PERSISTENCE AND FATE OF ACIDIC HYDROCARBONS IN AQUATIC ENVIRONMENTS: NAPHTHENIC ACIDS AND RESIN ACIDS

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PREFACE

This thesis is a "manuscript-style thesis" as noted in the *Policy and Procedure Manual* September 1996 (Revised September 1998, November 2002) of the College of Graduate Studies and Research (CGSR), University of Saskatchewan and has been formatted to conform to the *Guide for the Preparation of a Thesis* May 2000 published by the CGSR, U of S.

The first chapter (Chapter 1) provides a general introduction to the thesis and outlines the background and main objectives of the research. Chapter 1 describes the remediation methods applied in this research. The industries involved in the research project are outlined in general terms. As well, the environmental impacts evaluated in the study (i.e., bioavailability and toxicity) are described.

Chapters 2 and 3 describe the research conducted with respect to the naphthenic acid hydrocarbons including:

2. development of a negative-ion electrospray ionization LC/MS analytical method and
3. an investigation and evaluation of the photolytic fate and persistence of naphthenic acid compounds and mixtures in the aquatic environment.

Chapters 4 through 6 describe the research conducted with respect to the fate and transport of pulp and paper-based resin acid hydrocarbons. This includes:

4. evaluation of a negative-ion electrospray ionization LC/MS analytical method for four selected resin acids;
5. observation of the fate, transport and persistence of resin acids and their distribution in the River Saale, Germany downstream of a large pulp and paper mill and
6. investigation of the photolytic and biological fate and persistence of aqueous resin acids in the environment.

The final chapter (Chapter 7) comprises a general discussion summarizing the results of the combined research, discussing the ecological and industrial significance of the results, assessing the research approach and proposing directions for future research. The final chapter is a compilation of all references for the previous chapters as required by the University of Saskatchewan guidelines for preparation of a thesis.

Each of Chapter 2 through 6 includes its own introduction, experimental section, subheadings and acknowledgements in accordance with the Guidelines for Preparation of a Thesis published by the College of Graduate Studies and Research, University of Saskatchewan. Avoidance of redundancy has been attempted in all instances. References have been collated at the end of thesis in Chapter 8.

The authorship and current publication status of each scientific manuscript-chapter is indicated on the first page of the respective chapter.

- Chapter 1 naphthenic acids review manuscript is to be published under joint authorship with John V. Headley (in press)
- Chapter 2 was published with co-authors John V. Headley, Kerry M. Peru and Marcus Winkler
- Chapter 3 was published with co-authors John V. Headley, Duane A. Friesen and Jon A. Gillies
- Chapter 4 was published under joint authorship with John V. Headley, Kerry M. Peru, Marcus Winkler and Jon A. Gillies
- Chapter 5 was published under joint authorship with Wolf von Tümpling, John V. Headley and Jon A. Gillies
- Chapter 6 was published with co-authors John V. Headley, Thomas R. Neu and Duane A. Friesen

Co-authors have provided technical assistance, helpful suggestions on research design or editorial recommendations on earlier drafts of the manuscripts. The content and ideas of all manuscripts and chapters are mine.

ABSTRACT

The novel application of combination, or two stage, photochemical and microbial degradation systems for removal of resin acids from natural river water and single stage photolysis for degradation of naphthenic acids in natural river water was investigated. The organic compounds included in this project comprise naphthenic acid model compounds and mixtures as well as four resin acids. Naphthenic acids are crude oil-derived and accumulate to significant concentrations (>100 mg/L) in tailings pond water at oil sands extraction facilities. Resin acids are pulp and paper mill-derived compounds that tend to persist at low levels in receiving waters.

For each compound group, analytical methods utilizing liquid chromatography negative ion electrospray ionization mass spectrometry (LC/ESI/MS) were developed. The main hurdle to developing analytical methods for the naphthenic acids and resin acids are related to their polarity, complexity, and lack of available standards for the various individual components. As well, co-extractives, such as humic and fulvic acids, tend to interfere with the detection of naphthenic acids in aquatic samples (Headley *et al.,* 2002a). Resin acid mixtures are not as complex as the naphthenic acids, although each group of hydrocarbon acids may include several isomeric compounds.

The application of photochemical degradation prior to biodegradation was proven to be effective here for rapid degradation of the resin acids. In general, the resin acid precursors were more susceptible to the photolysis than were the naphthenic acids. Through thermal maturation and increased complexity, the naphthenic acids seemingly become more resistant to degradation, as evidenced by their commercial use as anti-microbial agents and the observed resistance to photolysis noted in this research. The results of this research may be significant for the design of staged treatment for reduced microbial shock loading and increased bioavailability (defined here as the ability of

microbial organisms to degrade the target contaminants) in both bioremediation systems and receiving waters.

Specifically, four selected pulp and paper mill-associated resin acids were exposed to several ultraviolet/visible (UV/vis) spectrum radiation sources in water collected from the River Saale in Germany. Background resin acid concentrations were observed in water collected in 2001 and 2002 from various locations along the well-forested River Saale and a manuscript detailing these results published. Analyses of water samples collected in the pulp and paper milling region of the river (in the state of Thuringia) indicated that resin acids persist through biodegradation treatment systems and for several hundred kilometres downstream. All four resin acids were degraded by facile photochemical and microbial degradation with pseudo-first-order kinetics. Half-life values were in the ranges of 18 to 200 minutes for photolysis applications, 8 to 40 hours for biodegradation applications and 3 to 25 hours for two-stage photochemical-microbial degradation processes, in which photolysis was limited to three hours. From these results, it was shown conclusively that photolysis pre-treatment is a viable and efficient method for reducing both resin acid concentrations and the associated acute toxicity.

The naphthenic acids investigated in this study were not effectively degraded via UV/vis radiation, including UV-A/UV-B radiation between 300-400 nm, near-monochromatic UV_{254}-radiation, full spectrum artificial solar radiation and natural sunlight. The photochemical degradation potential of three model naphthenic acid compounds and three naphthenic acid mixtures (one extract from the Athabasca Oil Sands and two commercial mixtures) were examined in Athabasca River water. Photolysis at UV_{254} was the most successful degradation source in all instances, although most naphthenic acids were not significantly degraded by any of the radiation sources. Therefore, it was determined that photolysis is not likely to contribute significantly to environmental degradation and attenuation in the aquatic ecosystem. The results observed from the various naphthenic acids photodegradation processes, coupled with their low affinity for adsorption to soils, reveal that naphthenic acids are likely to persist in the water column. However, UV/vis radiation is capable of significantly changing the composition of

mixtures in the aquatic ecosystem, but not reducing overall naphthenic acid concentrations. This may not be a beneficial as there is the potential for increased toxicity toward the lower molecular weight naphthenic acids.

ACKNOWLEDGEMENTS

This research was conducted in support of several programs including those associated with the Canada-Germany Bilateral Agreement. The Program of Energy Research and Development (PERD) provided funding for naphthenic acids studies. Further financial assistance was provided by University of Saskatchewan Teaching Assistantship, devolved scholarship funding and NSERC through Professor J.A. Gillies. Technical and research support was provided by the National Water Research Institute (NWRI), Environment Canada, Saskatoon and the Umweltforschungzentrum (UFZ) Centre for Environmental Research Leipzig-Halle, Magdeburg, Germany.

Supervision of the research work contained herein was conducted by Dr. John V. Headley of NWRI, Saskatoon. The PhD committee chair was Professor Jon A. Gillies from the Division of Environmental Engineering at the U of S. The PhD committee was comprised of Dr. Duane Friesen at the Applied Environmental Research Laboratory of Malaspina College, Nanaimo, BC, Dr. Karsten Liber from the Toxicology Centre at the U of S, Dr. Yen-Han Lin from Chemical Engineering at the University of Saskatchewan and Professor Dr. Ulrich Stottmeister of the UFZ Centre for Environmental Research Leipzig-Halle in Leipzig, Germany.

Invaluable technical assistance and advice was provided by Kerry Peru (NWRI), Christine Akre (Canadian Food Inspection Agency), Marcus Winkler (UFZ), Wolf von Tümpling (UFZ), Thomas Neu (UFZ), Ute Kuhlicke (UFZ), Ines Locker (UFZ), Christina Hoffmeister (UFZ), Margarete Mages (UFZ), Hajo Dahlke (UFZ), Norma Ruecker (Safe Drinking Water Foundation), Shannon Braithwaite (WateResearch Corporation) and Malcolm Conly (Canadian Wildlife Service).

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LIST OF ABBREVIATIONS

AbA	abietic acid
Ah	aryl hydrocarbon
amu	atomic mass unit
AOAC	Association of Organic Analytical Chemistry
AOS	Athabasca Oil Sands
APCI	atmospheric pressure chemical ionization
APCI/MS	atmospheric chemical ionization mass spectrometry
BA	benzoic acid
BKM	bleached kraft mill
BOD	biological oxygen demand
CCPE	Canadian Council of Professional Engineers
CDOM	chromophoric dissolved organic matter
CEATAG	Conrad Environmental Aquatic Technical Advisory Group
CPRGP	chorophenol red β-d-galactopyranoside
DhA	dehydroabietic acid
DMSO	dimethyl sulphoxide
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
DVB	divinyl benzene
EDS	endocrine disrupting substance
EI/MS	electron impact mass spectrometry
ein/s	einsteins per second; a measure of light intensity
ERAC	Environmental Research Advisory Council
ESI	electrospray ionization
ESI/MS	electrospray ionization mass spectrometry
ESI/MS/MS	electrospray ionization tandem mass spectrometry
EU	European Union
FAB	fast atom bombardment
FAB/MS	fast atom bombardment mass spectrometry
FIAB	fast iodide anion bombardment
FI/MS	fluoride negative ion mass spectrometry
FT/IR	Fourier Transform infrared spectroscopy
GC	gas chromatography
GC/MS	gas chromatography mass spectrometry
GDR	German Democratic Republic
HA	humic acid
HBA	hydroxybenzoic acid
p-HBA	*para*-hydroxybenzoic acid
$HO^{\bullet}$	hydroxyl radical
HPLC	high pressure liquid chromatography
IC_{50}	inhibitory concentration for 50 % of a test population
IpA	isopimaric acid
k	pseudo-first-order reaction rate coefficient

K_d	partitioning coefficient
LC_{50}	lethal concentration for 50 % of a test population (96 hours)
LC/ESI/MS	liquid chromatography electrospray ionization mass spectrometry
LC/MS	liquid chromatography mass spectrometry
LC/MS/MS	liquid chromatography tandem mass spectrometry
LD_{50}	lethal dose for 50 % of a test population over (96 hours)
4-MCHAA	4-methylcyclohexaneacetic acid
3-MCHCA	3-methylcyclohexanecarboxylic acid
4-MCHCA	4-methylcyclohexanecarboxylic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
M.W.	molecular weight
MWS	Magnetic Water Systems
m/z	mass to charge ratio
NAQUADAT	National Water Quality Data Bank
NCASI	National Council for Air and Stream Improvement, Inc.
NERC	National Environment Research Council
NH_4Ac	ammonium acetate
NRBS	Northern River Basins Study
NWRI	National Water Research Institute, Saskatoon, SK
PA	pimaric acid
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PERD	Program for Energy Research and Development
pK_a	dissociation constant
PrOH	1-propanol
RAR	rotating annular biofilm reactor
ROS	reactive oxygen species
RSD	relative standard deviation
SIM	select ion monitoring
SPE	solid phase extraction
S/N	signal to noise ratio
TCDD	tetrachlorodibenzodioxin
TOC	total organic carbon
UFZ	Umweltforschszentrum (Centre for Environmental Research)
U.S.EPA	United States Environmental Protection Agency
UV	ultra-violet radiation
UV-A	ultra-violet radiation in the wavelength range 315 to 400 nm
UV-B	ultra-violet radiation in the wavelength range 280 to 315 nm
UV-C	ultra-violet radiation in the wavelength range 100 to 280 nm
UV_{254}	ultra-violet radiation at 254 nm (UV-C)
UV/vis	ultra-violet/visible radiation
WBL	weak black liquor

1. GENERAL INTRODUCTION

1.1 Introduction

Primary degradation pathways of organic substances in the environment include microbiological and photochemical degradation. Biodegradation has been widely investigated for the remediation of contaminated waters for a wide variety of organic compounds. The application and exploitation of photolysis separately and in combination with biodegradation has not been likewise investigated. Implementation of photochemical pre-treatment in existing industrial effluent treatment systems may increase bioavailability and reduce shock loading to the microbial communities responsible for degradation. For these reasons, processes using pre-photolysis followed by biodegradation may be effective for removal of compounds seemingly recalcitrant to biodegradation alone.

Within the scope of this project, the photochemical and biodegradation potential of two groups of hydrocarbon acids were examined under various conditions. The hydrocarbon acid groups comprise a diverse mixture of saturated aliphatic and alicyclic carboxylic acids called naphthenic acids and a group of selected unsaturated analogues called resin acids. Resin acids are significant components of the microbial defence systems of coniferous trees while naphthenic oils and oil sands naphthenic acids are primarily derived from the organic matter of higher plant material, such as coniferous trees (Whelan and Farrington, 1992; Frakman *et al.,* 1990; Strausz, 1988; Shanmugam, 1985; Tissot and Welte, 1984; Snowdon and Powell, 1982). Despite chemical similarities and their analogous nature, the significant complexity of naphthenic acids suggests that the fate and persistence of each group of hydrocarbon acids will differ with respect to photochemical and biodegradation. It was hypothesized, however, that the bioavailability (defined here and throughout as increased availability for microbial degradation) of each group of hydrocarbon acids would be enhanced following photolysis.

To best simulate conditions where each hydrocarbon acid group is prevalent, water was collected from sources proximate to the Athabasca Oil Sands (northern Alberta, Canada) and pulp and paper milling industries (Sachsen-Anhalt and Thuringia, Germany). The Athabasca River at Fort McMurray, Alberta and the River Saale at Calbe, Germany were selected for investigations of naphthenic acid and resin acid fate and persistence, respectively. The Athabasca River at Fort McMurray is proximate to the Athabasca Oil Sands and two large mining / refining operations, while the Saale flows through a historically dominant pulp and paper production region in southeastern Germany.

The analytical challenges posed by each hydrocarbon acid group are related to their polarity, complexity and the presence of isomers. In particular, many components of naphthenic acid mixtures are not resolved due to complexity, lack of structural knowledge of the individual compounds of interest and lack of availability of individual standards (Headley *et al.,* 2002a; Moralez-Izquierdo, 1999). As well, co-extractives, such as humic and fulvic acids, tend to interfere with the detection of naphthenic acids in aquatic samples (Headley *et al.,* 2002a). Resin acids are not as complex as naphthenic acids, although they do include several isomeric compounds. Resolution of co-eluting isomers in liquid chromatography applications was the key focus of analytical method development.

1.1.1 Description of Analytical Principles

Due to the interdisciplinary nature of this work, there are several research areas that are not traditionally part of engineering design, including the development of analytical chemistry methods. This section provides a brief overview of the instrumental configurations chosen for use in this research so as to introduce more traditional engineers to the subject area.

The analytical methods applied here make use of liquid chromatography and mass spectrometry. There are two main types of chromatography, categorized by the type

of mobile phase used: gas (GC) or liquid (LC). Liquid chromatography (LC) is a chemistry-based tool for quantifying and analyzing mixtures of chemical compounds in solvent (usually water). It's an analytical technique used to separate and quantify a given organic or inorganic chemical compound within a sample (whether biological, pharmaceutical, food, environmental, industrial, etc.). In an LC process a liquid permeates through a porous solid stationary phase (the column) and elutes the solutes into a flow-through detector. The majority (~ 90 %) of LC separations are accomplished using reversed phase separation in which organic molecules are separated based on their degree of polarity. There is a correlation between the degree of lipophylicity and retention on the column.

From the LC, samples are introduced to the mass spectrometer (MS). The MS is an instrument designed to separate gas phase ions according to their *m/z* (mass to charge ratio) value. The MS analyzer separates gas phase ions using electrical or magnetic fields, or combination of both. Since the motion and separation of ions is based on electrical or magnetic fields, it is the mass to charge ratio, and not only the mass, that is of importance. The analyzer is operated under high vacuum, so that the ions can travel to the detector with a sufficient yield. Prior to the detector, some tandem MS instrumentation are equipped with a collision cell where ions can be collisionally dissociated. Product ion scans or selected reaction monitoring is typically used to achieve better selectivity and sensitivity for quantitative analysis.

The ionization mode used for analysis of the two groups of hydrocarbon acids investigated was Electrospray Ionization (ESI). Here, the LC is connected to the electrospray probe consisting of a metallic capillary surrounded with a nitrogen flow. A voltage is applied between the probe tip and the sampling cone. In most instruments, the voltage is applied on the capillary, while the sampling cone is held at low voltage. The first step is to create a spray due to the difference in potential at very low flow rates (low μL/min). At the tip of the capillary (within the electrical field), the surface of the droplets containing the ionized compound will become either positively or negatively charged depending on the voltage polarity. Due to the

solvent evaporation, the size of the droplet reduces and, consequently, the density of charges at the droplet surface increases. The repulsion forces between the charges increase until there is an explosion of the droplet. This process repeats until analyte ions evaporate from the droplet. Ions are then mass filtered, focused and detected.

1.1.2 Application of Engineering Principles

The elements of engineering principles addressed in this research include those related to the development of processes, modeling and holding public safety above all else. The Code of Ethics for professional engineers states that professional engineers shall hold paramount the safety, health and welfare of the public and the protection of the environment (CCPE, 2003a; APEGS, 2000). The issue of protecting the public interest and the question of whether the public is at risk must be considered in the broadest terms. The component, product, device, system, process and others that are the outcome of the engineering undertaking must be viewed from its broader societal perspective (CCPE, 2003a). Environmental engineering includes the design of experimental protocols relative to the complex aquatic, terrestrial and atmospheric environment; evaluation of environmental conditions, design and, in this instance, kinetic results and models; development and application of processes and treatment schemes that are beneficial to the public or society at large and the environment (CCPE, 2003b).

1.2 Naphthenic Acids

The persistence and fate of naphthenic acids in aquatic environments is not well-documented due to a lack of adequate analytical methods to determine the concentration, composition and extent of these crude-oil based compounds in environmental samples. This lack of knowledge constitutes a critical gap in scientific understanding. Models for predicting the fate and transport of naphthenic acids in the water column have not been developed, although it is known that

naphthenic acids are often found hundreds of kilometres from identified sources (CEATAG, 1998). This study addresses the photochemical fate of naphthenic acids in water from the Athabasca Oil Sands region north of Fort McMurray, AB. *It was hypothesized that photolysis will affect the concentration and/or composition of naphthenic acid mixtures and model compounds, rendering the overall solutions more bioavailable.* It was anticipated that the results of this work should add to the understanding of the photochemical processes acting on naphthenic acids in tailing pond water at bitumen extraction sites, as well as those that may be applied in an industrial treatment system for reducing the potential environmental impact of those compounds in the event of an accidental discharge.

To that end, information was collected and laboratory experiments completed to determine the occurrence and fate naphthenic acids in aquatic environments. Although naphthenic acids are known to be persistent biomarkers used in identification of oil source maturation, little is established regarding their relative degradation pathways in aquatic environments. Published research indicates the potential for microbiological degradation (Meredith *et al.,* 2000; Lai *et al.,* 1996; Herman *et al.,* 1994, 1993), relatively high solubility in water with low affinity for adsorption to soils (Peng *et al.,* 2002). Therefore, naphthenic acids are likely to persist in the water column unless some level of intervention is achieved.

The culmination of gathering information to determine the environmental fate and persistence of naphthenic acids in aquatic environments is the following review article. This manuscript brings together some of those environmental persistence results, as well as detailed information regarding the origin of naphthenic acids in tailings ponds, chemistry and toxicological considerations, current analytical methods for aquatic sampling and some potential areas of future remediation research. In addition, a description of the potential pathways for naphthenic acids diagenesis is included. This discussion provides an important link between the two groups of hydrocarbon acids studied herein. A review manuscript was prepared and is currently in press at the *Journal of Environmental Science and Health: Part A* and

is cited as:

Headley, J.V. and **D.W. McMartin**. 2003. A review of the occurrence and fate of naphthenic acids in aquatic environments. *Journal of Environmental Science and Health: Part A*. In Press.

1.2.1 Introduction

The oil sands mining and extraction industry in the Athabasca Oil Sands (AOS) north of Fort McMurray, AB (Figure 1.1) is the world's largest producer of synthetic crude oil with potential output in the range of 645,000 barrels of bitumen per day (Department of Energy, 2001; AOSTRA, 1991). Currently, crude oil production in the AOS accounts for more than 25 % of Canada's total production figures and is rapidly expanding (Leung *et al.,* 2003). In Alberta, the provincial environmental legislation (Alberta Environmental Protection and Enhancement Act, Section 23, 1993) prohibits the release of potentially toxic waste streams and no oil sands tailings are deliberately released to ground or surface water supplies (Madill *et al.,* 2001). Significant environmental and regulatory attention has been focused on the naphthenic acids fraction of oil sands material due to its persistence in the environment and aquatic toxicity at the levels found in the tailings pond water of bitumen extraction facilities.

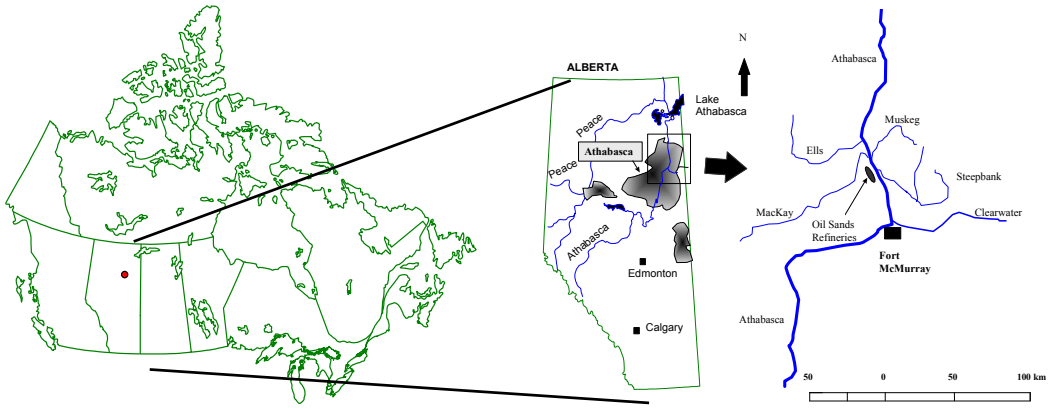


Figure 1.1. Map of Canada and Alberta showing the Athabasca Oil Sands region. (adapted from Tetreault *et al.*, 2003).

Naphthenic acids are natural constituents of bitumen and, during the oil sands extraction process, are solubilized and concentrated in tailings. In the natural setting, naphthenic acids may enter surface water systems through such processes as groundwater mixing and erosion of riverbank oil sands deposits (CEATAG, 1998; Brient *et al.,* 1995). Since naphthenic acids exist at low concentration in aquatic environments, have similar structure and behaviour as naturally occurring dissolved organic carbon components in surface water, and have significant complexity dependent upon oil source and geological factors, they present an analytical challenge. The absence of adequate separation and identification procedures for naphthenic acid mixtures has hampered studies to determine specific information on structural relationships, environmental reactivity and degradation pathways in the environment (CEATAG, 1998; Robbins, 1998; Brient *et al.,* 1995; Seifert and Teeter, 1970).

1.2.2 Naphthenic Acid Diagenesis

The diagenesis, or recombination or rearrangement of a mineral resulting in a new mineral, of naphthenic acids is a topic of some debate. Various organo-chemical parameters carry the fingerprint of the original organic matter or are formed during

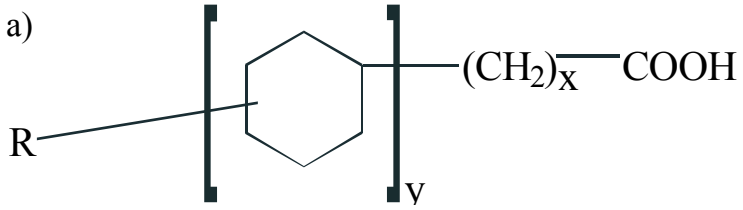
early diagenesis and reflect other aspects of the depositional environment. The question of naphthenic acids diagenesis reflects the fact that in petroleum, all acids are components of the resin fraction, even though some exist within the asphaltene fraction as co-precipitated resins they are not truly part of the asphaltene (Frakman *et al.,* 1990; Strausz, 1989, 1988). It is thought that naphthenic acids are by-products of original plant transformation to oil or maybe oxidative products of petroleum hydrocarbons. Resins of higher plants have often been proposed as significant contributors to naphthenic oils (Feinstein *et al.,* 1991; Shanmugam, 1985; Tissot and Welte, 1984; Snowdon and Powell, 1982). Diterpenic compounds, including resin acids derived from softwood tree bark, are common in oils and their presence is an indicator of generation from organic matter enriched in higher plant material (Grimalt *et al.,* 1989).

Chromatographic studies suggested that the naphthenic fraction of oil in the Gippsland Basin, Australia was derived chiefly from resin (Shanmugam, 1985). The geochemical similarities between laboratory-generated and natural oils in that study supports this hypothesis. Although resins are somewhat resistant to chemical attack, they tend to break down at low levels of thermal exposure, to produce naphthenic oils (Shanmugam, 1985; Tissot and Welte, 1984; Snowdon and Powell, 1982). Others have pointed to the presence of diterpenoid biological markers in both oils and extracts confirming resinite as a source for certain Canadian oil deposits (Snowdon and Powell, 1982). The general distribution of oils in the Beaufort-Mackenzie basin suggests that hydrocarbons derived from resinite-rich sediments yield naphthenic oils at very low levels of thermal maturation and condensates at slightly higher levels (Snowdon and Powell, 1982). Thermal products contributing to conventionally sourced crude oils would be primarily naphthenic compounds derived from the breakdown of diterpenoids in the resinites, such as resin acids (Lewan and Williams, 1987). The chemical affinity of labile long-chain monocarboxylic acids to basic sites of the minerals in oil sands can protect against their removal by water washing, biodegradation or thermal alterations. The historical action of microbial processes in the oil sands from Alberta, Canada, was

confirmed by the presence of certain carboxylic acids in the chemisorbed fraction and the virtual absence of those same compounds in the bitumen (Cyr and Strausz, 1984). Again, such processes affect the nature and concentrations of naphthenic acids in the oil sands, and thus also the bioavailability and toxicity of those compounds in the aquatic environment.

1.2.3 Chemistry of Naphthenic Acids

Naphthenic acids are a family of carboxylic acid surfactants, primarily consisting of cyclic terpenoids used in source and geochemical characterisation of petroleum reserves (CEATAG, 1998; Brient *et al.,* 1995). The compound group is composed predominately of alkyl-substituted cycloaliphatic carboxylic acids with smaller amounts of acyclic aliphatic (paraffinic or fatty) acids. Aromatic olefinic, hydroxy and dibasic acids are also present as minor components of naphthenic acids. The cycloaliphatic acids include single rings and fused multiple rings. As shown in Figure 1.2, the carboxyl group is usually bonded or attached to a side chain rather than directly to the cycloaliphatic ring (CEATAG, 1998; Fan, 1991; Dzidic, 1988).



