencies for the organic constituents within the metalworking fluid (practically complete removal of DIPA, DGBE and saturated fatty amides and 67.3% ± 2.0% for oleamide-DIPA). There were no significant differences in the removal rates between 1000mg/L of biocide, and 5000 mg/L of biocide. These results showed that the fixed-film reactor was able to remove pollutants in the presence of biocides and was thus capable of treating hazardous wastes.

The biocide Na-OPP was also removed by the bioreactor, but removal efficiencies significantly declined as the concentration of Na-OPP increased. At 1000mg/L, a removal efficiency of 81.2% ± 0.9% was achieved, but this declined to 45.8% ± 1.9% at the initial concentration of 5000 mg/L. Thus, the microbial treatment by itself is

not sufficient at removing large concentrations of biocides, prompting the need for pre-treatment or post-treatment.

7.2 Recommendations for Future Work

7.2.1 On the Direct Treatment of Oil-containing Metalworking Fluids

- It was shown in Chapter 3 that main removal mechanism for carbon removal in the bio-treatment of oil-containing metalworking fluids was demulsification and oil/water separation. There needs to be a study to understand the simultaneous kinetics of surfactant degradation, coalescence and oil-water separation. Developing this model can lead to optimized reactor designs, as it is likely that removal rates are significantly affected by the geometry of reactors.
- Since the direct biotreatment of oil-containing metalworking fluids results in an oily-sludge, further treatment options need to be explored. Incineration or disposal to a landfill may not be environmentally acceptable, thus a more sustainable means of disposing of this sludge is required. One possible means of disposal is long-term biodegradation using micro-organisms that have an affinity for degrading mineral oil.
- A feasibility study of directly comparing the costs of utilizing the direct treatment of metalworking fluids against the Utilisation of a pre-treatment programme would need to be done in order to gauge whether the direct treatment process is a feasible option.

7.2.2 On coagulation/coalescence as a pre-treatment

- There is a need to develop a means of recycling coagulant to counter the disadvantage of constant chemical addition. If a process utilises coagulation

and the biotreatment process, then a possible means of recycling dissolved coagulant is to employ a micro-filtration unit after the biotreatment process. This will retain the micro-organisms, but will allow for coagulant to pass through. Studies on the accumulation of recalcitrant material, as well as the potential savings of adding a membrane process need to be conducted in order to see if this is a feasible action.

- There is a need to study if mixing soluble oils, (which have high concentrations of mineral oil) and waste streams with little or no mineral oil, is an effective means of removing oil-partitioning biocides from semi-synthetic wastes. The soluble oil metalworking fluids would in effect act as an extractant liquid which may be able to reduce biocide and organic concentrations from waste streams that are difficult to treat biologically.

7.2.3 On the Removal of Biocides

- The coagulation/coalescence process was not effective at completely removing Na-OPP and Benzisothiazolinone from the simulated metalworking fluid waste streams. An alternative treatment strategy would need to be identified either as a pre-treatment to the biological process, or as a post-treatment succeeding the biotreatment process. One possible means solution for removal is to employ surfactant-modified bentonite as an adsorbent targeting the phenolic groups present on these compounds. Should this work, it would serve to complement the treatment process that is already developed.

7.2.4 On the Recovery of Amides

- It is shown that the biofilms are capable of accumulating unsaturated fatty amides. Research into the recovery and reuse of these surfactants may prove useful in making the use of metalworking fluids a closed cycle.

7.2.5 On the Application to Real Waste

- All results and trends here need to be repeated on real waste streams in order to ensure that the results are valid for industry. A real metalworking fluid waste stream should be sourced, and the trends, especially with regards to nutrient stimulation, should be validated before recommendations are applied to real waste streams

7.2.6 On the Resilience of Biofilms to Toxic Shocks

- The data and results found during this project show that biofilm biomass can be controlled using physico-chemical and nutritional factors. A study showing how biomass can be manipulated in a reactor, especially after receiving toxic shock loads, would be useful in providing operators information on what would need to be done in the unexpected inhibitory event.

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