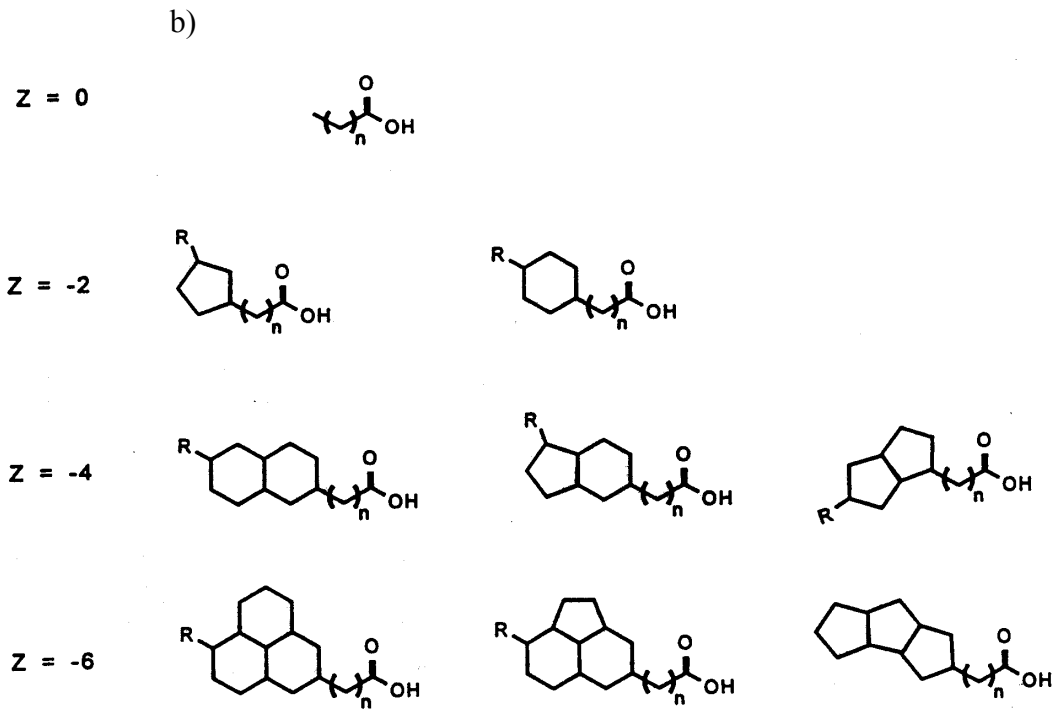


Figure 1.2. Typical structures of naphthenic acids.

The components of naphthenic acids are commonly classified by their structures and the number of carbon atoms in the molecule. The compounds are represented by the general formula $C_mH_{2m+z}O_2$ where *m* indicates the carbon number and *z* represents the number of hydrogen atoms that are lost as the structures become more compact. This is the most useful classification system and is based on the same definition of characterizing petroleum compounds or group type used for hydrocarbon analyses (CEATAG, 1998; Lai *et al.,* 1995). The *z* value is equal to 0 for saturated linear or branched hydrocarbon chains, and changes to -2 in monocyclic naphthenic acids, -4 in bicyclic, -6 in tricyclic and so on. The molecular weights change by 14 mass units (CH_2) between *m* series and by 2 mass units (H_2) between *z* series (CEATAG, 1998; Brient *et al.,* 1995; Herman *et al.,* 1993). Previous studies indicated that the *z* = -4 series predominates in oil sands tailings pond wastewater (Lai *et al.,* 1995). The relationship between *z* series, *m* families and molecular weights is shown in Table 1.1 (CEATAG, 1998).

Table 1.1. Molecular weights (M.W.) of different *z* series and *m* families of naphthenic acids ($C_mH_{2m+z}O_2$).

Number of Carbon Atoms	M.W. $z = 0$ (open chain)	M.W. $z = -2$ (1 ring)	M.W. $z = -4$ (2 rings)	M.W. $z = -6$ (3 rings)
10	172	170	168	166
11	186	184	182	180
12	200	198	196	194
13	214	212	210	208
14	228	226	224	222
15	242	240	238	236
16	256	254	252	250
17	270	268	266	264
18	284	282	280	278
19	298	296	294	292
20	312	310	308	306

The polarity and non-volatility of naphthenic acids increases with molecular weight, giving individual compounds various physical, chemical and toxicological properties (CEATAG, 1998; Brient *et al.,* 1995; Herman *et al.,* 1993). In general, however, as a group the naphthenic acids have physical and chemical characteristics that can be used to describe the overall mixture as shown in Table 1.2.

Table 1.2. Physical and chemical properties of naphthenic acids.

Parameter	General Characteristic
Colour	Pale yellow, dark amber, yellowish brown, black
Odour	Primarily imparted by the presence of phenol and sulphur impurities; musty hydrocarbon odour
State	Viscous liquid
Molecular Weight	Generally between 140 and 450 amu
Solubility	< 50 mg/L at pH 7 in water; Completely soluble in organic solvents
pK_a	Between 5 and 6
Boiling Point	Range between 250 to 350 ºC

(Brient, 1998; CEATAG, 1998; Brient *et al.,* 1995; Herman *et al.,* 1993; Headley *et al.,* 2002a)

It is possible for naphthenic acids to exist at higher concentrations in tailings pond water (such as the 110 mg/L value noted for Athabasca Oil Sands tailings ponds

earlier) for a number of reasons, including the formation of micelles in which the more hydrophobic parts of compounds cluster inward exposing their slightly more hydrophilic parts to the surrounding aquatic environment, or increased in pH within the tailings pond water. The pH of naphthenic acids directly correlates to solubility (Headley *et al.,* 2002a; CEATAG, 1998). Chemically, naphthenic acids behave like typical carboxylic acids with acid strengths similar to those of the higher fatty acids. Naphthenic acids are slightly weaker than low molecular weight carboxylic acids, such as acetic acid (Whelan and Farrington, 1992; Tissot and Welte, 1984; Snowdon and Powell, 1982).

Some naphthenic acids are solubilized to produce metal salts that have industrial applications (Table 1.3) (Brient, 1998; St. John *et al.,* 1998; Herman *et al.,* 1994; Davis, 1967). Over two-thirds of the naphthenic acids produced are converted to metal salts, the largest component of which is made into copper naphthenate used for the preservation of wood products (Brient *et al.,* 1995). Although the major commercial use of naphthenic acids has been in the production of metal soaps, they can also react to form esters, amine salts, amides, imidazolines and other derivatives (Stajner *et al.,* 1998; Whelan and Farrington, 1992; Tissot and Welte, 1984).

Table 1.3. Industrial uses for naphthenic acids.

Naphthenic Acid Metal Salt	Industrial Applications
Na salt	emulsifying agent for agricultural insecticides additive for cutting oils emulsion breaker in oil industry
Ca naphthenate	additive for lubricating oil
Fe and Mn naphthenates	fuel additives for improved combustion and reduced
Pb & Ba salts	catalysts for oil-based paints
Cu & Zn naphthenate	wood preservatives
Co naphthenate	curing agent in rubbers and resins adhesion promoter of steel cord to rubber
Mn, Pb, Co, and Ca soaps	oxidative catalysts

(Brient, 1998; St. John *et al.,* 1998; Herman *et al.,* 1994; Davis, 1967)

In a recent investigation of several commercial naphthenic acid mixtures and those extracted from oil sands, significant differences amongst four commercial mixtures and between the extracts from various oil sands ores and tailings ponds were established (Clemente *et al.,* 2003a). The data from GC/MS analyses was divided into plots describing the relationship between the abundance of specific ions versus carbon number and *z* family. The naphthenic acids analysed ranged from carbon numbers between 5 and 30 and *z* families between 0 and -12. Clemente *et al.* (2003a), noted in their analyses that naphthenic acids concentrations and composition were highly varied amongst commercial sources, oil sands ore and tailings pond sources. Their results indicate significant differences especially in the C22+ cluster (i.e., those compounds containing more than 22 carbons), which was essentially not present in two of the commercial mixtures, but comprised approximately 23 % of one oil sands sample (Athabasca Oil Sands, Fort McMurray, Canada). Further results indicated significant differences in naphthenic acid composition based on two different ore samples from the same region. For example, one sample taken from the Athabasca Oil Sands contained a C22+ composition of only 6 % compared to the 23 % result described earlier (Clemente *et al.,* 2003a). The mass spectra shown in Figure 1.3 illustrate similar results for two commercial mixtures and one extract from the Athabasca Oil Sands (Toxicology Centre, University of Saskatchewan, Saskatoon, Canada). In the figure, three naphthenic acid mixtures were analysed by negative-ion electrospray ionization-mass spectrometry (Headley *et al.,* 2002a) and, although each is labelled “naphthenic acids”, none has the same complexity or composition. Each peak in the figures represents a mass, which may contain several or hundreds of naphthenic acid compounds. Figure 1.3a of the Athabasca Oil Sands extract shows a very complex mixture with the majority of the naphthenic acids compounds in the mid-mass range (150 to 350 amu), while Figures 1.3b and 1.3c indicate much less complex mixtures with few compounds above 300 amu. The compositions of these three naphthenic acids mixtures are clearly significantly different.

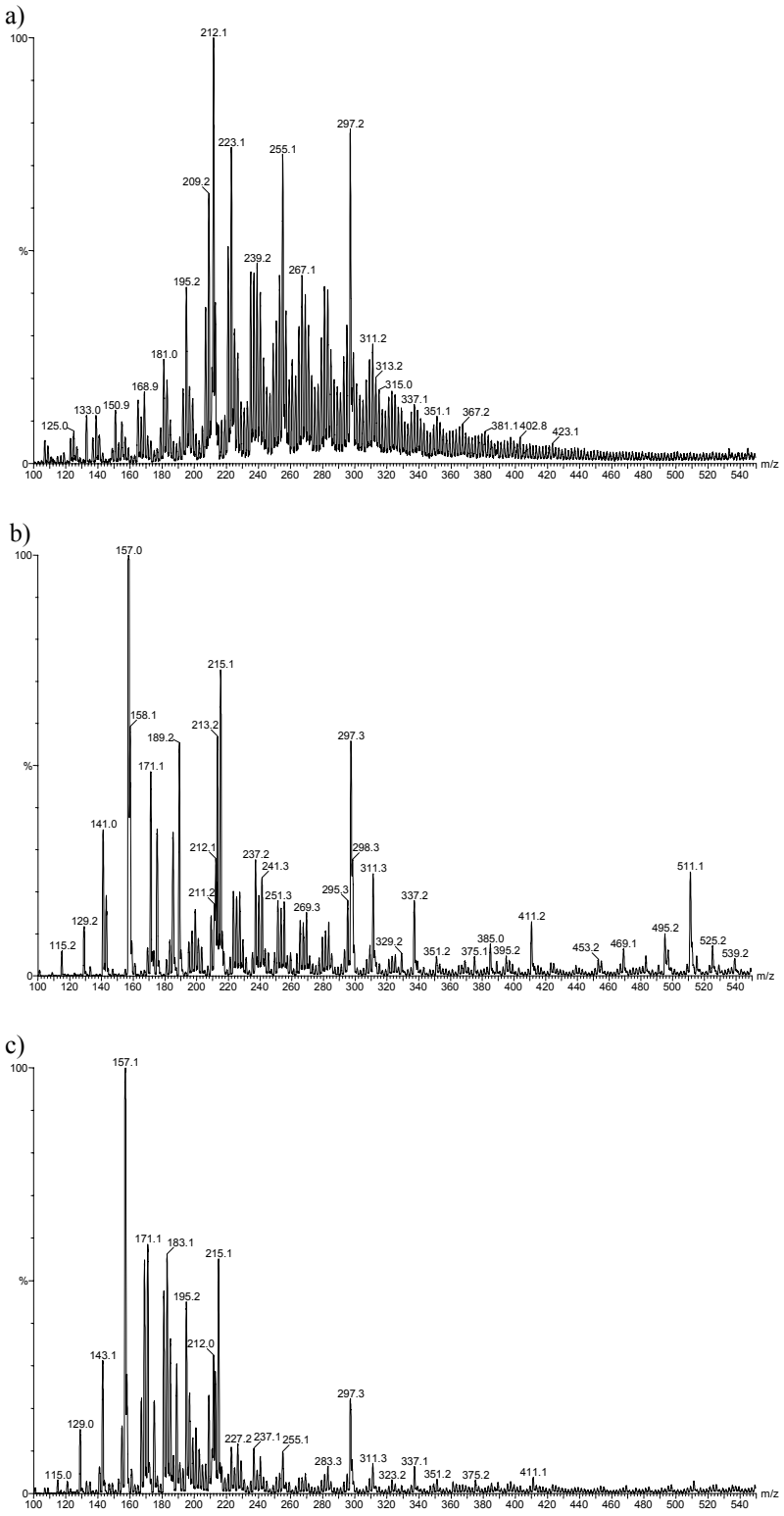


Figure 1.3. Mass spectra of naphthenic acids in a) Athabasca Oil Sands; b) Acros commercial mixture and c) Fluka mixture resulting from loop injection to a Micromass Quattro Ultima mass spectrometer (McMartin *et al.*, 2004).

1.2.4 Aquatic Toxicity of Naphthenic Acids

The reported toxicity of naphthenic acids is often associated with their surfactant characteristics (Rogers *et al.,* 2002a,b; MacKinnon and Boerger, 1986; Dokholyan and Magomedov, 1983). The most significant environmental contaminants and toxic components in oil sands deposits and tailings pond water are naphthenic acids of low molecular weight generated during the bitumen extraction process (Rogers *et al.,* 2002b). However, since literally hundreds of these compounds are found in oil sands materials, it is not currently established which specific naphthenic acids are the most toxic. Since no two oil sands formations are exactly the same, the content and complexity of naphthenic acids in the bitumen are also not exactly the same. Thus, toxicity does not necessarily correlate directly to the naphthenic acid concentration, but is more a function of content and complexity (CEATAG, 1998; Lai *et al.,* 1996; Brient *et al.,* 1995). By the same token, however, it has been shown that, when conducting experiments with one naphthenic acid mixture, decreased naphthenic acid content does correlate to decreased toxicity (Holowenko *et al.,* 2002).

Both oil sands development and natural exposure to oil sands deposits may result in concentrations of hydrocarbons and other substances, in both the water and sediment, which cause stress to fish and other biota (Conly *et al.,* 2002; Conly, 2001). Fresh tailings water from oil sands processing is acutely toxic to aquatic organisms (LC_{50} < 10 % v/v for rainbow trout) (MacKinnon and Boerger, 1986) and mammals (oral LC_{50} = 3.0 g/kg body weight) (U.S.EPA, 1984). The most recent mammalian toxicological results indicate that while acute toxicity in wild mammals is unlikely under worst-case exposure conditions, repeated exposure may have adverse health effects (Rogers *et al.,* 2002b). Naphthenic acids are toxic to aquatic algae and other micro-organisms (CEATAG, 1998; Brient *et al.,* 1995). Herman *et al.* (1994) concluded via the Microtox® test that naphthenic acid sodium salts are toxic to micro-organisms with an EC_{50} value (i.e., the percentage required to produce a 50 % loss of bioluminescence of the test organism) of 30 % (v/v).

Although there is little information about mammalian toxicity, the human lethal dosage was reported as 1 L (Rockhold, 1955). For rats, the oral LD_{50} is between 3.0 and 5.2 g/kg with death caused by gastronintestinal disturbances (Lewis, 2000). Further evaluation of the effects of naphthenic acids on mammals (including rats, dogs and rabbits) have indicated increased vascular permeability in capillaries; notable effects on the formation of red and white blood cells and platelets; and efflux of potassium from cells causing first stimulation then inhibition of cellular respiration (Rogers *et al.,* 2002b; Lai *et al.,* 1996). Further tests with Wistar rats indicate that the liver is the target organ in both acute and sub-chronic dosing experiments (Rogers *et al.,* 2002b). In tests with rainbow trout all naphthenic acid compounds and mixtures tested were cytotoxic to varying degrees in four rainbow trout cell lines (cytotoxicity of commercial naphthenic acid mixtures was enhanced with salinity) (Lee *et al.,* 2000) and it was noted that the compound, cyclohexane carboxylic acid, was toxic only at concentrations greater than 0.1 mg/mL, while at low dosages (< 50 μg/mL) cell proliferation was stimulated (Lee *et al.,* 2000). According to the Ames mutagenicity test (Pordes and Bangert, 1967), naphthenic acids are not mutagenic nor are they listed as carcinogenic by the International Agency for Research on Cancer and other health agencies (Brient *et al.,* 1995).

1.2.5 Naphthenic Acids in the Oil Sands Extraction Process

Holowenko *et al.* (2000) calculated that for each cubic meter of mined oil sands, a volume of 3 m^3 of water and approximately 4 m^3 of slurry waste consisting of sand, clays, organics, residual bitumen and process water result as a by-product of the bitumen extraction process. Petroleum components that often exist in the tailings pond water include classes as porphyrins, isoprenoid, terpenoid and steroid hydrocarbons, as well as carboxylic acids (fatty, isoprenoid, steroid, amino, naphthenic, etc.), some PAHs (polycyclic aromatic hydrocarbons) and a wide variety of cations and anions (Holowenko *et al.,* 2000; Seifert, 1975). Naphthenic acids, when separated from the oil sands material, become a significant part of the

tailings pond water and its toxicological characteristics (Rogers *et al.,* 2002a,b; Strausz, 1989b).

The fate of naphthenic acids throughout the bitumen extraction process must be known in order to develop appropriate source control measures and wastewater treatment methods. Naphthenic acids constitute as much as 50 % by weight of the total acidic proportion in crude oil and therefore are present at significant concentrations in tailings ponds. In unrefined Athabasca bitumen (northern Alberta, Canada), the carboxylic fraction is about 2 %, of which approximately 90 % is comprised of the tricyclic acids that primarily make up the naphthenic acid fraction (CEATAG, 1998; Strausz, 1988; Cyr and Strausz, 1984). This predominance of tricyclic compounds in the unrefined bitumen differs from the overall heterogeneity of the naphthenic acids in the tailings pond water following bitumen extraction; no one *z* group between 0 and -6 is more or less prevalent that the other with *z* from -8 to -12 also constituting a large portion of the total naphthenic acid fraction (McMartin *et al.,* 2004; Rogers *et al.,* 2002a). Oil sands are mined from various depths and locations and contain different distributions of naphthenic acids, both in terms of composition and concentration (Clemente *et al.,* 2003a).

Bitumen is extracted from oil sands material using alkaline hot water extraction. During this process, the naphthenic acids are removed as dissolved naphthenates and stored along with the other waste products in tailings ponds (CEATAG, 1998; Hsu *et al.,* 1998; Lai *et al.,* 1996; Brient *et al.,* 1995). The separation process uses caustic hot water flotation producing large volumes of wastewater, fine tails (water and solids) and non-recovered bitumen in a ratio of approximately 50:50:1. The caustic extraction of naphthenic acids from petroleum distillates is performed at temperatures between 200 and 370 °C. During this process, other acidic fractions from petroleum, including phenol and cresols, mercaptans and thiophenols, are also removed (Brient *et al.,* 1995). In addition to removing corrosivity, the caustic wash improves the burning qualities, storage stability and odour of the finished products. In view of these advantages, caustic extraction is the industry standard, although

non-caustic processes for recovery of naphthenic acids from petroleum distillates have been attempted, but not commercialized. These include the use of ammonia, triethylene glycol, ion-exchange resins and aluminosilicate zeolites (Danzik, 1987; Ayres, 1960).

Residues of naphthenic acids in effluents or those present in the native oil sands materials may contaminate surface and ground water systems directly by effluent release, or indirectly by groundwater mixing and riverbank erosion. Of the possible environmental receptors (i.e., air, soil, and water), the most significant is water due to direct contact with oil sands material. Naphthenic acids concentrations in northern Alberta rivers in the Athabasca Oil Sands near Fort McMurray are generally below 1 mg/L, but may be as high as 110 mg/L in tailings waters (CEATAG, 1998). Natural groundwater levels of naphthenic acids in the region range from < 4 mg/L in near-surface aquifers to > 55 mg/L in basal and limestone aquifers (CEATAG, 1998). Reclamation of tailings into terrestrial and aquatic landscapes at operations shut-down must, therefore, address residual levels of naphthenic acids and their fate and transport in the environment.

1.2.6 Analytical Techniques

Owing to the aquatic environmental persistence of naphthenic acids, concerns about accidental discharge of tailings pond water to nearby surface water sources and a need to learn more about the processes and pathways by which naphthenic acids are degraded in aquatic environments, interest in developing sensitive and robust analytical methods for detection and quantitation of naphthenic acids has grown in recent years. Although naphthenic acids were first reported as oil sands components during the late 1960s, environmental assessment of naphthenic acids has been hampered due to a dearth of published analytical methods (Holowenko *et al.,* 2002; Moralez-Izquierdo, 1999; Nascimento *et al.,* 1999; Seifert *et al.,* 1969). The lack of routine quantitative analytical methodology is due to the complexity of the mixtures. To date, researchers have attempted such analytical procedures as mass

spectrometric analysis and fast atom bombardment (FAB/MS), electrospray ionization (ESI/MS), atmospheric chemical ionization (APCI/MS), gas chromatography (GC/MS) with derivatization, and Fourier transform ion cyclotron resonance (FTICR/MS) (Barrow *et al.,* 2003; Lo *et al.,* 2003; CEATAG, 1998; Fan, 1991; Headley *et al.,* 2002; Holowenko *et al.,* 2002; Moralez-Izquierdo, 1999; Seifert *et al.,* 1969). Recent studies also indicate success using high performance liquid chromatography (HPLC) (Clemente *et al.,* 2003b).

Adaptation of Fourier Transform Infrared (FT/IR) spectroscopy for the quantitative analysis of naphthenic acids has been implemented by Syncrude (Holowenko *et al.,* 2001). Historically, FT/IR has been used for qualitative analyses, however, in recent years it has been applied quantitatively (Holowenko *et al.,* 2001; CEATAG, 1998). Other recent methods employing negative ion electrospray ionization MS have been shown to be more amenable for the quantitative analysis of naphthenic acid compounds in aqueous solutions than such methods as LC/ESI/MS (Headley *et al.,* 2002a; Holowenko *et al.,* 2002). Since co-extractives, such as humic and fulvic acids, tend to interfere with the ESI/MS detection of naphthenic acids in aquatic samples, the application of this procedure is generally limited to natural waters with relatively low to moderate concentrations of DOC or with masses that are beyond the naphthenic acid mass envelope range (Headley *et al.,* 2002a). Techniques such as APCI in negative-ion mode can produce very clean spectra with good sensitivity compared to other techniques (Hsu *et al.,* 2000). Analytical techniques such as negative ion Fast Iodide Anion Bombardment (FIAB), Positive Ion Chemical Ionization using isobutane reagent gas (iC_4-CI) and Electron Impact MS (EI/MS) met with less success (Dzidic *et al.,* 1998; Hsu *et al.,* 1998; St. John *et al.,* 1998).

Preparative solid phase extraction (SPE) methods for concentrating naphthenic acids from water samples and reducing matrix interferences have also been investigated (Headley *et al.,* 2002a; Jones *et al.,* 2001). Large volumes can be concentrated using SPE as illustrated in Figure 1.4. ESI/MS analyses may be performed on extracts with no further clean-up or derivatization steps (Headley *et al.,* 2002a). The SPE method of extraction has been shown to be reproducible and quantitative for the

analysis of naphthenic acids using relatively low solvent volumes (Headley *et al.,* 2002a; Jones *et al.,* 2001).

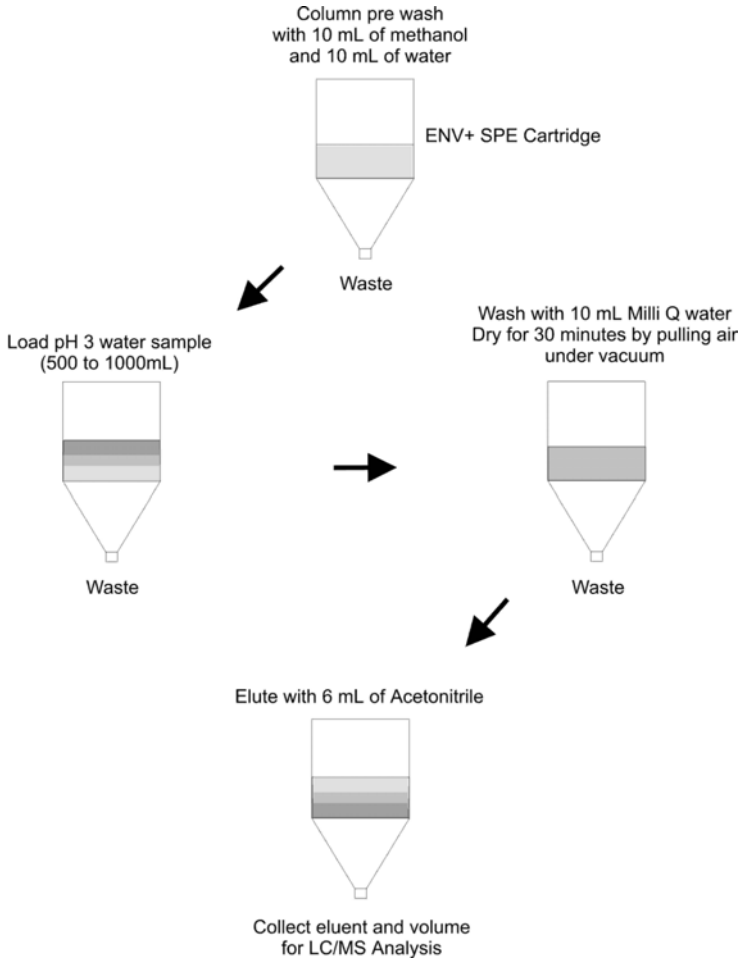


Figure 1.4. Solid Phase Extraction procedure developed by Headley *et al.* (2002a).

1.2.7 Adsorption of Naphthenic Acids to Soils and Sediments

Modelling the occurrence and fate of organic compounds in aquatic systems, as well as engineered bioremediation, relies on knowledge of the sorption characteristics of the compounds in question. In particular, degradation processes may be decreased through enhanced adsorption (Peng *et al.,* 2002; Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998; Mittelmann, 1998). Bioavailability (i.e., availability for microbial degradation) of organic compounds is affected by sorption

in two important ways. First, sorption diminishes the organic concentration in the bulk water such that only a small fraction of the compound may actually be in the water phase. Separation of organic compounds by sorption from the aqueous phase is likely to reduce the rate and extent of biotransformation since micro-organisms tend to be most efficient in utilising substrates dissolved in the aqueous phase. Second, because desorption and immobile zone diffusion must occur before biodegradation can proceed, the overall rate of bioremediation is limited or even controlled by these mass transfer processes, not by the activity of the degrading micro-organisms (Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998; Mittelmann, 1998).

Few studies have investigated the adsorptive properties of model naphthenic acids onto oil-affected soils, such as riverbank materials and sediments. However, two publications indicated that model naphthenic acid compounds have low K_d values and are, therefore, not likely to partition to soils and sediments (Peng *et al.,* 2002; Zou *et al.,* 1997). The potential factors influencing the adsorption isotherms of the model naphthenic acids investigated in these studies on oil-affected soils include molecular structure of the sorbate, organic carbon content of the sorbent, temperature, concentration of solution salt and pH (Peng *et al.,* 2002).

1.2.8 Microbial Degradation of Naphthenic Acids in Aquatic Environments

Because naphthenic acids are relatively water soluble (50 mg/L) they are not likely to persist for extended periods in the water column. For biodegradation to occur, the microbial communities in the water column must be adequately exposed to the target compounds, in this case the naphthenic acids. If compounds are adsorbed from the aqueous phase, bioavailability is dramatically reduced (Bryers, 2000; Providenti *et al.,* 1993). Therefore, because of the relatively high water solubility and low K_d values noted for model naphthenic acids, then, naphthenic acids are most likely to partition to the water column and are available to micro-organisms for degradation processes.

Despite these factors, as a group, naphthenic acids are known to be weakly biodegradable and are, thus, well suited for use in identification of oil source maturation (Headley *et al.,* 2002b; Meredith *et al.,* 2000; Lai *et al.,* 1996; Herman *et al.,* 1994; Davis, 1967). Such resistance to microbial degradation is reflected in the fact that the naphthenic acids are often present as metal salts and are effective commercial antimicrobial agents as shown in Table 1.3 (Lai *et al.,* 1996; Herman *et al.,* 1994, 1993; Davis and Raymond, 1961). It is generally accepted that the resistance to microbial degradation is related to the surfactant nature of the naphthenic acids, as was proposed for the aquatic toxicity of naphthenic acids.

Published studies of applied microbiological treatment processes for removal of model naphthenic acids and toxicity in oil sands tailings and downstream receiving waters suggests that highly robust and diverse microbial populations are required to thrive in the complex and toxic environment associated with naphthenic acids (Lai *et al.,* 1996; Herman *et al.,* 1994; MacKinnon and Boerger, 1986). Both aerobic and anaerobic cultures of indigenous microbial communities from oil sands tailings water are moderately capable of degrading naphthenic acid mixtures in aquatic systems given appropriate nutrient conditions (Holowenko *et al.,* 2001, 2000; Lai *et al.,* 1996; Herman *et al.,* 1994, 1993; MacKinnon and Boerger, 1986). One study involving three model naphthenic acids indicated that, of temperature, pH and DOC, temperature had the most significant effect on microbial degradation kinetics with an observed ten-fold increase in the first-order rate constant between 10 and 30 °C (Headley *et al.,* 2002b). In this instance, the *trans*-isomer of each model naphthenic acid was less resistant to microbial degradation than the *cis* isomer, suggesting that the more closed geometry corresponds to lower bioavailability of the *cis* isomer. The difference in this bioavailability was attributed to differences in intramolecular hydrogen bonding in the respective isomers (Headley *et al.,* 2002b).

The persistence of model naphthenic acids in aquatic systems appears to be related to the structure of the respective compounds in the same way that toxicity is structurally dependent (Headley *et al.,* 2002b; Herman *et al.,* 1993; MacKinnon and

Boerger, 1986). Indigenous cultures of *Acinetobacter, Alcaligenes, Pseudomonas,* and *Nocardia* bacteria have successfully degraded carboxylated cycloalkanes, although compounds with methyl substituents on the cycloalkane ring have been shown to be more resistant to microbial degradation (Herman *et al.,* 1993). Furthermore, there appears to be a difference in persistence of model naphthenic acids to microbial degradation based on carbon number. Some studies indicate that naphthenic acids with odd carbon numbers are less resistant to biodegradation (Lai *et al.,* 1996; Herman *et al.,* 1994; Davis and Raymond, 1961). However, there are also documented instances in which a naphthenic acid compound with 6 carbons (cyclopentane carboxylic acid) was more rapidly biodegraded than a 7 carbon naphthenic acid (cyclohexane carboxylic acid) (Herman *et al.,* 1993).

Biodegradation of naphthenic acids also occurs within oil reservoirs as the crude matures (Koike *et al.,* 1992). Linear and carboxylic acids of lower molecular weight are removed more rapidly than the corresponding hydrocarbons by biodegradation and water washing. Although the biodegradation migration path or reservoir conditions were different for each crude basin investigated, the results clearly indicated biodegradation of the low molecular weight carboxylic acid fraction (Koike *et al.,* 1992).

1.2.9 Current and Future Trends in Naphthenic Acids Remediation Research

The trend toward exploitation of natural systems and surroundings is integrated into the degradation technologies of algal biodegradation, photolysis using solar radiation, phytoremediation, land application and wetlands.

Published algal biodegradation investigations have been limited to date. One unpublished study (National Water Research Institute, Saskatoon, Canada) noted that several algae species were capable of surviving and metabolizing three single-ring model naphthenic acids (4-MCHAA, 4-MCHCA, 3-MCHCA), while others were quickly eradicated. In particular, green algae species and diatoms thrived in the

model naphthenic acid solutions up to approximately 100 mg/L. Further research is required to determine whether or not algae-mediated degradation is effective for decreasing the concentrations of model naphthenic acid mixtures along with the associated toxicity in aquatic environments.

Another potential remediation method for naphthenic acids in aquatic environments is photolysis. Photolysis occurs when light-absorbing molecules (chromophores) undergo chemical change as a direct consequence of absorbing photons of UV/VIS radiation. Chromophores may be either the target organic compounds (direct photolysis) or a sensitizer/catalyst that subsequently transfers energy to the target compound for degradation (indirect photolysis) (Mozumder, 1999; U.S.EPA, 1998; Suppan, 1994; Zafiriou *et al.,* 1984; Wells, 1972; Stein, 1968). Several petrochemical compounds absorb sunlight effectively and react with significant quantum yields. In fact, the susceptibility of crude oil to biodegradation was noted to increase subsequent to photolysis (Dutta and Harayama, 2000; Grzechulska *et al.,* 2000; Green *et al.,* 1985). However, the results of initial research indicate that photolysis does not efficiently degrade naphthenic acids on its own. Since the penetration of highly energetic radiation wavelengths (such as those in the UV portion of the optical spectrum) is most often limited to the top several millimetres of surface waters (depending on turbidity, colour and other physical properties), natural photolysis of naphthenic acids is likely to be severely limited. Further studies involving photo-Fenton reactions and other catalytic processes may demonstrate enhanced photodegradation and increased bioavailability. As an applied treatment, UV_{254}-radiation has the most potential for both removing naphthenic acids and increasing their bioavailability (McMartin *et al.,* 2004; Dutta and Harayama, 2000).

Phytoremediation is plant-assisted bioremediation that may be utilized to treat hydrocarbon-contaminated soils. This method exploits the fact that plants have extensive root systems that are in contact with large volumes of soil, support large bacterial populations in the rhizosphere and secrete compounds that are capable of

affecting the activity of the rhizobacterial populations (ERAC, 2002; Farrell *et al.,* 2000). The removal processes involved in phytoremediation include (a) the stimulation of rhizobacterial transformations, (b) the slowing of contaminant transport from the root zone due to adsorption and/or increased evapotranspiration and (c) plant uptake, followed by metabolism, volatilization or accumulation (Farrell *et al.,* 2000). To date, concerns of leaching to groundwater, heavy metal build up, and long-term plant productivity have limited the application of these technologies on a large-scale (ERAC, 2002, 2001, 2000; Farrell *et al.,* 2000; Knight *et al.,* 1999). No published studies have specifically addressed naphthenic acids remediation although it is possible that the diverse and rich rhizobacterial populations of phytoremediator plants are capable of metabolizing naphthenic acids and minimizing the associated toxicity.

To achieve an environmentally stable landscape at and near oil sands extraction areas, a system that requires minimal long-term management and inputs is highly favourable. The use of constructed or natural wetlands may be used to reduce the mobility and concentration of petroleum hydrocarbon contamination from wastewaters (Madill *et al.,* 2001; Leung *et al.,* 2003; ERAC, 2001, 2000; Knight *et al.,* 1999). Natural attenuation processes in wetlands include sorption, aerobic and anaerobic biodegradation, volatilisation, plant uptake and dilution. Although volatilisation is the primary removal process, biodegradation and dilution are also responsible for reducing hydrocarbon concentrations (Madill *et al.,* 2001; Leung *et al.,* 2003; ERAC, 2001; Farrell *et al.,* 2000; Knight *et al.,* 1999). Since naphthenic acids are not volatile, sufficient hydraulic retention times are required to allow for acclimation of microbial populations for adequate biodegradation and phytoremediation to occur. One published study directly related to the ecological effects of naphthenic acids on phytoplankton noted that the naphthenic acids affect the composition of phytoplanktonic communities in wet landscapes, but have minimal effect on total biomass (Leung *et al.,* 2003).

1.3 Resin Acids

Evaluation of the persistence and fate of resin acids in natural aquatic systems is critically lacking. The potential for photolysis of resin acids in rivers downstream of pulp and paper mills and as a pre-treatment to biodegradation at pulp and paper mills has not been evaluated. In addition, biodegradation of resin acid solutions using biofilm reactors that simulate riverine shear are required to determine the persistence and fate of resin acids in natural water systems. *It is hypothesized that the application of a degradation scheme involving photolysis treatment prior to biodegradation will reduce the concentration of pulp and paper-associated resin acids and eliminate the associated acute toxicity from natural surface water.*

Resin acids are tricyclic, diterpenic, carboxylic acids often discharged in pulp and paper mill effluents. Resin acids are found in the bark and wood of coniferous trees, which contain as much as one order of magnitude more resin acids than deciduous trees. As such, wood composition plays a significant role in the concentration of resin acids in pulp and paper mill effluents and, thus, in the environment (Corin *et al.,* 2000; Liver and Hall, 1996; Robinson *et al.,* 1994; Leach and Thakore, 1977). Overall effluent toxicity, to which it is believed resin acids are a substantial contributor, is closely scrutinized and regulated. Several researchers have linked between 20 and 70 % of the toxicity of untreated effluents to resin acids (Wang *et al.,* 1995; Munkittrick *et al.,* 1994; Volkman *et al.,* 1993; Zanella, 1983; Leach and Thakore, 1977).

1.3.1 Resin Acids in the Pulping Process

The elementary principle of pulping is the process in which wood chips are converted into fibrous raw material called pulp. The most common pulping process is the kraft or sulphate process in which wood chips are digested at high temperature (160 - 180 °C) and pressure (800 kPa) with white liquor (a mixture of hot caustic soda (NaOH) and sodium sulphide (Na_2S)) (Biermann, 1996). The lignin and wood extractives, including resin acids, are solubilized in the digestion chemicals, leaving

the less soluble cellulose fibres for pulp (McCubbin and Folke, 1992; MacLeay and Associates, 1987; Kringstad and Lindström, 1984). The pulp fibre is separated from the residual weak black liquor (WBL) that is a complex mixture of waste lignin, digestion chemicals and wood extractives (Biermann, 1996). To recover the digestion chemicals, WBL is cycled through a recovery process designed to recover between 96 and 99.5 % of the spent chemicals (McCubbin and Folke, 1992). When the WBL is evaporated in the chemical recovery cycle, the vapours are condensed to a solution contaminated with volatile organic compounds. This condensate is then either sewered or recycled for use as wash water in other areas of the pulp mill (Biermann, 1996; Blackwell *et al.,* 1979).

Bleached kraft pulp mills (BKM) produce large volumes of wastewater. Discharges may amount to between 90 and 130 million litres per day into surface receiving waters (i.e., lakes, rivers, estuaries, oceans) (Walden, 1976). Hundreds of compounds may be released including wood-derived carbohydrates, lignin derivatives, organochlorine compounds (resin and fatty acids, chlorinated phenols, catechols, guaiacols, dioxin and furan) and extractive compounds (resin and fatty acids, phytosterols and phenols) (LaFleur and Barton, 1997; Suntio *et al.,* 1988; Kringstad and Lindström, 1984). Despite treatment of BKM effluents prior to release to surface waters, many chemicals remain in the discharged wastewater. In the 1980s and 90s, following implementation of environmental protection regulations, many pulp and paper mills in Canada, the USA and Scandinavia installed secondary effluent treatment systems exploiting biodegradation processes (Kovacs *et al.,* 1996, 1997; Folke, 1996; Smook, 1994; NCASI, 1989). Since the advent of secondary effluent treatment, negative environmental impacts have been significantly reduced.

Pulp and paper products are fundamental to modernized societies and the industry plays a vital role in North American and European markets. Forty-six thousand people in Germany were employed in the pulp and paper industry and sales of paper

products grew by DM 20.6 billion (approximately $18 billion Cdn) in 1999 from the 1998 figures (European Pulp and Paper, 2000).

1.3.2 Chemistry of Resin Acids

Resin acids can be broken into two structural groups: *abietanes* with conjugated double bonds and an isopropyl substituent at C-13 and *pimaranes* with no conjugated double bonds and both methyl and vinyl substituents at C-13 (Figure 1.5). Dehydroabietic (DhA) and abietic (AbA) acid are abietanes that are predominant in pulp and paper mill effluents. The toxicity and solubility of both are strongly affected by pH, with toxicity increasing and solubility decreasing as pH is lowered (Werker and Hall, 1998; Liver and Hall, 1996). Unlike most abietanes, DhA possesses an aromatic ring and displays contrasting toxicological and environmental persistence characteristics from other abietanes (Patoine *et al.,* 1996; Liver and Hall, 1996; Wang *et al.,* 1995; Zender *et al.,* 1994; Volkman *et al.,* 1993).

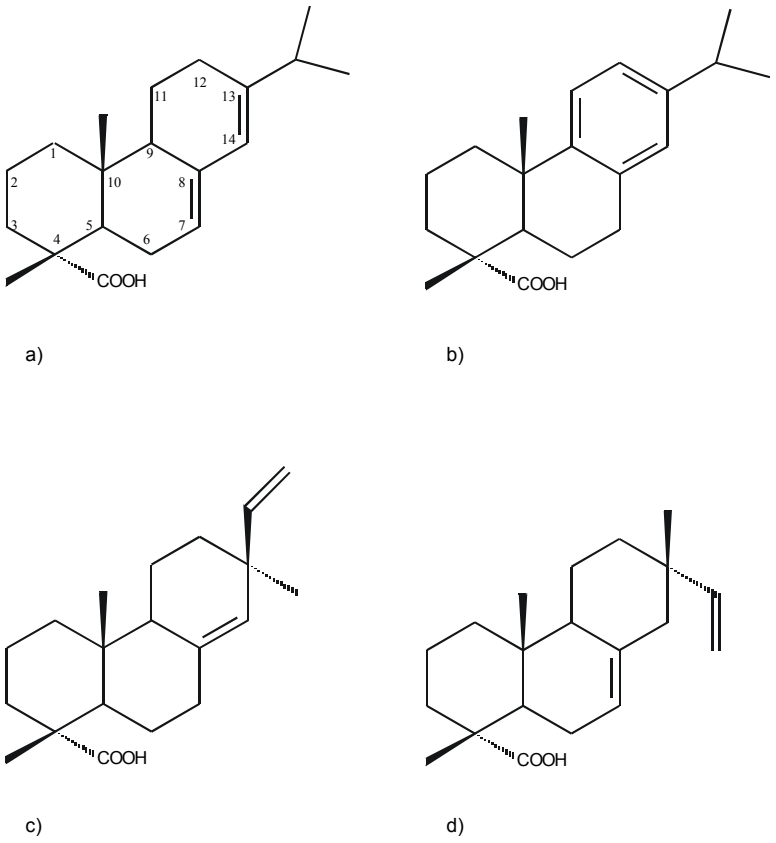


Figure 1.5. Chemical structures of the individual resin acids: a) abietic, b) dehydroabietic, c) isopimaric and d) pimaric acids.

Dehydroabietic acid concentrations in some pulp and paper wastewater represent 34 % of the total resin acid concentration while DhA concentration in the receiving water may represent up to 66 % of the total resin acid content. This may reflect the higher persistence of DhA relative to other resin acids in natural waters and thus its potential for use as a tracer for pulp and paper mill-derived organic materials in receiving waters (Volkman *et al.,* 1993). One group of researchers measured apparent DhA half-life values in natural water and sediment of 0.12 and 21 years, respectively (Volkman *et al.,* 1993) and others have noted similar results (Stuthridge and Tavendale, 1996; Zender *et al.,* 1994; Kutney *et al.,* 1988, 1981). Abietic acid is generally the second most prevalent of the resin acids in natural waters. Degradation

of AbA by either aerobic or anaerobic means is rapid, in contrast to DhA, which does not significantly degrade under anaerobic conditions (Guiot *et al.,* 1998; Patoine *et al.,* 1996).

Like the naphthenic acids, the toxicity and solubility of the resin acids are strongly affected by pH (Liver and Hall, 1996). In general, non-dissociated resin acid species (low pH conditions) have higher toxicological properties than their dissociated counterparts (high pH conditions) while at high pH their water solubility increases. Table 1.4 provides information on the physical and chemical properties of the four resin acids examined in this research.

Table 1.4. Physical and chemical properties of resin acids.

Parameter	Resin Acid	General Characteristic
Colour	All four	White or pale yellow
Odour	All four	Odourless
State	All four	Solid
Molecular Weight	AbA, IpA, PA	302 amu
	DhA	300 amu
Water Solubility	AbA	2.75 mg/L
(at pH 7)	DhA	5.11 mg/L
	IpA	1.70 mg/L
	PA	1.82 mg/L
pK_a		Between 5.7 and 6.4

(Peng and Roberts, 2000; Liss *et al.,* 1997)

1.3.3 Aquatic Toxicity of Resin Acids

Resin acids are part of the microbial defence mechanism in the bark of softwoods and, to a lesser extent, hardwoods (Morgan and Wyndham, 1996; Wang *et al.,* 1995; Leach and Thakore, 1977). Since resin acid concentration is directly related to the softwood content of pulp and paper operations, environmental concentrations also reflect the composition of wood used in processing (Guiot *et al.,* 1998; Liver and Hall, 1996; Volkman *et al.,* 1993; Leach and Thakore, 1977). At trace concentrations (parts per million or billion) resin acids can pose a potential hazard to animal, human and plant life as acutely toxic compounds with toxicity increasing as

pH is lowered (Werker and Hall, 1998; Liver and Hall, 1996; Patoine *et al.,* 1996; Mohn, 1995; Munkittrick *et al.,* 1994; Robinson *et al.,* 1994; Volkman *et al.,* 1993; Leach and Thakore, 1977). The toxicity of resin acids correlates inversely with their solubility (i.e., the pimarane resin acids are the least soluble and the most toxic of the resin acids) (Peng and Roberts, 2000). In addition to acute toxicity, resin acids may bioaccumulate in freshwater mussels and fish tissues (Corin *et al.,* 2000; Stuthridge *et al.,* 1997; Burggraaf *et al.,* 1996; Oikari *et al.,* 1983).

In testing the acute toxicity of the effluents from pulp and paper mills, rainbow trout (*Salmo gairdneri*) has been most commonly used. However, a further study with *Daphnia magna* shows significant differences in the toxicity results for pimaranes using these two common indicator species (Table 1.5). For both IpA and PA, the acute toxicity (reported as 96-h LC_{50}) is at least one magnitude higher for *Daphnia magna* (i.e., much lower concentrations will produce the same lethality results as reported for rainbow trout). The values for the abietanes tend to be closer in value using the two tests (Table 1.5). Besides this, abietic acid has been suggested that it might be the specific etiologic agent in the development of acute and chronic lung disease in workers exposed to resin derived from pinewood (Aranda and Villalaín, 1997; Lee *et al*, 1990; Volkman *et al.,* 1993).

Table 1.5. 96-h LC_{50} values for the four resin acids investigated for rainbow trout (*Salmo gairdneri*) and *Daphnia magna*.

Resin Acid	Toxicity Range (mg/L)	
	D. magna	**Rainbow Trout**
Abietic Acid	0.40	0.7 to 1.5
Dehydroabietic Acid	1.01	0.8 to 1.7
Isopimaric Acid	0.02	0.4 to 1.0
Pimaric Acid	0.06	0.7 to 1.5

(Peng and Roberts, 2000; Werker and Hall, 1998; Liver and Hall, 1996; Taylor *et al.,* 1988; Leach and Thakore, 1976)

The difference in acute toxicity, as well as biodegradability, between the pimaranes and abietanes is related to structure and has been attributed primarily to the C-13

substituents which may either prevent or inhibit degradative enzymes (Peng and Roberts, 2000; Morgan and Wyndham, 1996; Mohn, 1995; Zender *et al.,* 1994).

1.3.4 Analytical Techniques

To date, resin acids quantification and quantitation have been limited primarily to conventional high performance liquid chromatography with ultraviolet absorbance detection (HPLC/UV) (Volkman *et al.,* 1993; Kutney *et al.,* 1988; Morgan and Wyndham, 1996) and gas chromatography (GC) (Corin *et al.,* 2000; Robinson *et al.,* 1994; Werker and Hall, 1998; Voss and Rapsomatiotis, 1985). The GC applications commonly used for resin acid analysis require time-consuming extractions followed by derivatization. Despite this, environmental and effluent concentrations of resin acids have traditionally been quantified by GC methods. Gas chromatography has also been used for measuring degradation kinetics in laboratory experiments, although exhaustive sample preparation limits the quantity of samples generated (Guiot *et al.,* 1998; Bicho *et al.,* 1995; Söderberg *et al.,* 1990). The primary benefit of GC quantification of resin acids in environmental samples is that separation of the three structural resin acid isomers (AbA, IpA and PA) can be readily accomplished (Corin *et al.,* 2000; Robinson *et al.,* 1994; Werker and Hall, 1998).

It is desirable to eliminate the extraction and derivatization requirements of GC analysis while still retaining adequate detection limits for quantitation of resin acids by direct injection of natural water samples. Due to dilution effects in natural waters resin acid concentrations in receiving waters downstream of the effluent may be at or below the μg/L range. At these concentrations HPLC/UV detection limits are not adequate. At this project's beginnings, there were no liquid chromatography mass spectrometry (LC/MS) methods available in the literature, prompting the initiation of research and development of a rapid LC/MS technique that is robust and rugged. The lack of such an analytical method was seen to constitute a critical gap in scientific knowledge and application. In Chapter 4, the utility of liquid chromatography negative ion electrospray ionization mass spectrometry

(LC/ESI//MS) for fast and sensitive quantification of the four selected resin acids in laboratory and natural water samples was evaluated (McMartin *et al.,* 2002). This method minimized the number of sample preparation steps with no extraction or derivatization requirements. In general, detection limits of the LC/ESI/MS method were comparable or lower than GC methods reported in the literature. Unlike the majority of GC methods however, the three structural resin acid isomers (AbA, IpA and PA) do not separate sufficiently using LC under the various conditions evaluated in this work. Therefore, LC/ESI/MS may not be suitable for environmental monitoring in instances where measurement of individual isomeric resin acids is required.

1.3.5 Adsorption of Resin Acids to Soils and Sediments

The binding coefficient (K_p) for dehydroabietic acid in natural freshwater is approximately 500 L/kg indicating that associations between DhA and humic materials or particulates are unlikely (Kukkonen and Oikari, 1991). Binding coefficient values for the other three resin acids studied are not available in the literature. In general, resin acids are weak acids with pK_a values in the range of 5.7 to 6.4 (Liss *et al.,* 1997), indicating that at ambient River Saale pH (approximately pH 8) most of the resin acids are in the ionized, more hydrophilic form that do not tend to accumulate well in sediments (Liss *et al.,* 1997; Kukkonen and Oikari, 1991). Despite this, Crosley (1996) reported resin acid concentrations in suspended sediments of the Athabasca River, AB exceeding those observed in the water by more than three orders of magnitude; transport on suspended sediments for distances in excess of 200 kilometres from the source effluent was also reported.

1.3.6 Microbial Degradation of Resin Acids in the Aquatic Environment

Published biodegradation studies indicate that resin acids tend to be biodegradable by select microbial organisms including some species of fungi and bacteria (Guiot *et al.,* 1998; Liss *et al.,* 1997; Liver and Hall, 1996; Morgan and Wyndham, 1996;

Patoine *et al.,* 1996; Stuthridge and Tavendale, 1996; Bicho *et al.,* 1995; Mohn, 1995; Wang *et al.,* 1995; Zender *et al.,* 1994; Kutney *et al.,* 1988, 1981). Both aerobic and anaerobic biodegradation processes have been studied and shown successful for degrading resin acids in pulp and paper mills from mg/L concentrations to those in the µg/L or lower levels in discharged effluents (Martin *et al.,* 1999; Tavendale *et al.,* 1997a,b; Liver and Hall, 1996; Morgan and Wyndham, 1996; Patoine *et al.,* 1996; Stuthridge and Tavendale, 1996). Figures 1.6 and 1.7 describe proposed aerobic and anaerobic biodegradation pathways, respectively, for abietane resin acids and pimaric acid.

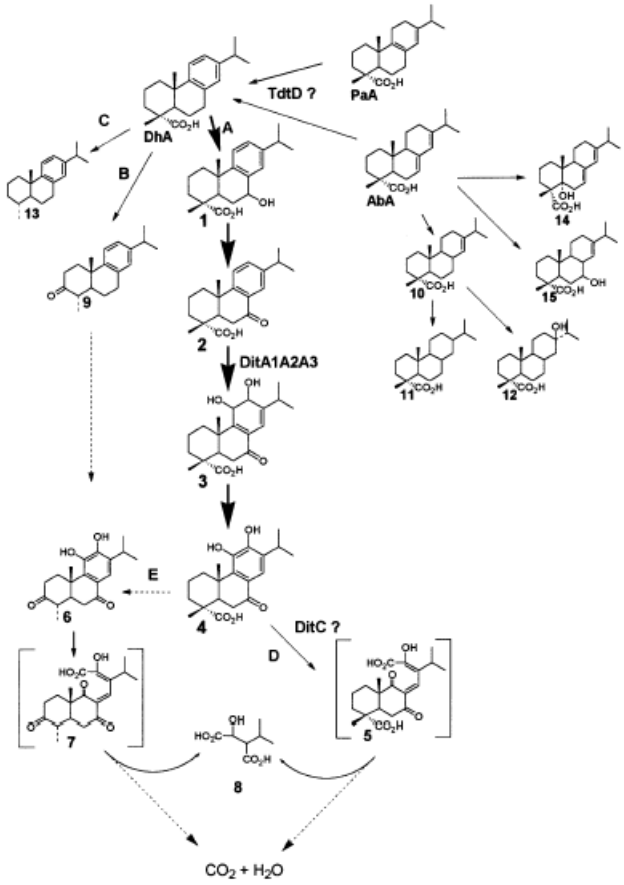


Figure 1.6. Summary of proposed biochemical pathways for aerobic degradation of abietane resin acid degradation or transformation by *Flavobacterium resinovortum*, *Pseudomonas abietaniphila* BKME-9, an *Alcaligenes* and microbial pulp mill effluent treatment systems. Compounds in brackets represent proposed intermediates; dashed arrows represent several potential hypothetical steps in the pathway with unidentified intermediates (reproduced from Martin *et al.,* 1999).

The pathways proposed for aerobic resin acid degradation in Figure 1.6 may be generalized as follows (Martin *et al.,* 1999):

- Pathway A: hydroxylation at C-7 to form the alcohol (**1**) followed by its oxidation to the ketone (**2**) may be achieved using all four of the microbial strains listed in the figure caption.
- Pathway B: oxidation at C-3 to form the ketone (again formed from the alcohol as listed in pathway A) followed by decarboxylation to the corresponding 3-oxodehydroabietin (**9**) achieved by *Flavobacterium resinovortum*.
- Pathway D: dioxygenation of the aromatic ring leading to the formation of a diol, thought to be dihydrodiol (**3**) to form 3,7-dioxo-11,12-diol (**6**) by *Flavobacterium resinovortum* or 7-oxo-11,12-diol (**4**) by *Alcaligenes* and *Pseudomonas*.
- Pathway E: similar result as pathway B, but oxidation of C-3 occurs following dioxygenation.

Further compounds shown in the figure but not listed in the above pathways include: two unidentified ring-cleavage reaction products, **5** and **7**; 2-isopropyl malic acid, **8**; 13-abietenic acid, **10**; abietenic acid, **11**; 13β-hydroxyabietenic acid (kinleithic acid), **12**; dehydroabietin, **13**; 5α-hydroxyabietic acid, **14** 7β-hydroxyabietic acid, **15** (Martin *et al.,* 1999).

Several researchers have noted that the anaerobic degradation (Figure 1.7) of resin acids primarily generates aromatized and decarboxylated products (such as retene) that are thought to persist in the environment (Martin *et al.,* 1999; Tavendale *et al.,* 1997a,b).

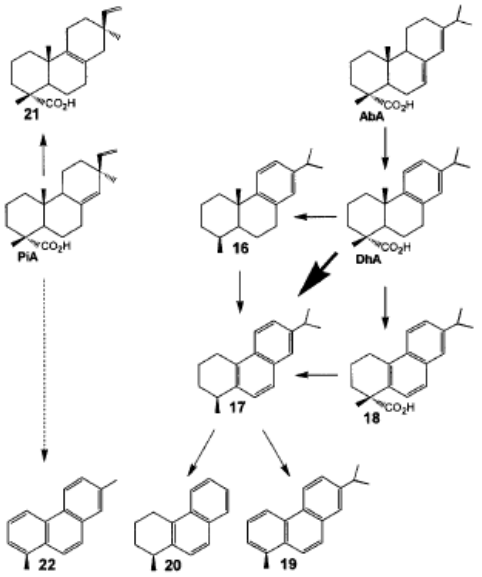


Figure 1.7. Proposed pathways of anaerobic transformation of dehydroabietic (DhA), abietic (AbA) and pimaric (PiA) acids, adapted principally from Tavendale *et al.* (1997a,b) and reproduced from Martin *et al.* (1999).

The proposed transformation products for anaerobic resin acid degradation in Figure 1.7 include the following (Martin *et al.,* 1999):

- The minor and major transformation products of DhA are dehydroabietin (**16**) and tetrahydroretene (**17**), respectively.
- Tetrahydroretene may involve the formation of 20-norabietapentaenoic acid (simonellite) (**18**) that is a short-lived intermediate.
- A small proportion of **17** has been observed to transform to retene (approximately 1 %) (**19**) and methyltetrahydrophenanthrene (**20**), although the majority of the transformation products of 17 are as yet unidentified.
- The degradation of pimaric acid (denoted as PiA in Figure 1.9) may involve transformation to 8-pimarenic acid (**21**) and other acids. As well, pimanthrene (**22**) may result from a multi-step degradation process (although the specific steps have not been identified).

It is generally agreed that abietic acid is the resin acid least resistant resin acid to biodegradation and that its first metabolite is dehydroabietic acid (whether degradation occurs via aerobic or anaerobic means), accounting at least partially for the prevalence of dehydroabietic acid in both effluents and the aquatic environment (Martin *et al.,* 1999; Patoine *et al.,* 1996; Zender *et al.,* 1994). In general, the pimaranes are more persistent to microbial degradation than the abietanes because of structural differences between the two compounds groups – primarily the C-13 substituents that may prevent enzyme induction or inhibit degradative enzymes (Peng and Roberts, 2000; Morgan and Wyndham, 1996; Mohn, 1995; Zender *et al.,* 1994).

1.3.7 Photolysis of Resin Acids in the Aquatic Environment

Irradiation of aquatic organics generates oxygen species (such as hydroxyl radicals and hydrogen peroxide) that may have toxic effects on aquatic organisms. Conversely, solar light and UV-radiation cleave high molecular weight recalcitrant compounds into smaller fragments that are more easily utilized by micro-organisms. Consequently, there are two counteracting effects: bacteria may be either stimulated or harmed by photolysis of aquatic organic compounds (Corin, 2000).

Corin *et al.* (2000) published the single previous investigation pertaining to the potential for photo-transformation of the resin acid, dehydroabietic acid (DhA). These results indicated that DhA might be removed from aquatic samples via exposure to either UV_{254}-radiation or artificial solar radiation with half-life values ranging between 35 and 231 minutes (Corin *et al.,* 2000). The results of the research conducted herein support these findings and further describe the facile pseudo-first-order kinetic photodegradation of a group of four resin acids, including DhA (McMartin *et al.,* 2003). Whether exposed to UV_{254} or broadband UV/vis radiation, photolysis is a powerful degradation method for removing resin acids and their associated toxicity (as measured with Microtox® luminescence assays). Photolysis was also shown to be an effective pre-treatment method for resin acid

biodegradation, with the speed of degradation doubled in bioreactors spiked with photolysis-treated resin acids versus those containing un-photolyzed samples (McMartin *et al.,* 2003).

1.3.8 Summary

Resin acids are natural components of softwood, and to a lesser extent hardwood, trees. Like the naphthenic acids, individual resin acids display contrasting solubility, toxicity and resistance in aquatic environments. Although they are biodegradable, resin acids often persist through the biodegradation treatment systems at pulp and paper mills and are discharged to receiving waters (usually rivers or lakes). Recent research shows that the addition of photolysis pre-treatment to existing microbial pulp and paper mill effluent treatment is likely to be sufficient for reducing resin acids to concentrations well below those required to eliminate acute toxicity. For systems treating large effluent volumes, the implementation of such a combination treatment process utilizing biodegradation subsequent to photolysis at UV_{254} may be cost-effective. Economies of scale must be considered to justify the capital, maintenance and operation costs associated with adding photolysis to existing biodegradation treatment systems.

1.4 Description of the Applied Degradation Technologies

The removal of a given organic substrate in a natural aquatic system is governed by complex interactions between physical, chemical and biological processes – among which are the primary pathways of microbial and photochemical degradation. Both microbial and photochemical degradation processes are functions of environmental factors including temperature, incidence and duration of radiation, inorganic nutrients (such as nitrate-nitrogen, phosphate and others), dissolved organic carbons and supply of dissolved oxygen (Robbins, 1998; Kroschwitz, 1995; Herman *et al.,* 1993; Fan, 1991).

1.4.1 Biofilm Development and Microbial Degradation

In this study, rotating annular reactors (RAR) that simulate the shear and turbulent flow of a natural river system were used for microbial degradation experiments with the resin acids. The hydrodynamics of RARs are controlled by the constant rotational speed of the inner cylinder. As a result of hydrodynamic forces and stresses exerted across the biofilm, there can be a continual erosion of cells and extracellular material from the biofilm back to the bulk fluid. In rotating annular reactors, high surface area to volume ratio and defined flow dynamics in the majority of the reactor allow for estimation of biofilm frictional resistance by directly measuring changes in torque (Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998; Mittelman, 1998).

The RARs provide an environment in which biofilms consisting of microbial cells (algal, fungal, or bacterial) and extracellular biopolymers may be grown in a controlled setting. Surfaces, nutrients and hydrodynamics may all influence biofilm structure, but it remains unclear whether the biofilm structure is merely a consequence of the environmental conditions and forces acting upon it, or whether the biofilm can organize its structure to optimize growth conditions in a certain environment (Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998; Mittelman, 1998). Biofilm development is most rapid in flowing systems where adequate nutrients are available. Many surfaces attract and concentrate nutrients and several micro-organisms have the capacity to detect and move toward high concentrations of nutrients (Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998; Atlas and Bartha, 1987). Three primary factors are necessary for the growth and reproduction of bacteria and thus control the initial thickness of the biofilm, including (1) carbon and energy source; (2) nutrient source and (3) nitrogen source (Bryers, 2000; Stoodley *et al.,* 1999; Madsen, 1998; Nyholm and Peterson, 1997). Other secondary factors that do not contribute to biofilm growth unless the three primary factors are satisfied include: ambient water temperature, flow rate, concentration of inorganic material, pH, surface/substratum (Lookis, 2001; Stoodley *et al.,* 2000; Stoodley *et al.,* 1999; Atlas and Bartha, 1987; Padan, 1984; Ehrlich, 1978; Stotsky and Norman,

1964). Further, there are factors that control biofilm thickness such as hydrodynamic characteristics including mixing, flow regime (turbulent or laminar), shear stress and flow velocity (Bryers, 2000; Griebe and Flemming, 2000).

Bacteria at various depths within a biofilm carry out physiological processes at local conditions not equal to those processes elsewhere in either the biofilm or the bulk fluid (Bryers, 2000; Griebe and Flemming, 2000; Stoodley *et al.,* 1999). The effects of a continuously growing biofilm on the degradation of a substrate (or target compound) are dependent on:

1. Thickness, density and reactivity: Taking into account both external and internal molecular diffusion coupled with a simultaneous microbial reaction rate and
2. Diffusion path: Which allows prediction of the concentration profile of the targeted substance (and by stoichiometry all other nutrients) with biofilm depth and the maximum substrate uptake or flux to the biofilm.

1.4.2 Photolysis and the Hydroxyl Radical ($HO^{\bullet}$)

Photolysis occurs when *chromophores* (the part of a molecule responsible for light absorption) undergo chemical change as a direct consequence of absorbing photons of ultraviolet/visible (UV/vis) light. Solar radiation is comprised of a wide range of wavelengths, the vast majority of which are invisible to the human eye (Figure 1.8). The optical portion of the solar spectrum consists of ultraviolet (100 - 400 nm), visible (400 - 770 nm) and infrared (770 – 1,000,000 nm) radiation. Ultraviolet (UV) radiation is responsible for only 1 % of the total solar irradiance, but is important because it is highly energetic and influences several biological, physical and chemical processes. UV radiation is capable of initiating chemical reactions at the atmospheric, aquatic and terrestrial levels.

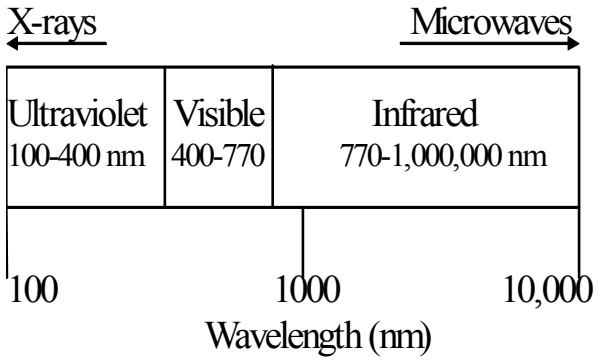


Figure 1.8. The optical portion of the electromagnetic spectrum.

Since optical radiation is capable of initiating biological, physical and chemical processes, it affects organic compounds in the atmosphere, aquatic systems and terrestrial landscape. The UV portion of the optical spectrum has been arbitrarily broken down into three bands, UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm), according to anecdotal effects. UV-A is the least harmful and most commonly occurring UV light at earth's surface; it also has the least energy. UV-B is typically the most destructive form of natural UV light since it has enough energy to damage biological tissues, but not quite enough to be absorbed completely by the atmosphere. The shortest UV wavelength range, UV-C, is almost completely absorbed by ozone and diatomic oxygen atoms in the earth's upper atmosphere (Suppan, 1994; Rabek, 1982; Parker, 1968). One of the most commonly utilized UV-C wavelengths in commercial production is 254 nm (UV_{254}) which has been shown effective for disinfection of air and drinking water supplies, rendering such pathogenic organisms as *Giardia* and *Cryptosporidium* ineffective. Of late, UV_{254}-radiation has been adapted for wastewater treatment for the degradation or removal of organic compounds and pathogens surviving the first three levels of treatment (i.e., City of Regina wastewater treatment plant).

Several photochemical systems, including annular quartz photochemical reactors and Teflon bottles with known transmittance values, and radiation sources,

including UV_{254}, limited UV/vis (two laboratory sources with differing wavelengths) and natural and simulated solar, were investigated in this study. Photochemical processes most often include bond cleavage, production of metabolites and reduction of toxicity. The process may occur in a variety of environments, including the atmosphere, aquatic systems and surface soils. The efficiency of photochemical reactions can be assessed by calculating the quantum yield (Φ) of a reaction. The quantum yield is a more universal measure of photodegradability than is a half-life calculation, because the quantum yield value is independent of the experimental conditions whereas the half-life value is not. The quantum yield of a reaction is given by the following:

$$\Phi = \frac{(\Delta C)(V)}{(I)(t)} \quad (1.1)$$

where: ΔC = change in concentration of the given organic compound (mol)
V = volume of solution (L)
I = intensity of absorbed light (absorbance unit)
t = irradiation time (s)

Understanding the mechanism and course of a photochemical reaction relies on the following rules:

(i) when Φ = 1, every absorbed quantum produces one photochemical reaction;

(ii) when Φ < 1, other reactions (e.g., luminescence or deactivation processes, etc.) compete with the primary photochemical reaction and

(iii) when Φ > 1, a chain reaction takes place.

Photodegradation may proceed via direct and indirect reactions. Direct photolysis occurs when the absorbing molecules undergo chemical reaction and involves energy and electron transfer after absorption of photons by chromophoric dissolved organic matter (CDOM) and chromophoric contaminants. Indirect (sensitized) photolysis involves reactions with photochemically generated intermediates such as reactive oxygen species (ROS) (Goldstone *et al.,* 2002). Included in ROS are

hydrogen peroxide, ozone, nitrate, nitrite, dissolved organic matter (DOM), $FeOH^{2+}$ and reaction of Fe^{2+} with H_2O_2 (the Fenton reaction), but several studies point to nitrate as an important source of hydroxyl radical ($HO^{\bullet}$) in natural waters (Brezonik and Fulkerson-Brekken, 1998; Brezonik, 1994; Haag and Hoigné, 1985). The high reactivity of $HO^{\bullet}$ is advantageous because it degrades many pollutants. However, $HO^{\bullet}$ also reacts with non-pollutant species that are present at higher concentration. This matrix interaction is a major limitation of Fenton degradation in contaminated soils, natural waters and industrial waste streams. In addition, the matrix affects the efficiency of $HO^{\bullet}$ formation. The net results are poor remediation efficiency and higher costs (Lindsey and Tarr, 2000a,b,c,d).

Measurement of $HO^{\bullet}$ radical concentrations during photochemical reactions in natural water makes it possible to differentiate between direct and indirect photodecomposition processes as well as to determine matrix effects on hydroxyl radical formation. The ideal radical scavenger qualitatively gives one product that is easily measured, does not react with sibling radicals, is infinitely soluble and has a diffusion-controlled rate coefficient for the scavenging reaction. Several scavenger options have been reported for determining $HO^{\bullet}$ formation in natural waters exposed to solar radiation including formic acid, ferrocyanide ion, benzoic acid, 1-propanol (n-PrOH), bicarbonate, chloride and 1-chlorobutane (butyl chloride) (Goldstone *et al.,* 2002; LaVerne, 2000a,b; Lindsey and Tarr, 2000a,b,c,d; Brezonik and Fulkerson-Brekken, 1998).

1.5 Critical Gaps in Knowledge

Although it is well-established that a variety of environmental processes are responsible for the attenuation of organic contaminants in natural water courses, examination of photochemical and microbial systems in the laboratory and further exploitation of these processes by industry is not common, nor well-understood. Wastewater treatment plants often use mechanical and chemical filtration removal

processes, and several have implemented microbial degradation as tertiary treatment for removal of organic and inorganic nutrients from discharged effluents. Although widely accepted as efficient and effective degradation systems, the economics, retention periods and spatial issues associated with microbial treatment are often prohibitive for any but large urban centres and industrial facilities. The high hydraulic retention times for microbial processes imply the need for treatment cells of significant volume where settled and filtered wastewater may be allowed sufficient contact time with acclimated micro-organisms.

Photolysis is an emerging water treatment technology adaptable to pre-treatment of wastewaters. However, implementation of photochemical degradation systems, either for disinfection or organics removal, remains novel. Photolysis is acknowledged as an effective method for reducing the toxicity of organic compounds in most instances, often rendering target pollutants more bioavailable (Corin, 2000; Finkel and Irwin, 2000; Atlas and Bartha, 1987). The implementation of aqueous wastewater treatment systems in which microbial processes occur subsequent to photolysis pre-treatment may reduce the potential microbial shock loading as well as the required hydraulic retention times and, therefore, reduce space requirements while improving degradation efficiency. Although there are a very few municipal water and wastewater photolysis systems in place, there is no published research to date that identifies the efficacy of photolysis pre-treatment for organics removal in industrial, municipal or natural water systems.

In aquatic environments, photolysis may act as a natural pre-treatment for the biodegradation and attenuation of organic contaminants. However, since penetration of UV light into river water may be restricted to the top 5 mm, exposure to sunlight is minimal in some natural aquatic ecosystems. However, even short exposure periods may result in photon absorption or activation and structural change to allow for facile biodegradation by natural river biofilms. Since there is a dearth of published literature regarding applications of biodegradation followed by photolysis for the removal of specific target pollutants, research is required to adequately

assess the potential benefits and implications of such combination treatment systems. Critical gaps in knowledge exist with respect to the application and evaluation of pre-photolysis and biodegradation.

1.6 Applications to Remediation

The research presented herein provides a contribution regarding the environmental behaviour and potential for remediation of specific persistent organic hydrocarbon acids in aquatic environments. Through environmental monitoring and evaluation, it may be possible to develop and implement appropriate engineering measures to minimize negative impacts of industry-related organic compounds in the water column. Examination of kinetic behaviour is vital to environmental engineering and prediction of the fate and persistence of contaminants in aquatic systems. In addition, the exploitation of microbial and photochemical degradation techniques for the removal of complex chemical compounds prior to release to aquatic environments is essential in engineering practice to minimize negative environmental impacts of industrial operations. Determination of kinetic behaviour relative to primary degradation processes in aquatic and engineered environments is essential for design of efficient treatment systems for specific industrial applications.

At present, several industries utilize microbial wastewater treatment subsequent to primary treatment that generally entails settling or filtering of effluent streams. Microbial degradation is an inexpensive alternative that can be applied to a wide variety of organic contaminants and a variety of effluent qualities. The implementation of photolysis between primary and secondary wastewater treatment is an option that may significantly increase bioavailability of the target organic contaminants in the waste stream. Again, reliable data on median effluent quality as well as photodegradation kinetics are requisite to the optimal design and operation of such photolysis treatment systems. Extrapolation from laboratory results for each of these degradation processes enables modelling of contaminant fate and transport

within the industrial treatment system, as well as in natural aquatic environments.

1.7 Research Objectives

To examine and test the hypotheses of the proposed research, development of analytical methods was first accomplished. Since few rugged, fast and straightforward methods were available in the literature, development of analytical methods for both naphthenic acids and resin acids was a preliminary objective. For the purposes of minimizing laboratory sample preparation time and potential losses through the sample preparation stage, enhancing detection limits and producing the best overall results, LC/MS was the best option in each instance.

The overall objective of this research was to identify and evaluate degradation processes capable of removing two groups of hydrocarbon acids (naphthenic acids and resin acids) from aquatic environments and to elucidate the persistence and fate of those compounds. To that end, both photochemical and microbial degradation systems were investigated. *It was hypothesized that the rate of degradation of each compound group using the combined transformation processes would result in increased contaminant removal and bioavailability*. The specific objectives of each chapter are outlined in Table 1.6.

Rivers were chosen for studying each compound group in areas of environmental relevance. Naphthenic acid research was conducted in Athabasca River water collected in the oil sands mining and extraction region north of Fort McMurray, Alberta in the Athabasca Oil Sands. Resin acid persistence was examined in water collected from various locations along the River Saale (Germany) water from a well-forested pulp and paper-milling region of that Elbe tributary.

Table 1.6. Research objectives by chapter.

Chapter	Objectives	Description of Chapter
1	Introduction and Background	Information on oil production and a scientific review manuscript of past research related to diagenesis, extraction, toxicity and degradability of naphthenic acids; review of the pulp and paper process and a literature review of resin acids; identification of critical gaps in knowledge, degradation systems and applications to remediation
2	To develop a robust, fast method for naphthenic acid quantification in natural water sources	Solid phase extraction and pH dependence were investigated in the development of an LC/MS method for naphthenic acids
3	To determine the efficiency of photolysis for the removal of naphthenic acid mixtures and compounds from natural water	Studies of hydroxyl radical formation, artificial and natural solar radiation and UV_{254} photolysis of compounds and mixtures
4	To develop a robust, fast method for resin acid quantification in natural water sources	A method requiring no sample preparation was developed for four selected resin acids
5	To provide information on the fate and transport of resin acids in a natural river system and discuss some of the flow regime and resin acid persistence issues observed	A field monitoring study was undertaken at various key points along the River Saale, Germany, to investigate the extent of resin acid contamination downstream of a large pulp and paper mill
6	To evaluate the potential for removal of resin acids from natural waters using photolysis pre-treatment followed by biological degradation using natural River Saale biofilms	Investigations of the applicability of UV_{254} treatment radiation and artificial solar radiation for resin acid removal in natural waters
7	General synthesis, discussion and conclusions	A synthesis of results and how these contribute to existing critical gaps in knowledge and a description of the ecological and industrial significance of the results
8	References	

2. DETERMINATION OF DISSOLVED NAPHTHENIC ACIDS IN NATURAL WATERS USING NEGATIVE-ION ELECTROSPRAY IONIZATION MASS SPECTROMETRY

As noted in the section discussing the research objectives, the preliminary requirements of fate and persistence studies include analytical method development. No assessment of naphthenic acids degradation was possible without first establishing a sensitive and robust analytical method. The following is a description of the development of a negative ion electrospray ionization mass spectrometry method (ESI/MS) that is an important milestone in the progress toward accurate quantification and quantitation of naphthenic acids in aquatic environments. Additionally, the manuscript describes the development as a fast and robust solid phase extraction (SPE) procedure for the determination of naphthenic acids in natural waters. The citation for the published manuscript is as follows:

Headley, J.V., K.M. Peru, **D.W. McMartin** and M. Winkler. 2002. Determination of dissolved naphthenic acids in natural waters using negative-ion electrospray mass spectrometry. *Journal of the AOAC International* **85**(1): 182-187.

In addition to the published analytical method described herein, a further method utilizing a MicroMass Quattro Ultima instrument was developed for day-to-day analyses at the National Water Research Institute (NWRI). The operating conditions for this second LC/MS method were developed subsequent to the publication of this chapter manuscript, and are subsequently described in the instrumental section of Chapter 3.

2.1 Introduction

Naphthenic acids are a complex mixture of aliphatic and alicyclic carboxylic acids (Figure 1.2) that are completely soluble in organic solvents and have water solubilities that are pH dependent. Typical pKa values for naphthenic acid components are between 5 and 6. Naphthenic acids are represented by the general formula described by $C_mH_{2m-z}O_2$ where m indicates the carbon number and z represents the number of hydrogen atoms that are lost as the structures become more compact. The z value is equal to 0 for saturated linear hydrocarbon chains, and changes to -2 in monocyclic naphthenic acids, -4 in bicyclic, -6 in tricyclic, and so on. The polarity and non-volatility of naphthenic acids increases with molecular weight, giving individual compounds within the naphthenic acid group with varying physical, chemical and toxicological properties (CEATAG, 1998; Kroschwitz, 1995; Herman *et al.,* 1993; Fan, 1991; Dzidic *et al.,* 1988).

Surface water systems may be contaminated with naphthenic acids via several processes including groundwater mixing and riverbank erosion. Of the possible environmental receptors (i.e., air, soil, and water), the most significant naphthenic acid concentrations are found in water due to direct contact with oil sands material (CEATAG, 1998; Herman *et al.,* 1994; Davis, 1967). Concentrations of naphthenic acids found in northern Alberta rivers unaffected by oil sands materials processing are generally below 1 mg/L, but may be as high as 110 mg/L in tailings waters. Natural groundwater levels in the region range from < 4 mg/L in near-surface aquifers to > 55 mg/L in basal and limestone aquifers (CEATAG, 1998).

Naphthenic acids are environmentally significant as they are toxic to aquatic algae and other micro-organisms and are suspected endocrine disrupting substances (EDSs) (CEATAG, 1998). Water from oil sands processing has been shown to be acutely toxic to aquatic organisms (LC_{50} < 10% v/v for rainbow trout (*Salmo gairdneri*) (MacKinnon and Boerger, 1986) and rats (oral LC_{50} = 3.0 g/kg body weight for commercial mixtures and 1.75 g/kg for a mixture of dimethylcyclohexane isomers) (U.S.EPA, 1987; Uzhdavini and Glukharev, 1984).

Further mammalian evaluations (including rats, dogs, and rabbits) showed increased vascular permeability in capillaries, notable effects on the formation of red and white blood cells and platelets, and an efflux of potassium from cells causing first stimulation then inhibition of cellular respiration (Lai *et al.,* 1996; Herman *et al.,* 1994).

In view that naphthenic acids occur as a complex mixture of compounds in the environment, there has not been conclusive identification of which specific compounds are the most toxic and/or corrosive; toxicity does not always correlate directly with the concentration of naphthenic acids. The presence of naphthenic acids in crude oils is of concern due to their corrosivity to oil sands extraction units, and thus they have been the focus of remedial activities in oil sands extraction (CEATAG, 1998; Hsu *et al.,* 1998; Lai *et al.,* 1996; Kroschwitz, 1995).

Owing to the prevalence and toxicity of naphthenic acids, there has been growing interest in recent years to develop sensitive and robust analytical methods to study the fate and transport of naphthenic acids in the environment (Nascimento *et al.,* 1999; Herman *et al.,* 1994). Although naphthenic acids were first reported as oil sands components during the late 1960s, environmental assessment of naphthenic acids has been hampered due to a void of published analytical methods (Seifert *et al.,* 1969). This lack of methodology is due to the complexity of the mixture, the lack of suitable extraction techniques and the lack of detection sensitivity. To date, quantitation has been limited to infrared methods while Fast Atom Bombardment Mass Spectrometry (FAB/MS) or GC/MS with derivatization have been used for qualitative analysis (CEATAG, 1998; St John *et al.,* 1998; Fan, 1991). Other unpublished methods utilized by Syncrude Canada include adaptation of Fourier Transform Infrared (FT/IR) Spectroscopy for quantitative analysis of naphthenic acids.

In this work, the utility of electrospray ionization with mass spectrometric detection (ESI/MS) is discussed, as is the development as a fast and robust solid phase

extraction (SPE) procedure for the determination of naphthenic acids in natural waters.

2.2. Experimental

2.2.1 Chemicals

Two naphthenic acid mixtures were studied using negative-ion ESI/MS. These included a standard mixture from FlukaChemicals (Fluka, Sigma-Aldrich Canada, Inc., Oakville, Canada) and an acidic fraction obtained from the tar sands of the Syncrude (Fort McMurray, AB, Canada) processing operation (isolated and described by Rogers *et al.,* 2002a). During the oil sands extraction process, the acidic compounds, including naphthenic acids, are extracted from the crude oil using a caustic scrubbing technique (CEATAG, 1998; Davis, 1967).

2.2.2 Standard Preparation

Commercial naphthenic acid standards were prepared at four pH values (3, 5, 7 and 9) in Milli-Q water using the Fluka naphthenic acid compounds to evaluate the effect of pH on ESI/MS mass profiles. Saturated solutions were prepared by suspending three to four drops of the Fluka naphthenic acid in 100 mL of phosphate (BDH, Inc., Toronto, Canada) buffered Milli-Q water and gently agitating overnight. Calibration was established using a set of four standards prepared from the pH 7 saturated naphthenic acid stock solution using appropriate dilutions with Milli-Q water. The concentration of saturated stock was determined prior to dilution by comparison to standards prepared in acetonitrile (HPLC grade, BDH, Inc.).

Standards of the Syncrude tar sand acidic fraction were used to determine the expected mass profile of naphthenic acids in water samples located near oil sands extraction sites and to measure recovery of the extraction procedure. Saturation was not achieved with this naphthenic acid fraction since the caustic hot water flotation

process required for the exctraction process had solubilized the naphthenic acids into sodium salts. Two sets of four standards were thus prepared at pHs 9.2 and 11.3 by measuring 1 mL of the Syncrude acid fraction into 100 mL of phosphate buffer and making the necessary dilutions with Milli-Q water. Final pHs were achieved with additions of phosphoric acid (BDH, Inc.).

To evaluate the recovery of naphthenic acids in natural waters, both Fluka and Syncrude naphthenic acid standard solutions were used to spike South Saskatchewan River (near Saskatoon, Canada) water and northern Alberta river water samples. This has proven to be a critical step in the method development as natural waters contained dissolved organic matter (DOC) of similar chemical and physical properties to the naphthenic acids.

2.2.3 Sample Extraction

Water samples were collected from several northern Alberta rivers located near crude oil refineries in the Fort McMurray area. In addition, samples from the South Saskatchewan River water, with a known low DOC content (~ 3.5 mg/L year round), were collected at Saskatoon, Canada for use in determining the effects of natural DOC on extraction efficiency and the detection limit of the naphthenic acids. A DVB (divinyl benzene) supported sorbent (ENV+, IST, UK) was used for solid phase extraction.

Triplicate water samples of 1 L were extracted using the SPE procedure summarized in Figure 1.4. In brief, for each spiked river water sample a 200 mg Isolute ENV+ cartridge was conditioned using 10 mL of methanol followed by 10 mL of Milli-Q water under gravity flow. The sample was acidified to pH 3 with formic acid (Fluka, Sigma-Aldrich Canada, Inc, Oakville, Canada) to maximize recovery. A specified volume of this acidified sample containing 5 mL/L methanol (HPLC grade, Fisher Scientific, Edmonton, Canada) (also to aid recovery) was drawn through the cartridge under vacuum suction at a rate of 10 mL/min. The cartridge

was rinsed with 10 mL of Milli-Q water under gravity flow (this step proved useful for desalting the extract and removing residual phosphate buffer) and subsequently dried under vacuum for at least 30 minutes. The sorbed naphthenic acids were eluted using 6 mL of acetonitrile by gravity flow into a graduated test tube and the extract evaporated with dry N_2 gas (HP grade) to 1 mL total volume. ESI/MS analysis was performed on this extract with no further clean-up or derivatization steps.

2.2.4 Instrumental

Analysis was conducted using an AutoSpec Q mass spectrometer (Micromass, UK) equipped with an electrospray ionization (ESI) interface operating in the negative ion mode. MS conditions were as follows: source temperature 80 °C, cone voltage setting 23 V, ring electrode setting 17 V, needle voltage setting 56.2 V, nebulizer gas N_2 at 15 L/hr, bath gas N_2 at 200 L/hr. Resolution was tuned to 1200 and the detector set to 415 V. Quantitative analysis was performed using full scan data. A Phoenix 20 (Fisons) syringe pump was employed for eluent delivery at a flow rate of 200 μL/min with a post column split allowing 30 μL/min to the ESI source. Eluent consisted of 50:50 acetonitrile:water plus 0.5 % ammonium hydroxide. Loop injection (10 μL) was performed using a Reodyne 7125 injector.

2.3 Results and Discussion

2.3.1 Effects of pH on the Solubility of Naphthenic Acids in Water

The fraction of dissolved naphthenic acids in natural water sources is dependent on the pH of the water. At pH 3.0 (Figure 2.1a), the main soluble components are non-cyclic naphthenic acids as confirmed by GC/MS analysis of derivatized extracts. Other components (i.e., cyclic naphthenic acids) become more soluble as the pH increases as shown in Figure 2.1b. At pH 7 and above, the most representative solubility is reached; further pH increases do not change the mass profile significantly as illustrated in Figures 2.1c and 2.1d. However, the total ion

abundance increases by 37 % from pH 7 to pH 9 reflecting that the relative total solubility of the naphthenic acid mixture is dependent on higher pH values. This correlates well with the caustic scrubbing procedure used by the industrial extraction process (CEATAG, 1998; Davis, 1967).

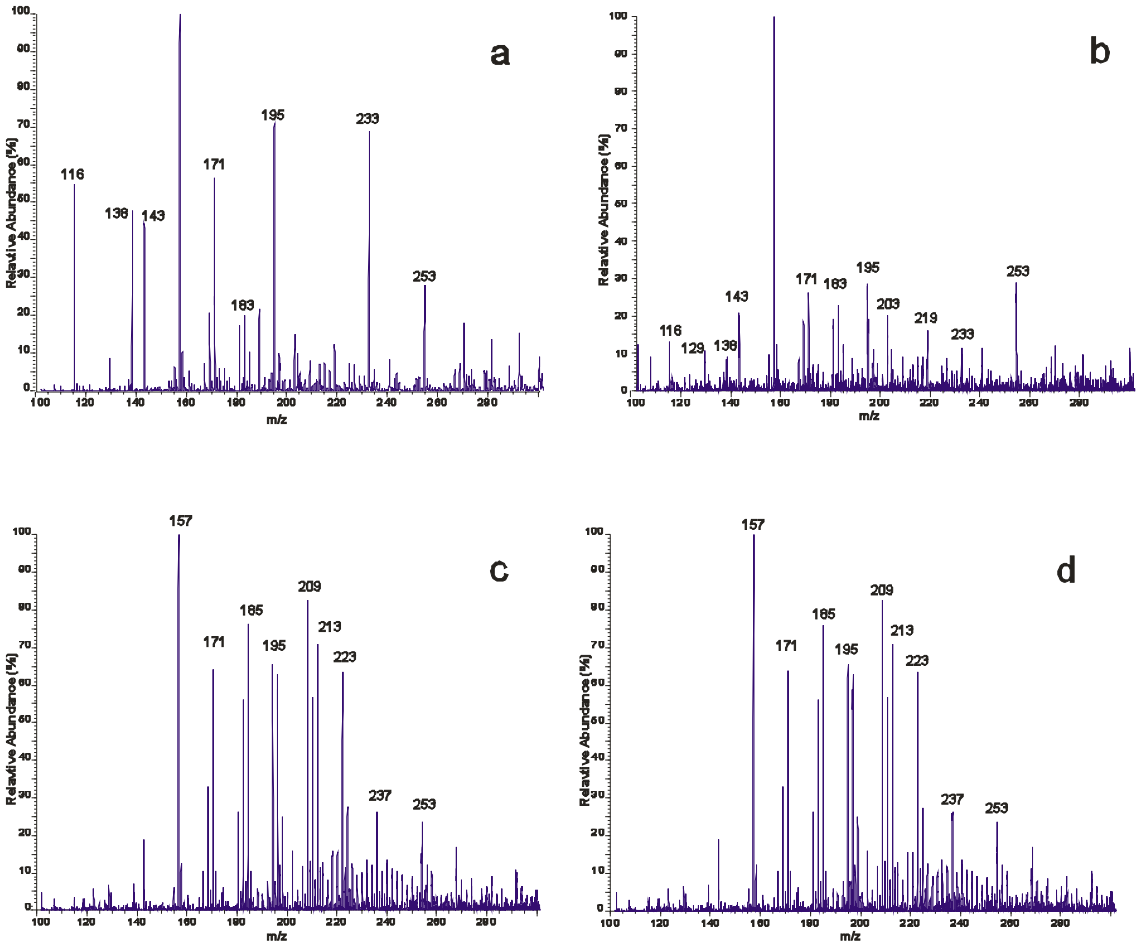


Figure 2.1. Mass profiles obtained from Fluka naphthenic acid saturated Milli-Q water at pH a) 3.0; b) 5.0; c) 7.0; and d) 9.0.

It is thus critical to measure the initial sample pH, which determines the expected mass profiles to identify naphthenic acids in natural waters. This is an essential step to determine the most suitable ions for selected ion monitoring analysis.

The mass profiles of the four Fluka naphthenic acid standard solutions indicated that the extraction efficiency (based on *m/z* = 237) for solutions of both 0.1 mg/L acetonitrile and buffered solutions was best at pH 3.0 with approximately 100 % recovery. At pHs 7 and 9, the recoveries were 63 % and 50 %, respectively. In unbuffered Milli-Q water, extraction efficiency was 79 %.

2.3.2 Calibration for Quantitative Analysis

To obtain a representative calibration, curves were constructed using the area summation of the five major naphthenic acid ions present (namely *m/z*: 205, 223, 237, 251 and 265) in the full scan mass spectra shown in Figure 2.2. At the resolution employed in the collection of the continuum mass spectra, all peaks were base-line resolved singlets. No correction was therefore needed for calibration.

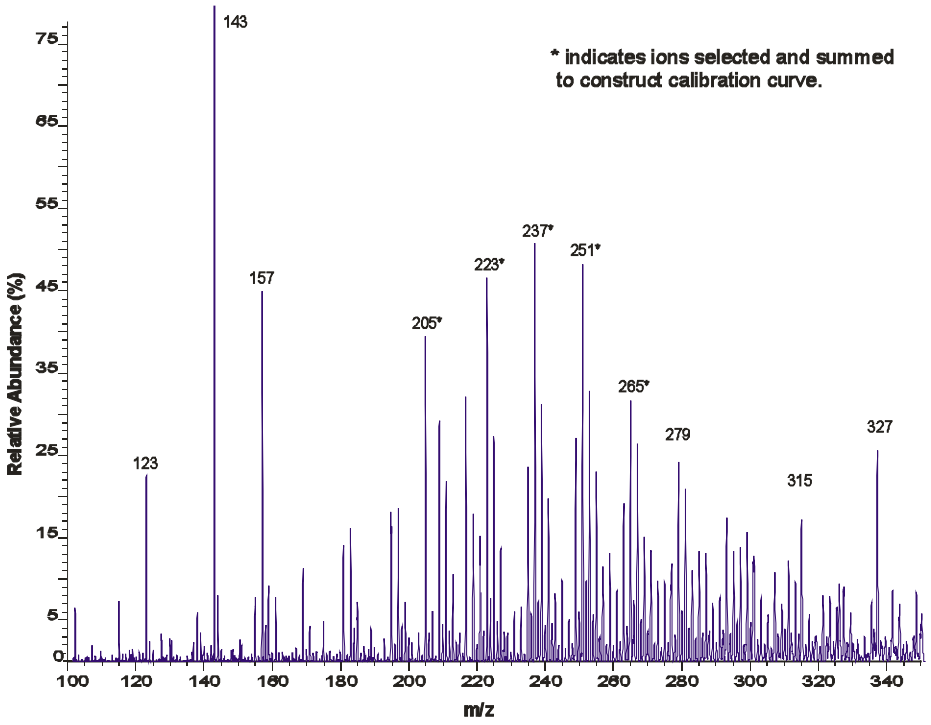


Figure 2.2. Mass spectra of Syncrude naphthenic acid fraction at pH 9.2.

Linear calibration curves (r^2 = 0.9998) were obtained from analysis of both Fluka and Syncrude mixtures with naphthenic acid concentrations of 10 mg/L through 100 mg/L. Limits of detection of the naphthenic acid mixtures in 500 mL water samples using the SPE procedure were calculated to be 0.01 mg/L using 3X S/N. However, the detection limits may be increased significantly in the presence of high amounts of other DOCs in the matrix as seen in Figure 2.3.

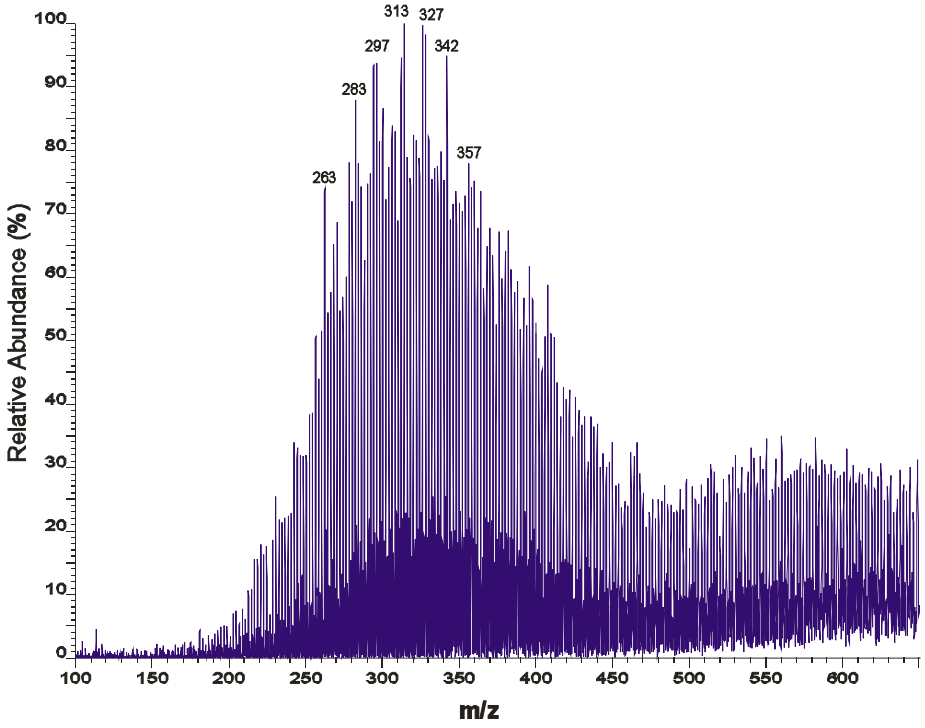


Figure 2.3. Mass spectrum of extracted water sample containing naphthenic acids and high DOC levels.

2.3.3 Natural River Water

To determine the extraction efficiency of the Syncrude naphthenic acid mixture, 500 mL water samples were spiked in duplicate to contain 0.16, 0.02 and 0.01 mg/L of the Syncrude naphthenic acid prior to SPE. Mean recoveries were 87, 114 and 120 %, respectively for each of the concentrations, with corresponding RSD values of 9, 13 and 15 %, respectively. As shown in Figure 2.3, mass interference was

problematic in those northern Alberta samples that contained high levels of DOC (10 to 20 mg/L) with masses in the same range as the naphthenic acid standards.

In contrast, for Syncrude naphthenic acid spiked at 10 to 150 mg/L in South Saskatchewan River water (DOC approximately 3.5 mg/L) there was little mass interference for extractions performed at pHs 3 and 5. Both naphthenic acid extraction efficiency and resolution of the naphthenic acids and DOCs were best at pH 3. Overall, samples with low to moderate DOC levels (2 to 5 mg/L) could be analyzed with reproducible recoveries, as shown in the mass spectrum given in Figure 2.4.

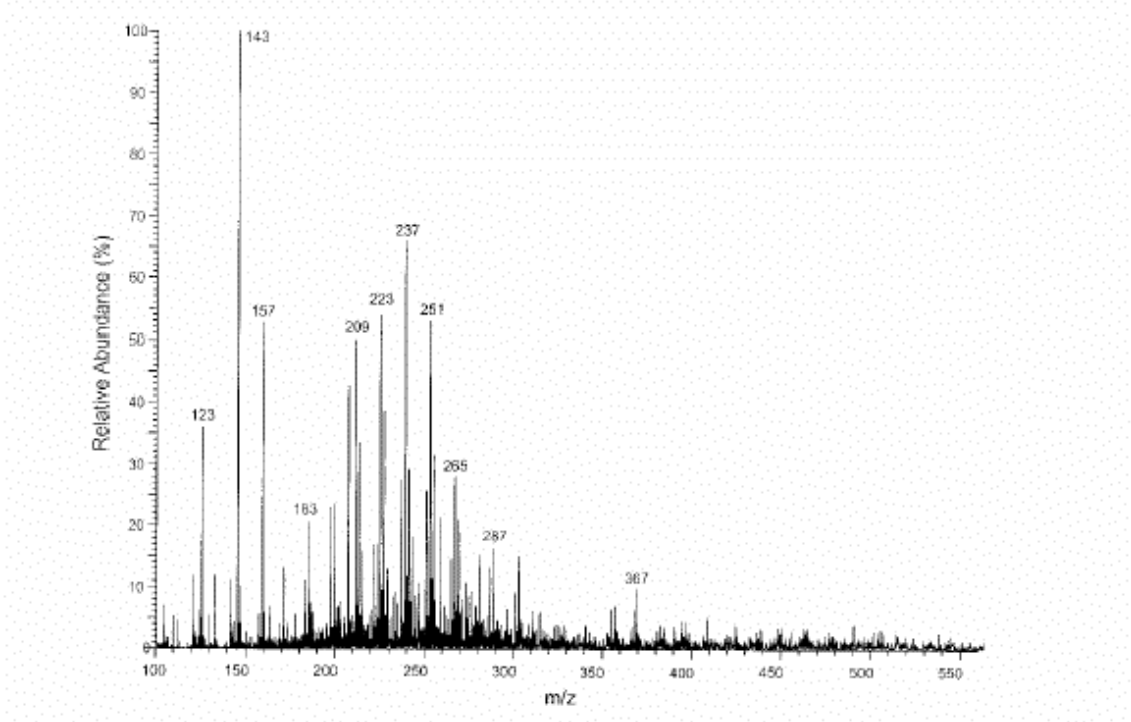


Figure 2.4. Mass spectrum of sample illustrating naphthenic acid mass envelope in spiked natural water.

The extraction and ESI/MS procedures were applied in a preliminary investigation of eight surface water samples from northern Alberta rivers and the South Saskatchewan River. Ambient naphthenic acids in these samples were found to be below the detection limit (< 0.01 mg/L).

Research is continuing to explore the use of liquid chromatography and high resolution MS to separate naphthenic acids from interfering substances in the natural water samples, and to extend the methodology to similar method development for soils and sediments. The latter is needed to provide a more detailed picture of the fate and transport of naphthenic acids in the environment.

2.4 Summary

The ESI/MS method proved to be a simple procedure for the quantitative analysis of naphthenic acids. The method was amenable for studying the naphthenic acid compounds at a detection limit of 0.01 mg/L. While full scan data was used in order to monitor and characterize the complete mass profile of the naphthenic acids, it is noted that the detection limits could be reduced significantly using selected ion monitoring (SIM). For application to SIM, the pH dependence of the dissolved naphthenic acid fraction must be accounted for in choosing suitable ions for quantification and confirmation of the naphthenic acids. However, as co-extractives, such as humic and fulvic acids, interfere with the detection of naphthenic acids, the application of this procedure is limited to natural waters with relatively low to moderate concentrations of DOC or with masses that are beyond the naphthenic acid mass envelope range.

2.5 Acknowledgements

Thanks to Malcolm Conly for sample collection and the Program of Energy Research and Development (PERD) for financial funding of this study.

3. PHOTOLYSIS OF NAPHTHENIC ACIDS IN NATURAL WATERS

The objective of the research described in this chapter was to determine whether or not photolysis is an effective method for the removal of naphthenic acid mixtures and compounds from natural water sources. Further, the effect of the naphthenic acids investigated on a specific toxicological pathway was evaluated as a measure of the success of the applied degradation method. *It was hypothesized that the use of photolysis as a pre-treatment to biodegradation will affect the composition and/or concentration of naphthenic acid mixtures, rendering the overall mixture more bioavailable to micro-organisms.*

This paper is in press as of November, 2003:

McMartin, D.W., J.V. Headley, D.A. Friesen, K.M. Peru and J.A. Gillies. 2003. Photolysis of naphthenic acids in natural surface waters. *Journal of Environmental Science and Health Part A*. In Press.

3.1 Introduction

Naphthenic acids are a diverse group of saturated aliphatic and alicyclic carboxylic acids where, in Figure 1.2a, y usually ranges from 0 to 6 (as $y > 6$ constitutes a very minor part of the acid content) and $x \geq 0$ (Fan, 1991; Dzidic *et al.,* 1988; CEATAG, 1998). They are surfactant-type compounds that are natural constituents of bitumen and other forms of petroleum (Frakman *et al.,* 1990; Strausz, 1989). Naphthenic acids form a complex group of compounds in the environment and, currently, there is no conclusive identification of which specific compounds are the most toxic. Toxicity does not always correlate directly with the concentration of naphthenic acids (Rogers *et al.,* 2002b; CEATAG, 1998; Hsu *et al.,* 1998; Lai *et al.,* 1996; Kroschwitz, 1995).

Since they are highly persistent in the environment and are contained at significant concentrations in oil sands extraction tailings pond water (as high as 110 mg/L), the potential for naphthenic acid contamination of surface water supplies is worth examining. Naphthenic acids are extracted to reduce corrosivity using a caustic hot-water treatment (CEATAG, 1998; Kroschwitz, 1995; Lai *et al.,* 1996; Hsu *et al.,* 1998). Surface waters may become contaminated with naphthenic acids via several processes including groundwater mixing and riverbank erosion (CEATAG, 1998; Herman *et al.,* 1994; Davis, 1967).

In this study, exposure to UV and UV/vis radiation was evaluated to determine the photolytic fate of select naphthenic acid compounds and mixtures. The radiation sources were chosen to represent both industrial remediation (UV_{254}-radiation) and environmental fate (natural and artificial solar full-spectrum radiation). Several factors impact on the photodegradation kinetics of organic molecules in aquatic environments including sunlight conditions, humics content, the presence of other chromophores such as ferric compounds and water quality factors such as pH and turbidity. Humic substances can have multiple photochemical roles including: (1) absorbing UV light and sometimes acting as photosensitizers, (2) acting as a source of hydroxyl radicals ($OH^{\bullet}$) and (3) serving as an $OH^{\bullet}$ scavenger in water solutions and therefore playing a dominant role in the aquatic photochemical processes (Balmer and Sulzberger, 1999; Clair and Sayer, 1997; Torrents *et al.,* 1997; Kieber *et al.,* 1990). Here, the production of hydroxyl radicals was assessed using a benzoic acid chemical probe monitored simultaneously with the naphthenic acids via electrospray ionization with mass spectrometric detection (LC/ESI/MS).

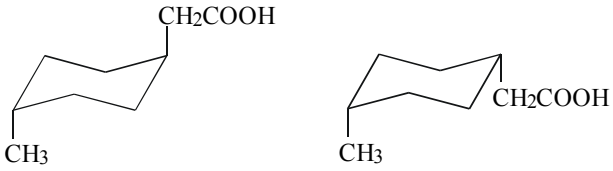
The anticipated increase in global UV-radiation and growing interest in UV water treatment processes require a better understanding of UV and sunlight-mediated chemical processes taking place in the environment. The purpose of the current study was to determine the effects of select naphthenic acids exposed to both UV_{254}-radiation and solar radiation.

3.2 Materials and Methods

3.2.1 Water Samples and Chemicals

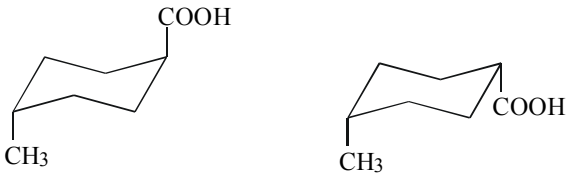
Water was collected from the Athabasca River at Fort McMurray (Figure 1.1) as previously described. Three naphthenic acids mixtures with varying composition and three individual isomeric compounds were assessed. Two commercial naphthenic acid mixtures were purchased from FlukaChemicals (Fluka, Sigma-Aldrich Canada, Ltd., Oakville, Canada) and Acros (Acros Organics, Geel, Belgium). The third naphthenic acids mixture was extracted from an Athabasca Oil Sands (AOS) tailings pond (Rogers *et al.,* 2002a,b), obtained from the Toxicology Centre, University of Saskatchewan, Saskatoon, Canada. The three individual model naphthenic acids (Figure 3.1), 4-methylcyclohexaneacetic acid (4-MCHAA), 4-methylcyclohexanecarboxylic acid (4-MCHCA) and 3-methylcyclohexanecarboxylic acid (3-MCHCA), were purchased from Sigma-Aldrich Canada, Ltd. (Oakville, Canada).

4-MCHAA



(a) *trans*-isomer *cis*-isomer

4-MCHCA



(b) *trans*-isomer *cis*-isomer

3-MCHCA

(c) *trans*-isomer *cis*-isomer

Figure 3.1. Chemical structures of the three model naphthenic acids investigated.

3.2.2 Photolysis

Several photolysis experiments were completed including those with natural sunlight, artificial solar radiation in growth chambers using a canopy of incandescent and fluorescent lamps, artificial UV-range solar radiation in quartz annular photochemical cells (Figure 3.2) and 254 nm (UV_{254}) ultraviolet lamps in quartz annular photochemical cells. A full listing of the experiments conducted is outlined in Table 3.1. All solutions were subjected to a delay of 15 minutes prior to

exposure to allow for adequate mixing of the spiked solutions. No commercial catalysts were added.

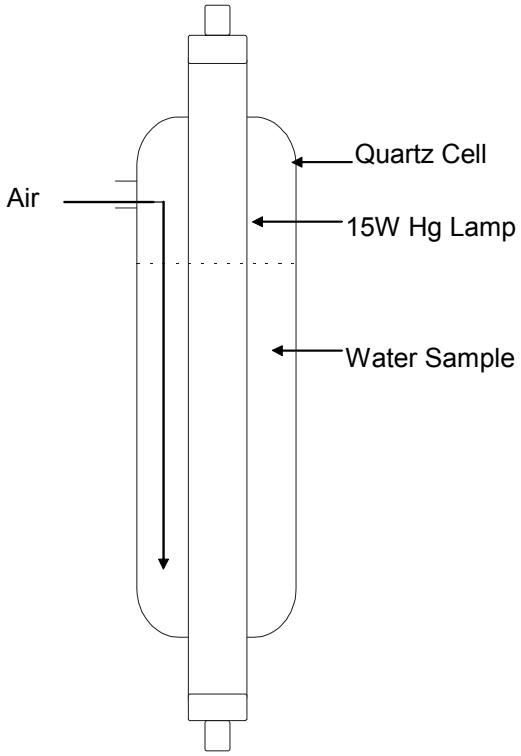


Figure 3.2. Schematic of the photochemical reactor apparatus.

To adequately expose naphthenic acid solutions in Athabasca River water to solar radiation, Teflon bottles with the spectral characteristics illustrated in Figure 3.3 were used. The bottles were placed in a shallow plexiglas tray constructed to maintain constant water flow around the bottles. These experiments were conducted for periods of 7 to 10 days on the roof of the National Hydrology Research Centre in Saskatoon, Canada between July and September, 2002.

Table 3.1. Experiments conducted.

Compound	Radiation Source	Concentration (mg/L)
4-MCHAA	NSR	0.5, 1
	BLB	0.5, 1
	UV_{254}	0.5, 1
4-MCHCA	BLB	0.5, 1
	UV_{254}	0.5, 1
3-MCHCA	BLB	0.5, 1
	UV_{254}	0.5, 1
Combination of 4-MCHAA, 4-MCHCA and 3-MCHCA	BLB	0.5, 1
	UV_{254}	0.5, 1
AOS Extract	NSR	0.5, 1, 5, 50
	BLB	50, 100
	Growth Chamber	50, 125
	UV_{254}	5, 10, 25, 50
Fluka Mixture	ASR	50, 100
	Growth Chamber	50
	UV_{254}	50, 100
Acros Mixture	BLB	50, 100
	Growth Chamber	50
	UV_{254}	50, 100

NRS = natural solar radiation; BLB = Philips 300-400 nm lamp; Growth Chamber = artificial solar radiation (lamp canopy)

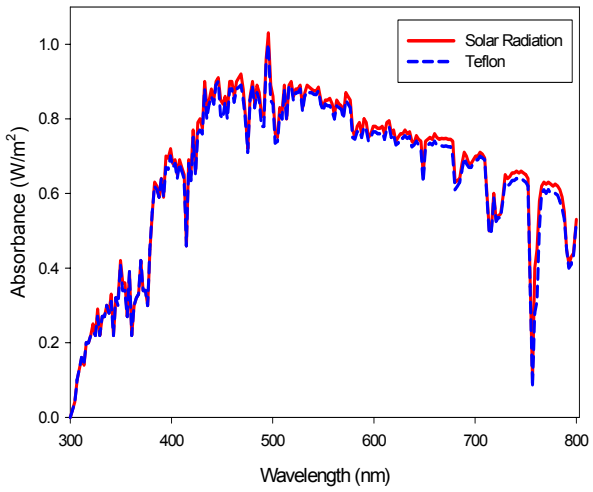


Figure 3.3. Comparison of Teflon bottle transmissivity to solar radiation.
(*Reprinted with permission from Amyot et al., 1997)*

Daily incident radiation was measured using a CM11 radiometer not more than 5 meters from the natural sunlight experiment. Mean incident radiation between the wavelengths of 305 and 2800 nm was collected and indicated a daily maximum of approximately 900 W/m^2 (Figure 3.4). The majority of solar UV wavelengths that reach the earth's surface were measured by the CM-11 (i.e., no UV-C was measured, but the majority of UV-B and all of UV-A were included). Measurements were collected every 5 minutes throughout the 24-hour cycle.

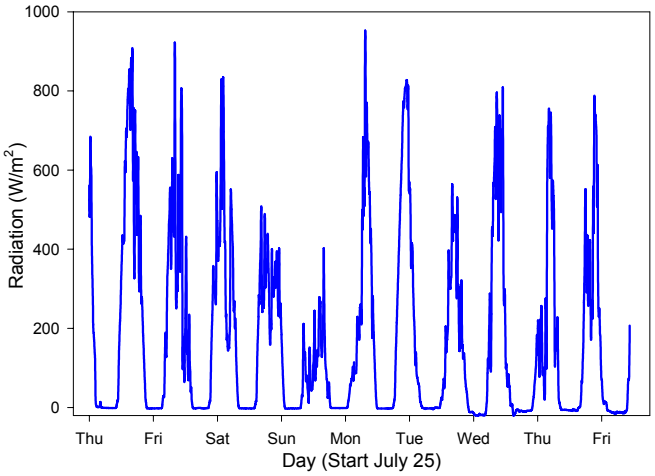


Figure 3.4. Example of measured UV/vis radiation using CM-11 radiometer.

One solar radiation mimic was used to determine photo-oxidation of naphthenic acids under controlled environmental conditions and 24-hour per day solar exposure. A growth chamber was used to simulate the full range of solar radiation including electronic environmental controls. Here, samples were exposed to artificial solar radiation through small volume (8 mL) Teflon bottles with the spectral characteristics shown in Figure 3.3. Electronic controls in the growth chamber maintained temperature at 20.0 ± 0.5 °C, while humidity was maintained near ambient levels. Light exposure was constant throughout the 24-hour day providing a light intensity of 420-440 μein/m^2/s at the water samples. The lamp canopy was comprised of incandescent and fluorescent lamps to simulate solar

radiation. The duration of exposure to the growth chamber-simulated solar radiation was, in general, one week. However, one experiment with the Athabasca Oil Sands extract was conducted for more than three months (97 days) to determine the effects of long-term exposure.

A broadband UV-A/UV-B radiation source comprised of a 15 W fluorescent blacklight (300-400 nm, max at 350 nm; Philips BLB medium-pressure Hg lamps) was used to differentiate between solar UV degradation potential and full-spectrum solar degradation kinetics. This "BLB radiation" does not represent the full solar spectrum; rather, the output constitutes the majority of the solar UV wavelengths that reach the earth's surface. Exposure of naphthenic acid mixtures was completed in annular quartz photochemical reactor cells (Figure 3.2). Here, suspensions of the aqueous contaminants were aerated and mixed by a steady stream of air forced through Teflon-coated capillary lines. The irradiation period for BLB exposure in photochemical reactor cells was eight hours.

Finally, a near monochromatic UV radiation source with output in the UV-C band at 254 nm was investigated. Here a Philips TUV low-pressure Hg UV_{254} lamp was used to irradiate naphthenic acid solutions in annular quartz photochemical cells under the same conditions noted for BLB experiments. The incident light intensity within the photocells was approximately 400 $\mu ein/m^2/s$, as determined previously by ferrioxalate actinometry (St. John *et al.,* 1998; Kirk and Namasivayam, 1983; Seifert *et al.,* 1969; Hatchard and Parker, 1956).

All aqueous solutions of the naphthenic acids were prepared in unaltered Athabasca River water and 1 mL aliquots collected at selected time intervals to assess photochemical degradation and toxicity changes. Experiments were completed at concentrations between 0.5 and 125 mg/L depending on the compound or mixture investigated. Control reactors were monitored simultaneously in the absence of UV light in natural water and in both the absence and presence of UV light in Milli-Q laboratory water.

The production of hydroxyl radical during photolysis was determined using a benzoic acid chemical probe. Benzoic acid (BA) reacts with hydroxyl radical in a predictable manner forming 3-hydroxybenzoic acid (HBA) when the hydroxyl radical is scavenged (Lindsey and Tarr, 2000a,b). By measuring the loss of the probe and formation of the product, hydroxyl radical concentration may be calculated and the primary method of photolysis determined (i.e., whether direct or indirect). Benzoic acid was added to selected samples at a concentration of 50 μM. Loss of BA and production of HBA were monitored using the same LC/MS conditions utilized for the analysis of naphthenic acids.

3.2.3 Instrumental

DOC concentrations of filtered river water samples were measured using a Technicon model AA2 TOC analyzer (Technicon Instruments Corporation, Terrytown, NY). Dissolved organic carbon (DOC) was determined using an automated persulphate-UV digestion with a phenolphthalein colour reagent as described by NAQUADAT (National Water Quality Data Bank) No. 06170L.

Calibration was established using sets of four standards. HPLC analysis was conducted using a Waters 2690 (Milford, MA) separation module. The selected method for the isomeric naphthenic acid compounds employed a 2.0 mm x 250 mm, 5 μm particle size Luna C8 reversed phase analytical column (Phenomenex, Torrance, CA) maintained at 45 ± 1 °C. For resolution of the three isomeric naphthenic acids compounds, the mobile phase consisted of 4 mmol ammonium acetate in a 30 % methanol aqueous solution at a flow rate of 200 μL/min. For evaluation of naphthenic acid mixtures, sampled were loop injected using a mobile phase consisting of 4 mmol ammonium hydroxide in a 70 % methanol solution at a flow rate of 200 μL/min. In each case, volumes of 10 μL sample and calibration standard were injected using a Waters 2690 auto-sampler.

Mass spectrometric analysis was conducted using a Quattro Ultima mass spectrometer (Micromass, UK) equipped with an electrospray ionization interface operating in the negative ion mode. MS conditions for analysis of the mixtures were set as follows: source temperature 90 °C, desolvation temperature 220 °C, cone voltage setting 62 V, capillary voltage setting 2.63 kV, cone gas N_2 158 L/hr, desolvation gas N_2 489 L/hr. Low mass resolution was 14.1 and high mass resolution was set at 14.3; ion energy was set at 1.7. Entrance voltage was 95 V and exit voltage 55 V. The multiplier was set at 650 V. Full scan MS was employed for extracts of the mixtures.

MS conditions for analysis of the individual compounds and hydroxyl radical scavenger were set as follows: source temperature 120 °C, desolvation temperature 220 °C, cone voltage setting 37 V, capillary voltage setting 3.32 kV, cone gas N_2 158 L/hr, desolvation gas N_2 492 L/hr. Low mass resolution was set at 13.7 while high mass resolution was 14.3; ion energy was 2. Entrance voltage was 55 V and exit voltage 72 V. The multiplier was set at 650 V. Quantitative analysis was performed using SIM of the *m/z* 154.8 for the naphthenic acid 4-MCHAA isomers and *m/z* 141 for 3- and 4-MCHCA. Mass to charge ratios utilized for the detection of BA and HBA hydroxyl radical chemical probe were 127 and 137, respectively. MassLynx version 3.4 software was utilized for all instrumental control and data manipulation.

3.2.4 Aryl Hydrocarbon Receptor Assay

The potential for toxicological triggering via a known and specific pathway was measured prior to and following photolysis experiments using an aryl hydrocarbon (Ah) receptor assay. This test was chosen to determine whether or not naphthenic acids are capable of binding to the Ah receptor and also to determine whether or not photolysis affects this capability and the corresponding specific toxicological response. Water samples from the experiments were withdrawn into sterilized vials (autoclaved at 121 °C for 20 minutes). The aryl hydrocarbon receptor assay utilizes

a specialized strain of yeast genetically modified to contain a human aryl hydrocarbon receptor (Ah). The Ah receptor gives a response to any molecule with an aryl group (TCDD, PCBs, PAHs, etc). (Miller, 1999, 1997; Miller *et al.,* 1998). This method for toxicological analysis is useful for evaluating potent ligands that activate the Ah receptor as the initial step in a toxic signalling pathway that leads to immune dysfunction, endocrine disruption, reproductive toxicity, developmental effects and cancers in vertebrates. Previous studies have shown that the Ah receptor plays a role in the toxic response to specific aromatic hydrocarbons (Miller, 1999, 1997; Miller *et al.,* 1998).

Triplicate water samples were assayed using the following procedure (yeast was obtained from and tests were completed at the Soil Science Department, College of Agriculture, University of Saskatchewan, Saskatoon, Canada). In brief, stock solutions were used to form a minimal medium solution that was autoclaved prior to use. This solution, along with a glucose stock solution, was mixed with yeast and left to culture for 2.5 days, with shaking at moderate speed in an orbital shaker between 28 and 32 °C. After the yeast was cultured, a second mixture was prepared using a minimal medium and galactose stock solution plus some of the glucose yeast culture. Volumes of these solutions were determined from the optical density of the yeast culture and the required dilution thereof; minimal medium and galactose solution were added in a 9:1 ratio. This second mixture was grown for 24 hours so that the optical density was approximately 0.2 at 630 nm. At 0.2 optical density the yeast was in exponential growth and most active with young cells.

Three series of 96-well sterilized plates were next prepared in a biosafety hood. Into each well of the first plate was added dimethyl sulphoxide (DMSO), excluding the first column that contained triplicate pure sample and two blank solutions to make 8 rows. From the first column, serial dilutions were completed, excluding the last column, which was the control. The second plate was prepared using small volumes of sample from the chemical plate plus yeast culture. This plate was incubated for 24 hours at 32 °C. The final plate was the β-galactosidase plate into which minimal

media, cyclohexamide and chorophenol red β-d-galactopyranoside (CPRGP) were added with a small volume of the incubated yeast culture. This plate was incubated at 37 °C and optical density recorded at 540 (red) and 630 (yellow) nm every 30 minutes to plot a concentration-response curve. In this final step, binding with the Ah receptor results in expression of Lac Z gene that codes for production of the β-galactosidase enzyme. This enzyme cleaves a galactose group from the CPRGP indicator changing the colour of the solutions in the 96-well plate from yellow to red. The greater the intensity of the red colour, the greater concentration of β-galactosidase (Miller, 1999, 1997; Miller *et al.,* 1998).

The Ah receptor assay provides insight to the effects of photolysis on one of many potential toxicological pathways that may be triggered by naphthenic acids. It represents one mechanism of action, but may not be representative of total toxicological response.

3.3 Results and Discussion

Based on a four-point calibration, a linear response was observed from 0.1 ng to 10 ng of naphthenic acid compounds on-column using 10 μL injections. Method detection limits based on a signal:noise ratio of 3:1 in river water samples were in the range of 4 to 8 μg/L for the *cis*- and *trans*-isomers of the three individual model naphthenic acids (4-MCHAA, 4-MCHCA and 3-MCHCA). The *cis*- and *trans*-isomers of individual naphthenic acid compounds were well resolved using the chosen LC method (Figure 3.5). The order of elution of the *cis*- and *trans*- isomers was determined based on both isomer polarity and relative composition in the authentic standards. Manufacturer specifications indicated that the ratio of 4-MCHAA *cis*- and *trans*-isomers was 55:45. The manufacturer did not specify the ratios for 3-MCHCA and 4-MCHCA. However, a pure mixture of 4-MCHCA *trans*-isomer was also analyzed to determine elution order and *cis:trans* ratio. Ratios of the three model naphthenic acids were calculated from LC/MS results to indicate

that the ratio of 4-MCHAA *cis*- to *trans*- isomers was 55:45; 3-MCHCA was 40:60; and 4-MCHCA was 80:20.

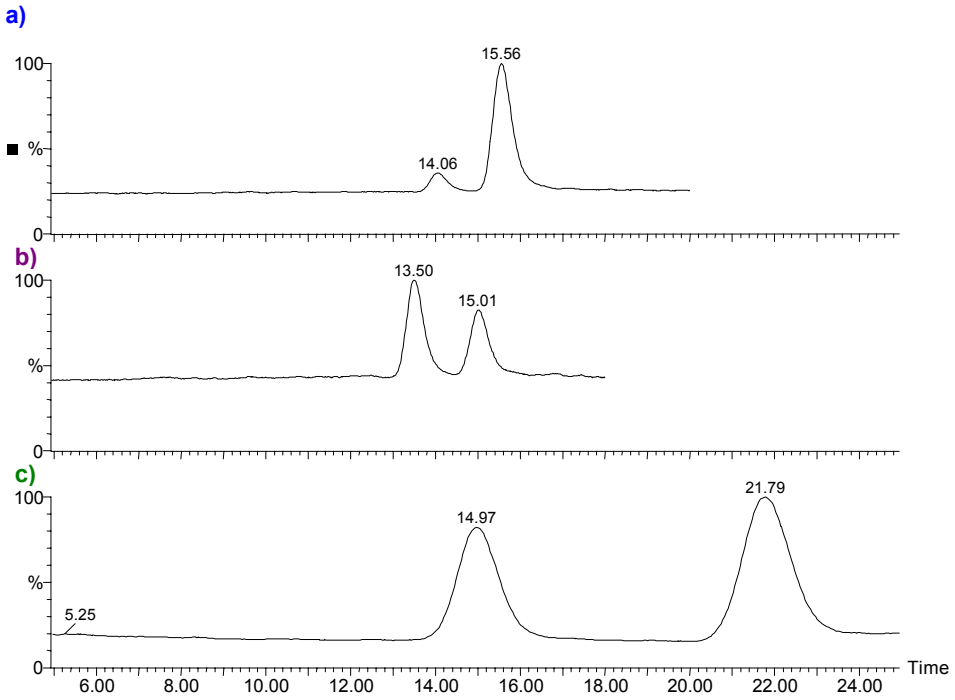


Figure 3.5. Chromatographic separation of each of the three model naphthenic acids a) 4-MCHCA, b) 3-MCHCA and c) 4-MCHAA.

3.3.1 Photolysis

Naphthenic acid photolysis resulting from exposure to natural and artificial sunlight was limited. Subsequent to more than one week of exposure to natural solar radiation, no individual compounds or mixtures were significantly degraded, although compositional changes were noted in the mixtures (i.e., higher molecular weight compound concentrations decreased, while lower molecular weight compound concentrations increased). Artificial solar radiation, in the form of growth chamber canopy lamps and Philips BLB lamps, was similarly ineffective over 168 and 8-hour exposure periods, respectively. Exposure to UV_{254}-radiation induced the most photolysis of the naphthenic acids, but was not, however, an efficient means for complete removal of complex mixtures from natural water

sources.

The 4-MCHAA was removed by facile photodegradation with pseudo-first-order kinetics following exposure to UV_{254}-radiation, but neither the 3-MCHCA nor 4-MCHCA was notably degraded. Although UV_{254}-radiation more effectively removed 4-MCHAA from solution, artificial and solar radiation methods were also effective at degrading this model naphthenic acid. Both 4-MCHAA isomers were readily degraded with half-life values of less than 4 hours when exposed to UV_{254}-radiation, 25 hours for laboratory artificial solar radiation experiments and approximately 700 hours in rooftop experiments conducted in Athabasca River water (Table 3.2).

Table 3.2. Half-life values for three individual naphthenic acid compounds in Athabasca River water.

Compound	Radiation Source	Half-Life (hours)	
		***cis*-Isomer**	***trans*-Isomer**
4-MCHAA	NSR	701 ± 14	434 ± 2
	BLB	18.9 ± 0.6	25 ± 4
	UV_{254}	3.6 ± 0.4	3.2 ± 0.3
4-MCHCA	UV_{254}	18230 ± 122	6560 ± 94
3-MCHCA	BLB	---	17629 ± 126
	UV_{254}	7056 ± 55	2585 ± 43

n = 15; based on duplicate samples at two concentrations; error limits are 95 % confidence levels of the mean of analytical results.

The same photodegradation trends were not noted for either 3-MCHCA or 4-MCHCA. In those experiments, the *trans*-isomer was more readily photolyzed than the *cis*-isomer, although neither was well photodegraded. Like the photolysis of 4-MCHAA, UV_{254}-radiation was more effective for removal of the compounds from Athabasca River water than the laboratory ASR experiments. However, half-life values were in the range of several weeks (Table 3.2) not hours or days as observed for 4-MCHAA.

Similar results were noted with respect to exposure of the three selected model naphthenic acid compounds to the Philips BLB radiation source (Table 3.2). Exposure to the BLB radiation resulted in minimal degradation of each MCHCA compound, with 4-MCHAA again the most susceptible. In all but four 3-MCHCA experiments, neither the *cis*- nor *trans*-isomers of 3-MCHCA and 4-MCHCA exposed to BLB radiation were appreciably degraded. In those four remaining 3-MCHCA experiments, the calculated half-life for the *trans*-isomer was approximately 735 days.

To best elucidate the effects of UV/vis radiation on the complex naphthenic acid mixtures investigated (Figure 1.3), calculation of half-life values in addition to an examination of compositional changes were completed. Evaluation of the overall degradation was used to determine net concentration changes, while mass spectra shifts within the three mixtures were examined to determine the extent of compositional changes following photolysis. Although any given *m/z* value may correspond to more than 100 naphthenic acids (Holowenko *et al.,* 2002; Rogers *et al.,* 2002a), changes in the mass spectra aid in determining which general mass families are predominantly chromophoric in nature.

Overall, minimal degradation of the naphthenic acid mixtures was noted from the analysis of chromatographic results. Half-life values indicated that none of the mixtures are likely to degrade significantly in the environment due to photolysis (Table 3.3). The calculated half-life values relate well with absorbance data (Figure 3.6). Since the ability to absorb photons is directly related to the potential for photochemical degradation, it may be seen that the absorbance data support general trends noted in the calculated photolysis kinetics. The Athabasca Oil Sands (AOS) extract is the most absorbing of the naphthenic acid mixtures tested, and was correspondingly the most readily photolyzed mixture for all UV/vis radiation sources tested. The Fluka naphthenic acids were more UV/vis absorbent than the Acros naphthenic acids (Figure 3.6), as mirrored in the calculation of half-life values (Table 3.3). From Figure 3.6, it is apparent that solar radiation is anticipated

to have the least impact on photolysis, followed by the BLB radiation source (300-400 nm), and finally the UV_{254}-radiation source at which point in the absorption spectrum each mixture has some potential for absorption and, therefore, photochemical reaction.

Table 3.3. Half-life estimates for naphthenic acid mixtures in Athabasca River water.

Mixture	Radiation Source	Concentration (mg/L)	Half-Life (hours)
AOS Extract			
	NSR	0.5	6860 ± 110
		1	5300 ± 140
		5	6485 ± 95
		50	5875 ± 70
	BLB	10	227 ± 4
		50	236 ± 7
	Growth chamber	50	6240 ± 105
		125	7000 ± 55
	UV_{254}	5	4.9 ± 2.1
		10	12.4 ± 1.7
		25	7.7 ± 2.3
		50	9.5 ± 1.9
Fluka Mixture			
	BLB	10	1050 ± 20
		50	1200 ± 40
	Growth chamber	50	8120 ± 100
	UV_{254}	10	50 ± 1
		50	52 ± 1
Acros Mixture			
	BLB	10	2100 ± 30
		50	2070 ± 50
	Growth chamber	50	8480 ± 150
	UV_{254}	10	61 ± 3
		50	65 ± 2

n = 6

Note: half-life values calculated for exposure to solar radiation are beyond the exposure time for experiments and thus must be viewed as extrapolations of anticipated photodegradation kinetics.

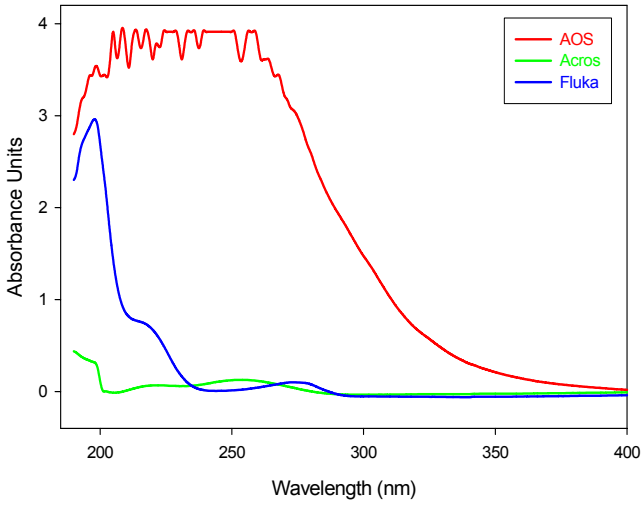


Figure 3.6. Absorbance of the three naphthenic acid mixtures investigated.

In experiments with Acros and Fluka commercial naphthenic acid mixtures, overall concentrations remained relatively unchanged following exposure to the artificial solar radiation (growth chamber) and artificial UV radiation (Philips BLB source). Application of UV_{254}-radiation was the most effective of the methods investigated for reducing the concentration of the two commercial naphthenic acid mixtures (Table 3.3). Since each of the two commercial naphthenic acid mixtures will absorb in the UV_{254}-radiation range, there is increased photochemical degradation potential at that wavelength. Similarly, since the commercial mixtures possess minimal overall UV/vis absorption potential, it may be anticipated that neither will be significantly impacted by solar radiation.

From the standard solutions of each naphthenic acid mixture tested, a selection of five masses for each of 7 *z* series (Table 3.4) was monitored for changes over the duration of the photolysis experiments. These 35 masses were chosen to represent each of the predominant *z* series and carbon families of typical naphthenic acid mixtures. Compositional shifts within the three mixtures investigated were noted in all instances following exposure to each radiation source. Compositional changes

were best observed for prolonged exposure experiments conducted at 125 mg/L of AOS extract (Figure 1.3a) in the growth chamber over a period of more than 3 months (97 days; 2328 hours). Evaluation of the kinetics indicated a half-life value of approximately 7000 hours, denoting that relatively no overall degradation had occurred. However, significant compositional changes were noted based on the peak areas of the 35 monitored masses.

The compositional changes generally indicate no correlation to *z* series, although the *z* = 0 and -12 series had more increased concentrations after photolysis. However, the picture becomes clearer when analysing from the point of view of photo-induced changes in carbon number. Those instances where an increase in concentration was noted correlated strongly to low carbon family numbers. For instance, all masses included in the analysis attributed to the C10 and C12 grouping increased in relative concentration during the experiments. In contrast, no increases in relative concentration were detectable between C16 and C25. Rather, half-life values generally ranging between 3000 and 10,000 hours were noted (Table 3.5). It is possible, however, that the lower molecular weight compounds are photo-degradation products of the higher molecular weight components.

Table 3.4. Selection of masses investigated to represent seven *z* series in the naphthenic acid mixtures.

Z Series	Mass Examined				
0	172	214	284	312	368
-2	212	282	254	338	366
-4	210	252	280	322	364
-6	208	250	278	320	376
-8	164	206	276	318	374
-10	162	218	274	316	372
-12	160	188	216	314	370

Table 3.5. Half-life values extrapolated for Athabasca Oil Sands naphthenic acid extract following 97 days of exposure to artificial solar radiation in a growth chamber and arranged according to carbon family.

Carbon Family	Mass	*Z* Series	Half-Life* (hours)	Carbon Family	Mass	*Z* Series	Half-Life* (hours)
10	172	0	N/A	20	312	0	4050
10	164	-8	N/A	21	322	-4	5135
10	162	-10	N/A	21	320	-6	5250
10	160	-12	N/A	21	318	-8	3825
12	188	-12	N/A	21	316	-10	3940
13	214	0	N/A	21	314	-12	4235
13	212	-2	14655	22	338	-2	5235
13	210	-4	5065	24	368	0	5470
13	208	-6	5490	24	366	-2	6200
13	206	-8	10325	24	364	-4	7230
14	218	-10	10710	25	376	-6	9560
14	216	-12	N/A	25	374	-8	6640
16	254	-2	10975	25	372	-10	6100
16	252	-4	5350	25	370	-12	6780
16	250	-6	3925				
18	284	0	4400				
18	282	-2	5695				
18	280	-4	5715				
18	278	-6	4190				
18	276	-8	3120				
18	274	-10	3035				

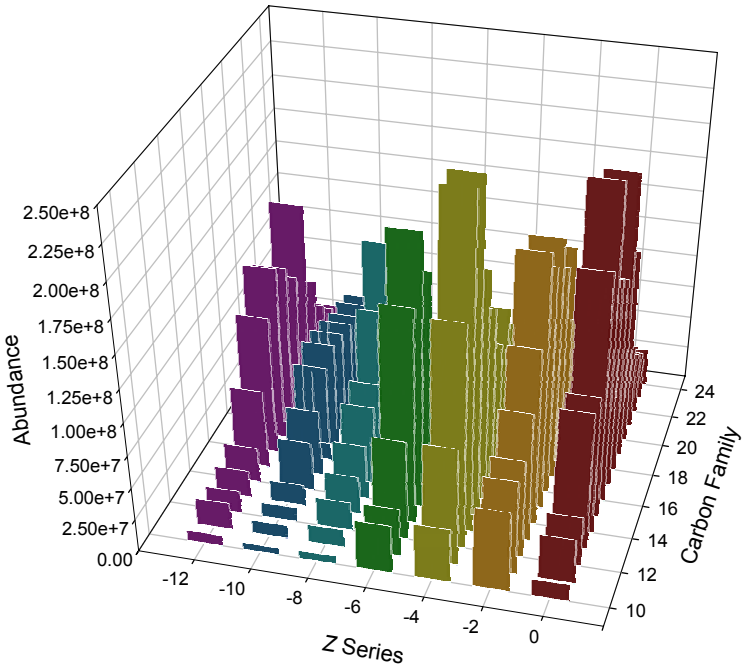
*based on duplicate results of two samples and rounded to nearest 5 hours.
N/A = not applicable; concentration of mass in question observed to increase during photolysis

Application of UV_{254}-radiation induced the most photolysis reducing the overall concentration and resulting in the most significant compositional changes within each of the three mixtures investigated, even after only 8 hours of exposure. However, the results for UV_{254}-radiation were somewhat different from those observed for solar radiation. Subsequent to UV_{254}-radiation exposure, all masses monitored in the three naphthenic acid mixtures investigated were reduced. The half-life values calculated for the lower molecular weight compounds in the C10 through C12 groupings were higher than those observed for the higher carbon numbers. However, degradation was noted in all instances, despite carbon number. Although naphthenic acid mixtures were only slightly degraded (i.e., most masses

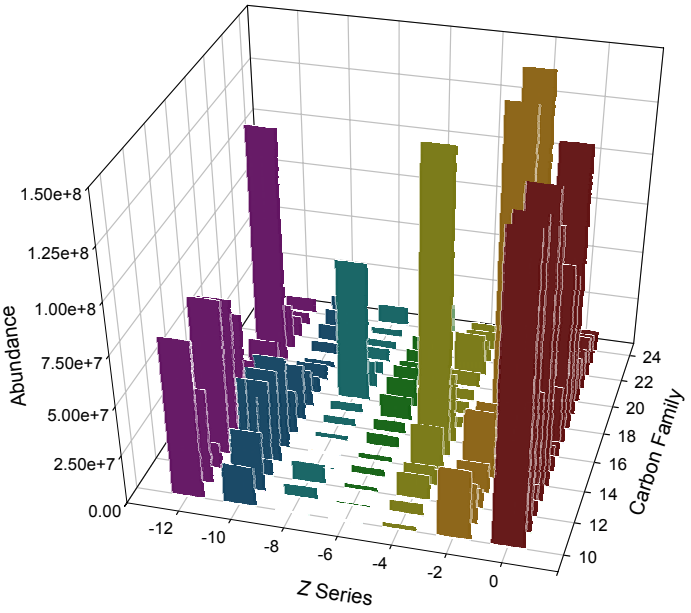
were photolyzed at less than 40 %) due to the photolytic exposure investigated, it is likely that in the long-term the compounds are partially mineralized to carbon dioxide and water, and partially degraded into compounds of lower molecular size, including a lower degree of conjugation.

From the results of photochemical degradation and mass spectra analyses, it was noted that compounds in the higher carbon families (such as those greater than C15) are not as common or abundant in the Acros or Fluka naphthenic acid mixture as compared to the AOS extract (Figure 3.7). Combining this knowledge with previous information that Acros naphthenic acids appear to be less UV absorbent than the AOS naphthenic acids, it was hypothesized that the molecules containing the most chromophoric parts are likely in the higher carbon family groupings.

a)



b)



c)

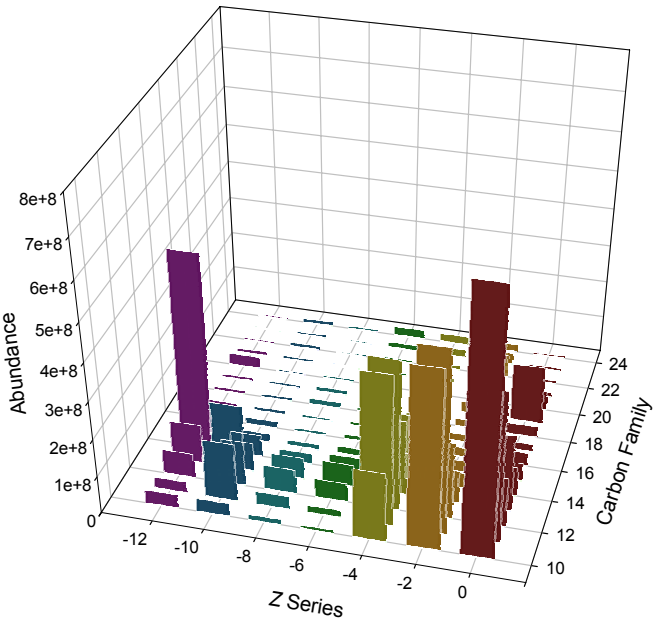
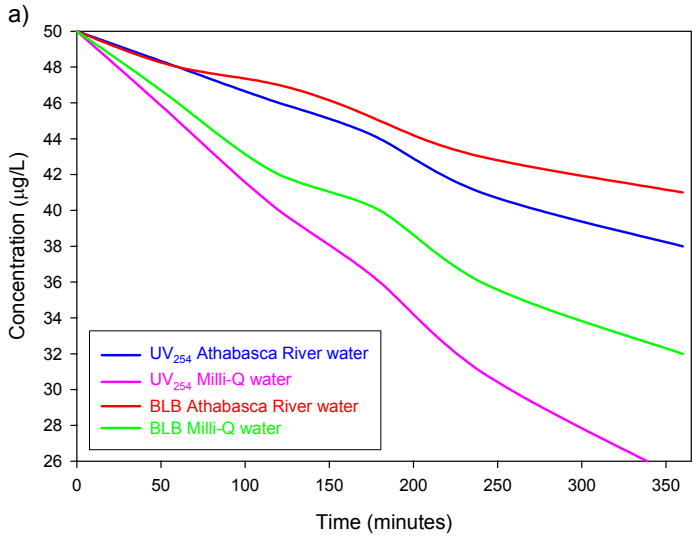


Figure 3.7. 3-D histograms of naphthenic acid mixture composition prior to photolysis for a) Athabasca Oil Sands; b) Acros and c) Fluka.

The final component of photolysis evaluation relative to naphthenic acid persistence in natural river water was that of hydroxyl radical formation and a hypothesis of the anticipated mode of photolysis (i.e., direct or indirect). To achieve this, a comparison of river and laboratory water was completed in photochemical reactor cells using UV_{254}-radiation and the Philips BLB lamp. For UV_{254}-radiation, the results indicated that the AOS extract was photolyzed by UV_{254}-radiation in Milli-Q water near 4 times faster than in Athabasca River water. For Philips BLB, the same trend was noted, although not to the same degree. In that instance, the degradation rate was not quite twice as rapid in Milli-Q versus Athabasca River water.

The enhanced removal efficiency of photodegradation of naphthenic acids in solution in laboratory Milli-Q water was hypothesized to be a result of the absence of absorption of photons by natural dissolved organic matter in the laboratory water in comparison to the natural river water source. Being a highland river, the Athabasca River has a relatively low DOC content (approximately 6.5 mg/L). However, even at that concentration the aromatic dissolved organic compounds compete for hydroxyl radicals. Results from the application of a benzoic acid chemical probe confirmed these points and indicated that the natural river water source did, in fact, inhibit the concentration of hydroxyl radicals available for photolysis of the naphthenic acids (Figure 3.8).



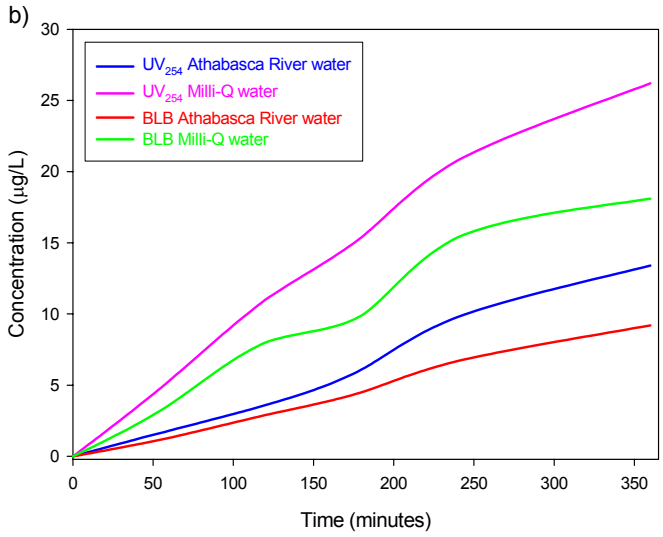


Figure 3.8. Concentration changes related to hydroxyl radical scavenging in AOS naphthenic acid aqueous solutions: (a) loss of benzoic acid chemical probe and (b) generation of 3-hydroxybenzoic acid.
Note: Seven samples were collected in duplicate over the 360 minute experimental period.

From the results shown in Figures 3.6 and 3.8, it is apparent that naphthenic acids do not appear to have significant direct photon absorption capacity and are competing for hydroxyl radicals in solution. Therefore, the scavenging of hydroxyl radicals may a more important process for the photolysis of naphthenic acid components than direct photon absorption.

3.3.2 Aryl Hydrocarbon Receptor Assay

Irradiation of aquatic organics generates oxygen species (such as hydroxyl radicals and hydrogen peroxide) that may have toxic effects on aquatic organisms. Conversely, solar light and UV-radiation cleave high molecular weight recalcitrant compounds into smaller fragments that are more easily utilized by micro-organisms. Consequently, there are two counteracting effects: bacteria may be either stimulated or harmed by photolysis of aquatic organic compounds (Corin, 2000). The changes in Ah receptor binding potential occurring during irradiation of the naphthenic acid mixtures and model compounds were monitored using the aryl hydrocarbon receptor

assay as noted. All assays of the three individual model naphthenic acid compounds were conducted at 1 mg/L initial concentration; naphthenic acid mixtures were each tested at 50 mg/L and AOS was further assessed at 125 mg/L using samples collected in growth chamber photolysis experiments. The chosen naphthenic acid mixtures and compounds indicated significant Ah receptor binding potential.

The results of Ah receptor assays indicated that this particular mechanism of toxicity is affected by naphthenic acid photolysis to differing degrees dependent on radiation source, type of naphthenic acids and period of exposure. In particular, changes in Ah receptor binding potential were noted following exposure of the selected naphthenic acids to UV_{254}-radiation, especially in the case of 4-MCHAA where significant photodegradation was observed and for the 97-day UV/vis exposure of the AOS naphthenic acid mixture. The results given in Table 3.6 describe the percent changes in the Ah receptor response at 540 nm (red) and 630 nm (yellow), as well as the UV/vis radiation treatment in each instance.

It may be noted that the Ah assay absorbance at 540 nm was slightly increased (i.e., positive percent change) in most experiments with the naphthenic acid mixtures following UV/vis exposure. This increase indicated heightened Ah receptor binding potential and, therefore, increased toxicity via this particular mechanism. This same conclusion may be drawn from the reduced absorbance (i.e., negative percent change) at 630 nm. Since compositional changes noted in all three mixtures investigated were toward the lower molecular weight naphthenic acids, a slight increase in toxicological response may be anticipated, as noted by previous researchers (Rogers *et al.,* 2002b; MacKinnon and Boerger, 1986). The lower molecular weight naphthenic acids are thought to be more toxic than their higher molecular weight counterparts.

Table 3.6. Percent change in absorbance of naphthenic acid samples using the aryl hydrocarbon (Ah) receptor assay.

Compound	Radiation Source	% Change 540 nm	% Change 630 nm
4-MCHAA	NSR	-46.8	42.8
	BLB	-58.2	63.3
	UV_{254}	-87.6	91.3
4-MCHCA	BLB	0.35	1.4
	UV_{254}	-10.2	8.5
3-MCHCA	BLB	-6.7	8.6
	UV_{254}	-22.0	17.9
AOS Extract	NSR	16.8	20.2
	BLB	44.9	-54.8
	Growth Chamber (97 days)	-11.3	11.9
	UV_{254}	64.4	-89.8
Fluka Mixture	BLB	37.2	-33.1
	Growth Chamber	38.5	-38.8
	UV_{254}	54.5	-59.0
Acros Mixture	BLB	22.7	-28.2
	Growth Chamber	15.3	-11.0
	UV_{254}	25.9	-21.4

NRS = natural solar radiation; BLB = Philips BLB radiation source (300-400 nm); Growth Chamber = full spectrum artificial solar radiation
Note: Results are based on the mean of triplicate analyses.

As noted for overall degradation of the selected naphthenic acids, UV_{254}-radiation was the most efficient photolysis method of those applied for reducing Ah receptor binding potential. The application of UV_{254} and BLB radiation to the three naphthenic acid compounds examined resulted in decreased Ah receptor binding potential commensurate with the photolysis of the parent compound in question.

In terms of developing treatment strategies for removal of naphthenic acids in oil sands extraction effluent streams and downstream receiving waters, rather than pursue complete mineralization in a single stage, it may be more effective to use photolysis as a viable pre-treatment to biofilms in the overall attenuation of aquatic organic contaminants. Applied UV_{254}-radiation may provide an effective method for

reducing naphthenic acids entering microbial treatment systems, thus eliminating potential shock effects.

3.4 Conclusions

Evaluation of degradation kinetics for three individual naphthenic acid compounds and the mass spectra within three naphthenic acid mixtures investigated indicated that some naphthenic acids are significantly more susceptible to photodegradation than others. None of the UV/vis radiation sources were effective at fully mineralizing the naphthenic acids compounds and mixtures investigated in Athabasca River water. Natural and artificial sunlight are not efficient at reducing the range of either naphthenic acid compounds or mixtures studied here in a relatively clear natural water source. Therefore, it must be anticipated that photolysis by natural means in coloured tailings pond water has negligible effect on both concentration and toxicity of naphthenic acids in the pond since light penetration beyond the upper 1 mm is attenuated (as supported by field observations at Syncrude, Rogers *et al.,* 2002a). However, incorporation of applied photolytic treatment at the highly energetic UV_{254}-radiation for naphthenic acid concentration reduction may prove more effective at the industrial level. The results of aryl hydrocarbon receptor assay testing for changes in toxicological response to a specific pathway indicated that exposure to UV_{254}-radiation is effective for reducing that one component of the inherent toxicity of the naphthenic acids investigated.

3.5 Acknowledgements

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maintenance of the CM11 radiometer; Sarah Armstrong and Steve Siciliano at the Soil Science Department, University of Saskatchewan for technical advice and laboratory support for the aryl hydrocarbon receptor assay; Shannon Braithwaite at WateResearch Corp for DOC analyses; and Tyler Birkham at the Department of Civil and Geological Engineering, University of Saskatchewan for Athabasca River water collection.

4. EVALUATION OF LIQUID CHROMATOGRAPHY-NEGATIVE ION ELECTROSPRAY IONIZATION MASS SPECTROMETRY FOR DETERMINATION OF SELECTED RESIN ACIDS IN RIVER WATER

A liquid chromatography negative ion electrospray ionization mass spectrometric (LC/ESI/MS) method was evaluated for detection of four prevalent softwood-derived resin acids in natural water. Method detection limits based on a signal:noise ratio of 3:1 in river water samples of 0.40, 0.40, 0.30 and 0.25 μg/L for abietic, dehydroabietic, isopimaric and pimaric acids, respectively, are comparable or lower than reported GC methods. Unlike the majority of GC methods, however, the three structural resin acid isomers (abietic, isopimaric and pimaric acids) do not separate sufficiently under the various LC conditions evaluated in this work. Therefore, LC/ESI/MS may not be suitable for instances where measurements of individual isomeric resin acids are required. However, the method is suitable for trace analysis of resin acids in natural waters where isomeric speciation is not required.

This manuscript was published as a short communication and is cited as follows:

McMartin, D.W., K.M. Peru, J.V. Headley, M. Winkler and J.A. Gillies. 2002. Evaluation of liquid chromatography-negative ion electrospray mass spectrometry for determination of selected resin acids in river water. *Journal of Chromatography A* **952**: 289-293.

4.1 Introduction

The four most prevalent resin acids occurring in pulp and paper mill effluents were included in this study: abietic (AbA), dehydroabietic (DhA), isopimaric (IpA), and pimaric (PA) acids (Figure 1.5). The present study was considered a first step

toward a complete solution for fast and sensitive quantification of the four selected resin acids in laboratory and natural water samples using liquid chromatography negative ion electrospray ionization mass spectrometry (LC/ESI/MS). Using this method minimizes the number of sample preparation steps with no extraction or derivatization requirements with detection limits comparable to, or lower than, reported GC methods (Corin *et al.,* 2000; Werker and Hall, 1998; Robinson *et al.,* 1994; Voss and Rapsomatiotis, 1988). Unlike the majority of GC methods however, the three structural resin acid isomers (AbA, IpA and PA) do not separate sufficiently using LC under the various conditions evaluated in this work. Therefore, LC/ESI/MS may not be suitable for environmental monitoring in instances where measurement of individual isomeric resin acids is required.

4.2 Experimental

4.2.1 Sample Collection

River Saale water samples were collected twice weekly over a five month period between April and August, 2001 (basic water quality information is provided in Chapter 5). The water was collected at Calbe, approximately 5 km from the confluence of the Saale and Elbe Rivers. Analysis was completed to evaluate the ruggedness of the LC/ESI/MS method and to determine the extent of resin acid contamination from local pulp and paper mills situated upstream. Spiked water samples of both River Saale and Milli-Q laboratory water were also analyzed. Aliquots of these water samples were spiked with each individual resin acid and combinations thereof.

4.2.2 Chemicals

Neat standards of the four resin acids were purchased from Helix Biotech (Vancouver, Canada). Dehydroabietic and isopimaric acids were above 99% purity. Abietic acid was between 90 and 95% purity; pimaric acid was between 85 and

90%, containing traces of sandopimaric acid. Ammonium acetate (NH_4Ac) of minimum 98 % purity was purchased from Riedel-de Haen (Germany). Glacial acetic acid and HPLC grade acetonitrile were purchased from Fisher Scientific (Edmonton, Canada).

4.2.3 Standard Preparation

Resin acid standards were prepared using a 10 mg/mL stock solution prepared in methanol and stored at 4 °C for no longer than 2 weeks. Calibration was established with four to six standards prepared from the stock solution using appropriate dilutions with a 50:50 mixture of eluent A and eluent B (see below).

4.2.4 Instrumental

HPLC analysis was conducted using a Waters 2690 (Milford, MA) separation module. The HPLC pumps were primed with fresh eluents on a weekly basis or sooner as required. The selected method employed a 2.0 mm x 250 mm, 5 μm particle size Luna C8 reversed phase analytical column (Phenomenex, Torrance, CA) maintained at 30 ± 1 °C. The mobile phase consisted of 10 mmol ammonium acetate in water (eluent A) and of 10 mmol ammonium acetate in acetonitrile (eluent B) at a flow rate of 200 μL/min. Volumes of 10 μL sample and calibration standard were injected using a Waters 2690 auto-sampler.

Mass spectrometric analysis was conducted using a Quattro Ultima mass spectrometer (Micromass, UK) equipped with an electrospray ionization interface operating in the negative ion mode. MS conditions were as follows: source temperature 90 °C, desolvation temperature 220 °C, cone voltage setting 90 V, capillary voltage setting 2.74 V, cone gas N_2 81 L/hr, desolvation gas N_2 265 L/hr. Low and high mass resolution was set at 14.1 and 14.3, respectively and ion energy was 1.4. Entrance voltage was 36 V, collision energy 16 eV, and exit voltage 66 V.

The multiplier was set at 650 V. At these conditions mass resolution was approximately 1 Dalton. Quantitative analysis was performed using selected ion monitoring of *m/z* 299.3 for DhA and 301.3 for AbA, IpA and PA. MassLynx version 3.4 software was utilized for all instrumental control and data acquisition.

4.3 Results and Discussion

4.3.1 Mobile Phase Evaluations

Several mobile and stationary phase conditions were evaluated to attain optimum method sensitivity and analysis time while maximising separation of AbA, IpA and PA. Both C8 and ABZ (Supelco, Bellefonte, PA; similar to C18 column chemistry) analytical columns were evaluated for retention and separation of the resin acids. Although the retention times were greatly increased using the ABZ stationary phase (> 20 minutes) compared to that of the Luna C8, separation was not improved.

The results obtained using various LC conditions indicated that AbA, IpA and PA are not adequately resolved using liquid chromatography in order for isomers to be quantified individually. The conditions for optimum analysis time, separation and method sensitivity of these resin acids were with the selected method in which a C8 stationary phase and 10 mmol NH_4Ac (as an ion pairing agent) in 20 % water and 80 % acetonitrile isocratic mobile phase was employed. Although good sensitivity and analysis time were achieved, these conditions still did not adequately separate the three isomers for reliable individual quantification (Figure 4.1c).

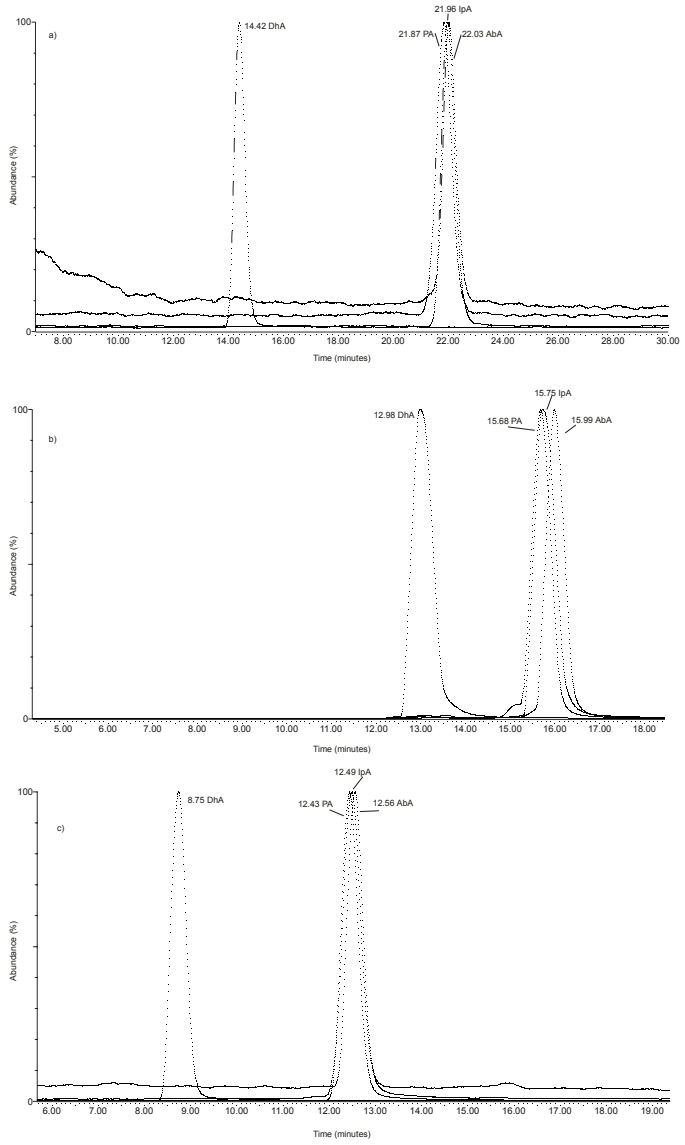

Figure 4.1. LC selected ion chromatograms of AbA, DhA, IpA and PA using a) acidified 80:20 acetonitrile, b) gradient of 20% to 80% acetonitrile/water containing ammonium acetate and c) isocratic 80:20 acetonitrile:water with ammonium acetate.

Results from preliminary trials using 20:80 water:acetonitrile indicated that under these eluent conditions the four resin acids were not retained by the C8 column and were eluted off in the void volume. This finding was not surprising as the pK_a's of resin acids are generally in the 5.5 range. Thus, the ionized species would have little affinity for the stationary phase.

Adjustment of the 20:80 water:acetonitrile eluent to a pH < 4 with acetic acid was completed to determine the effects on chromatographic resolution of the non-dissociated forms of the resin acid isomers. Under acidic eluent conditions and using the C8 analytical column, retention of the resin acids increased but no further separation of the isomers was observed under these conditions (Figure 4.1a).

An ABZ analytical column (similar to C18 column chemistry) was also evaluated for retention and separation of the resin acids. Although the retention times were greatly increased using the ABZ stationary phase (> 20 minutes) compared to that of C8, separation was not improved. Since analysis using the C8 column reduced the run time, it was employed for quantification of the individual resin acids in spiked samples and total resin acid values for environmental samples.

Gradient methods (linear gradient of 20 % acetonitrile to 80 % acetonitrile over 20 minutes) did not significantly improve the separation of the resin acid isomers (Figure 4.1b). It was noted that a preliminary gradient program consisting of a linear gradient ending with 100% acetonitrile greatly reduced method sensitivity to approximately 10 % of the final isocratic method of 20:80 water:acetonitrile. It was determined that in order to promote optimal ionization the resin acids must enter the ES source with an aqueous eluent. Considering these factors, an isocratic method with 20 % water and 80 % acetonitrile was chosen based on the analysis time of < 20 minutes with optimum sensitivity.

Neither gradient nor acidic elution significantly improved the separation of the resin acid isomers. Further, a preliminary gradient program consisting of a linear gradient ending with 100% acetonitrile greatly reduced method sensitivity to approximately 10 % of the isocratic method; optimal ionization requires an aqueous eluent. The conditions for optimum analysis time, separation and method sensitivity of these resin acids were with the selected method in which a C8 stationary phase and 10 mmol NH_4Ac (as an ion pairing agent) in 20 % water and 80 % acetonitrile isocratic mobile phase was employed. The four resin acids eluted between 8 and 13 minutes

under isocratic conditions of 20:80 eluent A:B (Figure 4.2). Although good sensitivity and analysis time were achieved, these conditions still did not adequately separate the three isomers for reliable individual quantification. This lack of adequate separation is due to the nearly indistinguishable chemistry and identical mass of the three resin acid isomers. Likely, the only possible method for adequate resolution lies in the application of gas chromatography.

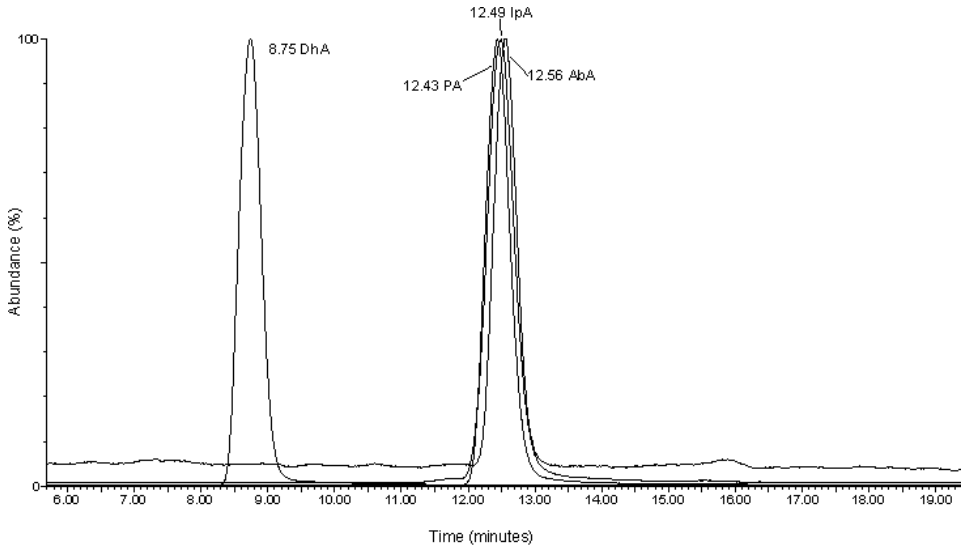

Figure 4.2. LC ion chromatogram of AbA, DhA, IpA and PA using isocratic 80:20 acetonitrile:water with ammonium acetate.

4.3.2 Mass Spectrometry

The four resin acids studied produced intense $(M - H)^-$ ions under negative ion electrospray ionization conditions (Figure 4.3). In order to optimize sensitivity, selected ion monitoring of the $(M - H)^-$ ions (corresponding to *m/z* 299.3 for DhA and 301.3 for AbA, IpA and PA) were used for all evaluations in this study.

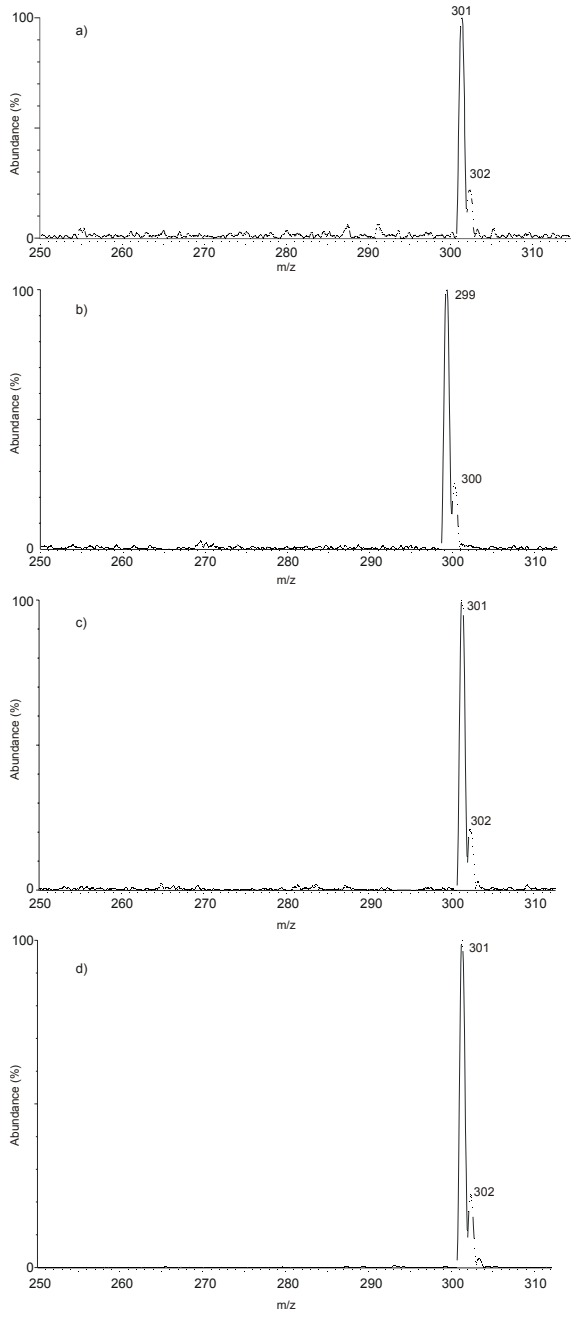

Figure 4.3. Full scan LC/ESI/MS negative ion spectra of a) AbA, b) DhA, c) IpA and d) PA.

Under the selected negative ion electrospray ionization conditions the four resin acids studied produced intense $[M - H]^-$ ions with no fragment ions being observed. MS/MS of the $[M - H]^-$ ions was performed in the aim that product ion spectra could be used to differentiate the three isomers in lieu of chromatographic separation.

However, $[M - H]^-$ precursor ions did not dissociate using argon as the collision gas at various collision cell energies, thus useful product-ion scans could not be obtained.

4.3.3 Detection, recovery and reproducibility

Based on a five-point calibration, a linear response was observed from 0.01 ng to 3.5 ng of resin acid on-column using 10 μL injections. Method detection limits based on a signal:noise ratio of 3:1 in river water samples were 0.40, 0.40, 0.30 and 0.25 μg/L for abietic, dehydroabietic, isopimaric and pimaric acids, respectively. In comparison, typical GC/MS detection limits for unconcentrated effluent and water samples are generally in the range of 5 μg/L (Corin *et al.,* 2000; Werker and Hall, 1998; Voss and Rapsomatiotis, 1985). In light of this, LC/MS was deemed a sensitive quantification method for the four individual resin acids while minimizing sample preparation with no extraction or derivatization requirements compared to reported GC methods (Corin *et al.,* 2000; Werker and Hall, 1998; Voss and Rapsomatiotis, 1985). The key limitation of the LC/MS method, then, is the inability to separate the three isomer resin acids as may be achieved with the more complex GC methods.

There was little or no matrix interference based on the observed recovery of matrix spikes. For example, 20 matrix spikes of 3.33 mg/L of resin acid resulted in values of 3.35 ± 0.01, 3.41 ± 0.03, 3.45 ± 0.01 and 3.39 ± 0.06 mg/L for AbA, DhA, IpA and PA, respectively. At lower matrix spike levels of 0.050 mg/L values of 0.053 ± 0.003, 0.048 ± 0.004 mg/L for AbA and DhA respectively were obtained, while slightly higher recoveries for IpA (0.066 ± 0.006 mg/L) and PA (0.075 ± 0.003 mg/L) were observed. The reproducibility of the method was ≥ 97 % based on analysis of laboratory standards and duplicate River Saale samples taken throughout a five-month period, in which more than 1000 determinations of resin acids were performed (Table 4.1).

Table 4.1. Recovery of the four individual resin acids using the chosen LC/MS method with spiked natural river water samples.

Resin Acid	Spike Concentration (mg/L)	Recovered Concentration (mg/L)
Abietic Acid	3.33	3.35 ± 0.01
	0.05	0.053 ± 0.003
Dehydroabietic Acid	3.33	3.41 ± 0.03
	0.05	0.048 ± 0.004
Isopimaric Acid	3.33	3.45 ± 0.01
	0.05	0.066 ± 0.006
Pimaric Acid	3.33	3.39 ± 0.06
	0.05	0.075 ± 0.003

n = 20; error values are based on 95 % confidence limits

Water samples indicate a background presence of DhA and the isomeric resin acids in the River Saale (Figure 4.2). For those samples in which resin acids were detected, overall mean and 95 % confidence interval values were 0.177 ± 0.022 and 0.150 ± 0.020 mg/L for DhA and resin acids isomers, respectively. Resin acid concentrations in natural water samples were below the detection limits for some 50 % of the natural water samples investigated.

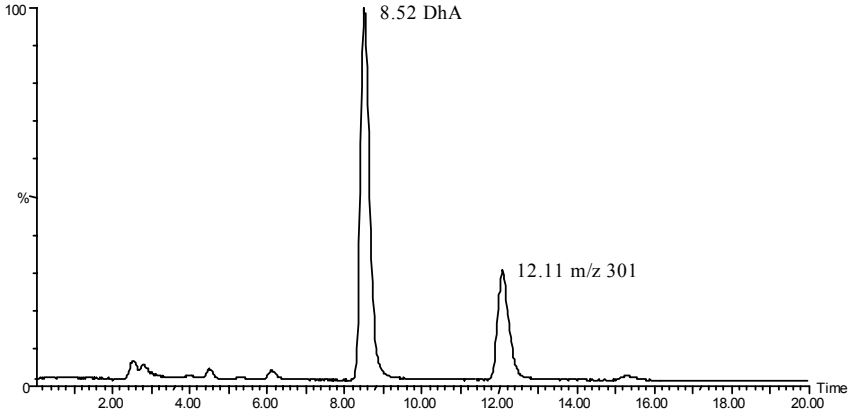

Figure 4.2. Chromatogram of DhA and the three resin acid isomers in a natural water sample from the River Saale.

4.4 Summary

The LC/ESI/MS method proved to be a rapid, highly sensitive procedure for the quantitative analysis of abietic, dehydroabietic, isopimaric, and pimaric resin acids in spiked natural waters at detection limits of 0.40, 0.40, 0.30 and 0.25 μg/L, respectively. However, the three structural resin acid isomers (AbA, IpA and PA) do not separate sufficiently under the various LC conditions evaluated. Therefore, LC/ESI/MS is not recommended for environmental monitoring in instances where measurement of individual isomeric resin acids is required. Application of LC/ESI/MS is, however, well suited for resin acids analysis of environmental water samples in which isomeric speciation is not required.

4.5 Acknowledgements

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5. OBSERVATIONS OF RESIN ACID DISTRIBUTION IN THE RIVER SAALE, GERMANY

The softwood-derived resin acids under investigation were found during 2001 and 2002 in the River Saale, sometimes several hundred kilometres from a known point source. It was through these monitoring field studies that it was established, therefore, that resin acids persist throughout the River Saale system. Although the likely sources of resin acids are primarily restricted to the forested state of Thuringia (via natural tree weathering processes, lumber processing and pulp and paper production), detectable concentrations of resin acids were noted in the most northerly reach of the river at Calbe, in the agricultural state of Sachsen-Anhalt. It was apparent from this preliminary observation study that a more in-depth analysis of the photochemical and microbial fate of resin acids in aquatic environments was warranted. Resin acid concentrations in 2002 were significantly lower compared to those observed in 2001, likely due to severe flooding throughout the River Elbe watershed in 2002.

The following manuscript is in press for September, 2003 and may be cited as follows:

McMartin, D.W., W. von Tümpling, J.V. Headley and J.A. Gillies. 2003. Observations of resin acid distribution in the River Saale, Germany. *Canadian Water Resources Journal* **28**(3): 359-374.

5.1 Introduction

Contamination concerns related to several industrial endeavours in the former German Democratic Republic (GDR) including abundant pulp and paper processing facilities surfaced following the reunification of Germany. Prior to reunification the economic policy of the GDR included over-industrialization and over-employment

directives. Some analysts have stated that this economic policy was directly responsible for deteriorating water quality and heightened production led to virtual complacency towards increasing environmental degradation (Stackpole, 1999; Picht and Schmidt, 1992). Environmental information and data in the former GDR tends to be incomplete for several environmental compartments including the watershed of the River Elbe and its major tributary, the River Saale (Figure 5.1). Only three percent of all rivers and one percent of still waters in the GDR were characterized as fully ecologically sound in 1989 (Umweltbundesamt, 1995; Rothkirch and Klinger, 1994). The current investigation was initiated to examine the spatial extent of pulp and paper processing-associated resin acids in the River Saale (sampling information listed in Table 5.1). Water samples for resin acid analysis were collected from mid-stream to provide a preliminary description of the fate and transport of the most abundant resin acids in the River Saale.

Table 5.1. Water sample collection information.

Town	Location	Collection Period	Collection Frequency
Calbe	Near confluence of the Rivers Saale and Elbe; agriculture and forestry in Sachsen-Anhalt	Apr-Aug, 2001 Apr-June, 2002	Twice weekly
Joditz	Near the headwaters of the River Saale; densely forested region in Thuringia	July, 2001 May, 2002	Once per year
Harra/Blankenstein	Downstream of the Rosenthal pulp and paper mill; densely forested region in Thuringia	July, 2001 May, 2002	Once per year
Eichicht	Downstream of two dammed reservoirs; densely forested region in Thuringia	July, 2001 May, 2002	Once per year
Freienorla	Downstream of industrial and urban discharges; densely forested region in Thuringia	July, 2001 May, 2002	Once per year
Porstendorf	Downstream of Jena (major urban centre); north boundary of dense forests in Thuringia	July, 2001 May, 2002	Once per year

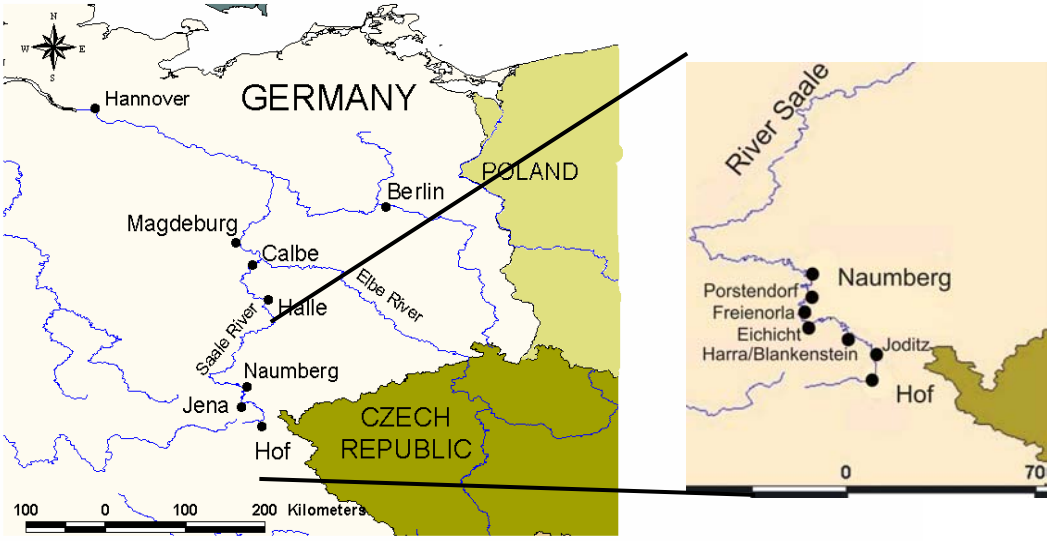

Figure 5.1. Map of the Rivers Saale and Elbe in the former GDR, including the five sampling locations near the headwaters of the River Saale.

5.2 Experimental

5.2.1 Chemicals and Standards

Chemicals and standard preparation were as described in Chapter 4.

5.2.2 Sampling Sites

During the periods of April through August, 2001 and April through May, 2002 more than forty water samples were collected from the River Saale at Calbe, approximately 5 km from the confluence of the Elbe and Saale rivers. Ten large volume (10 L) white plastic containers, from which triplicate small volume samples (2 mL) were taken for resin acids analysis, were filled twice weekly throughout the project duration. The containers were filled to leave no head space and were stored at 4 °C; no preservatives were added. Analysis of the triplicate raw water samples was completed on the day of sample collection.

In addition, duplicate 1 L grab samples were collected twice at five locations near the headwaters of the River Saale (Figure 5.1). These samples were utilized to

evaluate both the extent of resin acid input near the Rosenthal kraft mill and downstream resin acid concentrations. A Limnos water sampler was used to collect midstream samples from the river. The transfer tube was flushed twice and amber glass collection bottles and stoppers rinsed prior to sample transfer. Each bottle was filled, allowed to settle and stoppered without headspace. The samples were tested on-site for pH, temperature, dissolved oxygen content and conductivity. Dissolved oxygen (DO) was measured with a WTW Oxi 325 meter; pH and temperature with a WTW pH 340 meter. Both DO and pH measurements were temperature adjusted. From each sample, two 1 mL aliquots of raw and concentrated samples were analyzed for resin acid content. Sample volumes were reduced from 1 L to 100 mL using a rotovap for concentration of samples. Two 1 mL aliquots of each river water sample were transferred to amber vials and analyzed on the day of collection.

The five locations along the River Saale in the state of Thuringia chosen for inclusion in the sampling study are as follows:

1. Joditz: near the headwaters of the River Saale with no major upstream industrial or forestry facilities;
2. Harra: 2 km downstream of the Rosenthal pulp and paper mill at Blankenstein;
3. Eichicht: located immediately downstream of two dammed reservoirs. The primary purposes of the reservoirs are flood protection and energy production—a side benefit to the approximately two-week residence time is enhanced removal of organics due to biodegradation and other natural processes. It was anticipated that resin acids concentrations would be reduced significantly during the prolonged reservoir residence time. Since water is released from the bottom of the reservoir, it is possible that resin acid discharges are not representative of recent pulp and paper mill discharges due to the presence of a thermocline separating the warm pulp and paper mill effluent from the colder water at the bottom of the reservoirs. The deeper waters are likely to contain lower resin acid concentrations due not only to the presence of a thermocline, but also due to increased residence time allowing for longer exposure to natural degradation processes;

4. Freienorla: located downstream of a major cotton factory in the Saale Valley. The cotton factory discharge was identified as a potential influence on resin acid degradation in the river (whether to inhibit or promote degradation due to microbial action). As well, this location downstream of the double reservoir system at Eichicht may be used to evaluate the presence and effects of "plug" flow releases downstream from the reservoirs; and
5. Porstendorf: located downstream of the major urban centre of Jena. Several municipal and industrial inputs are added to the River Saale at Jena that may impact on the rate of resin acid degradation. For instance, the addition of alternative carbon sources may create competition or toxic conditions that, in turn, may result in reduced biodegradation.

5.2.3 Resin Acids Quantification

The liquid chromatography (LC) method utilized for environmental baseline data is as described in the Chapter 4 discussion of analytical method development.

Field blanks and laboratory standards were analyzed in series simultaneously with the resin acid laboratory solutions. A set of four to six standards plus a duplicate run of the field blank corresponding to the spiked River Saale solution were analyzed every 20 or fewer samples.

5.3 Results and Discussion

5.3.1 On-Site Environmental Conditions and Observations

Table 5.2 provides the results of water temperature, pH, DO and conductivity monitoring and reveals that the River Saale is slightly basic. At pHs greater than 7, the resin acids were anticipated to be present in their ionized form in the river. No odour was noted at any of the sampling locations during either 2001 or 2002.

Table 5.2. Results for water quality parameters recorded on-site.

Location	Secchi disk* (m)		pH		Temperature (°C)		DO (mg/L)		Conductivity (µS/cm)
	2001	2002	2001	2002	2001	2002	2001	2002	2002
Joditz	1.5	3	7.3	7.6	19.7	7.5	n/a	7.5	450
Harra/Blankenstein	0.09	0.1	7.9	7.8	20.4	7.7	6.2	7.5	520
Eichicht	2	3	8.2	7.8	11.0	6.8	10.6	9.3	415
Freienorla	0.2	0.5	8.3	7.9	18.4	7.4	8.4	8.6	482
Porstendorf	2.5	6	8.3	7.8	21.5	7.8	8.4	8.9	600

* Values for all sampling locations except Harra/Blankenstein are equal to the approximate depth to the riverbed; 2001 samples were taken in July; 2002 samples were collected in May.

The spring/summer of 2001 and spring of 2002 were rainy and cool with average temperatures of approximately 20 and 15 °C, respectively. Rainfall in the mountains near the head of the River Saale compounded significant snow melts in 2002 creating substantial flooding throughout the watershed. During separate observation excursions during the months of April and May, 2002 few locations were noted at which the river had not overflowed its banks. A subsequent report from the Infrastructure Development Institute (2002) indicated that the flood was of a magnitude that would be observed once every 300 years in terms of flow and every 200 years in terms of the water level throughout the River Elbe watershed.

At the Joditz control site in 2001 the River Saale was less than 10 meters wide and 1.5 meters deep with thick reeds and a swift current. In contrast, the river was more than 30 meters wide and 3 meters deep, having overflowed its banks, in April, 2002. A lumber mill approximately 50 meters downstream of the Joditz sampling site was determined to be a possible source of resin acids. However, it was assumed to have no impact on the resin acids concentration at the Joditz collection site upstream from the mill.

At Harra the river was more than 50 meters wide and at least 5 meters deep with a strong current. Flooding at this site was not as noticeable in 2002 as at other locations since the riverbanks here are quite steep and rocky making it difficult to

visually assess water level. The murky brown colour of the river at this location was assumed to be partially related to the coloured effluent discharge at the Rosenthal mill. At the Harra sampling location, the water was highly coloured as indicated by Secchi disk transparency to a depth of approximately 90 cm, although no associated odour was noted. Previous studies have noted that the colour of the effluent, and thus the river, has been significantly reduced since the Rosenthal mill was re-opened by Canadian/Swedish conglomerate Mercer International after 1990 (Richter and Stüber, 2001). The Rosenthal pulp and paper processing facility was identified as the sole significant resin acid source along the southern reach of the River Saale. Most of the smaller capacity pulp and paper processors in Thuringia were closed following the reunification of Germany. Other small sources of resin acids are the many lumber mills along the river.

At the remaining three sampling locations, the Saale was observed to be clear. At these locations the river was at least 20 meters wide and noticeably flooded in 2002. Water depth was lower in August, 2001 compared to April, 2002, although both years experienced above-average cumulative rainfall. At Eichicht, the temperature was much lower than that observed at any other location along the Saale as a result of deep-water release from the system of upstream reservoirs. Also, the DO content at Eichicht indicated near-saturation conditions resulting from aeration of the shallow water in extensive rapids less than 20 meters upstream from the sampling location. In 2001, the DO saturation level was 100 %, while in 2001 the level was significantly lower at 80 % saturation.

5.3.2 Detection of Resin Acids

Resin acids were detected using mass/charge ratio (*m/z*) of 299 for DhA (molecular mass 300). The unresolved resin acid isomers comprising the molecular mass group 302 (AbA, IpA and PA) were detected at *m/z* 301. All were detectable in many River Saale water samples. The chromatograms resulting from LC/MS analyses clearly indicated the presence of both resin acids mass groups (Figure 5.2). In

further testing it was determined that the resin acids were negligibly sorbed to suspended particles and recovery values between 82 and > 100 % were attained. There was thus negligible interference from the river water matrices. The binding coefficient (K_p) for DhA is approximately 500 L/kg in natural freshwater indicating that associations between the resin acid and humic materials or particulates in the water column are unlikely (Kukkonen and Oikari, 1991). Binding coefficient values for the isomer resin acids studied (AbA, IpA and PA) are not available in the literature. However, the recovery results indicate these may be similar to that published for DhA.

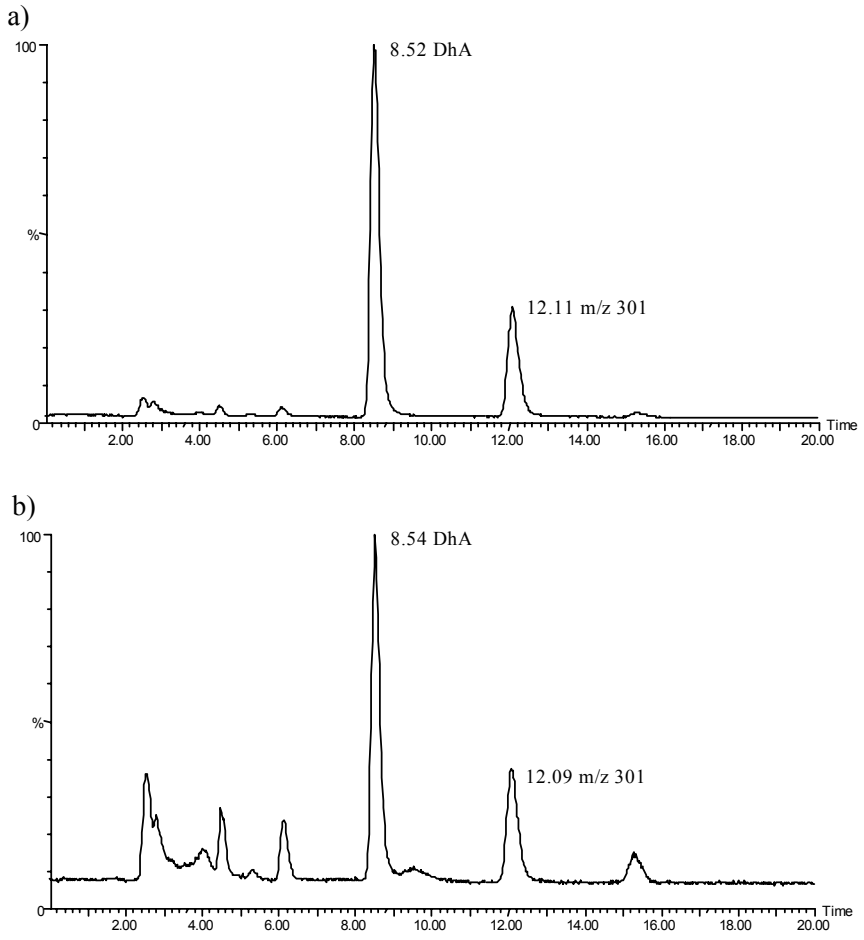

Figure 5.2. Chromatograms of the resin acids showing the retention time and identity of the compounds at two of the five sampling locations along the River Saale in Thuringia: a) Harra; b) Freienorla.

Although resin acids were frequently detected in natural water samples, concentrations were below the 0.40 μg/L detection limit for some 50% of those investigated. The notable difference in resin acid concentrations at Calbe in the state of Sachsen-Anhalt from 2001 to 2002 was attributed primarily to the 2002 flood conditions. The concentrations at Calbe indicated maximum background resin acids at approximately 18 and 34 μg/L for the resin acid isomers and DhA, respectively, during the five-month study period in 2001. The mean concentration of positive detections for the resin acid isomers between April and August, 2001 was 4.95 μg/L; the DhA mean concentration was 6.45 μg/L (Table 5.3). Maximum concentrations during the second study period in 2002 were 2.5 μg/L for both mass groups.

Table 5.3. Mean concentrations of the resin acids at each sampling location.

Location	AbA, IpA and PA* (μg/L)		DhA** (μg/L)	
	2001	**2002**	**2001**	**2002**
Calbe[a]	5.0 ± 2.4	2.0 ± 0.2	6.5 ± 1.8	2.0 ± 0.2
Joditz[b]	33.9 ± 3.1	nd	17.6 ± 3.1	nd
Harra/Blankenstein[b]	596 ± 28.0	94.3 ± 3.8	577 ± 19.9	101.1 ± 1.7
Eichicht[b]	69.6 ± 1.9	10.4 ± 0.0	112 ± 8.0	22.3 ± 0.4
Freienorla[b]	210 ± 35.4	32.2 ± 0.8	288 ± 14.9	44.0 ± 2.7
Porstendorf[b]	79.7 ± 4.1	7.2 ± 0.2	122 ± 8.2	13.1 ± 0.2

*concentration based on extracted ion at *m/z* 301.3 in LC/MS chromatogram
**concentration based on extracted ion at *m/z* 299.3 in LC/MS chromatogram
[a] n = 40; errors are based on 95 % confidence levels
[b] n = 8; errors are based on 95 % confidence levels
nd = non-detectable (i.e., < 0.40 μg/L)

One previous researcher noted resin acid concentrations more than 200 km from a known source in a prolonged study of the Athabasca River in Alberta, Canada (Crosley, 1996). Others in Ontario, Canada and New Zealand also noted that resin acids tend to persist in both the water column and sediments at lengths of at least 12 km downstream of pulp and paper mills (Munkittrick *et al.,* 1994; Robinson *et al.,*

1994; Volkman *et al.,* 1993) Therefore, the results of River Saale resin acid measurements generally match well with the literature. However, the values in the River Saale may be somewhat elevated as compared to those noted in, for instance, the Ontario study in which only 8 µg/L total resin acids were discovered downstream of a given pulp and paper mill with secondary wastewater treatment (Munkittrick *et al.,* 1994; Robinson *et al.,* 1994).

Grab samples collected near the headwaters of the River Saale in the state of Thuringia indicated the presence of resin acids at most locations in both sample periods (Table 5.3). Background resin acid levels at the Joditz control site indicated undetectable or low resin acid content, most likely related to bark or other tree remnants in the river arising from lumber mills or natural weathering processes along the riverbank. Upstream of the pulp and paper operation at Blankenstein/Harra, there is no apparent industrial resin acid source. Pulp and paper facilities, forest-products fabrication and natural forest-weathering processes are the major resin acids sources noted by previous researchers (Munkittrick *et al.,* 1994; Robinson *et al.,* 1994; Volkman *et al.,* 1993).

Immediately downstream of the Rosenthal pulp and paper mill the resin acid content increased dramatically by as much as 30 times the level noted at Joditz in 2001. Since the Rosenthal facility processes primarily softwoods from the Saale Valley, significant amounts of resin acids were anticipated in the effluents. Even so, the 2001 resin acids concentrations were higher than expected. This was likely due to recent conversion and expansion (i.e., increased production volume) of the mill from sulphite to a 280,000 tonne per year kraft operation (Mercer International, 2000; Ridder and Stüber, 1999). It was during this transitional period that environmental systems may not have been operating at optimal conditions. In 2002, the concentrations of resin acids 2 km downstream of the mill in the River Saale at Harra were similar to those noted by previous researchers (Munkittrick *et al.,* 1994; Robinson *et al.,* 1994; Volkman *et al.,* 1993). At these concentrations the resin acid contents are approaching levels at which they may be deemed hazardous to aquatic

ecosystem health. The concentrations reported here are within the range of the lowest reported 96-h LC_{50} values for fish but are well below those indicated by Taylor *et al.* (1988) in their analysis of data for the development of Ontario provincial water quality objectives and guidelines.

Taylor *et al.* (1988), have indicated that a maximum total resin acid concentration recommended in receiving water with pH values in the range of 7.5 to 8.5 (as for the River Saale) is between approximately 45 and 50 mg/L. Due to the slightly basic nature of the River Saale, acute toxicity is less likely to be an enduring issue since at more basic pHs the toxicity is significantly reduced (i.e., toxicity at pH 6.5 is between 15 to 30 times higher for DhA than that observed at pH 9) (Werker and Hall, 1998; Liver and Hall, 1996; Taylor *et al.,* 1988). Therefore, in the River Saale, both DhA and total resin acid concentrations were observed at levels well-below those required to mitigate fish-related acute toxicity.

Samples collected downstream of the double reservoir system at Eichicht indicated that total resin acid concentrations (Table 5.3) were reduced to concentrations well below those deemed acutely toxic. At this location, the DhA concentration was approximately twice that of the isomer resin acids (AbA, IpA and PA combined). It is probable that this heightened concentration is due to an additive effect of both persistent DhA released in pulp and paper effluent and also due to degradation of the isomer resin acids, particularly AbA, into DhA as previously suggested by Patoine *et al.* (1996) and Zender *et al.* (1994). A similar pattern of increased DhA concentrations was noted throughout the forested Saale Valley in Thuringia.

At Freienorla, both ion groups again increased in concentration to approximately two or three times those at Eichicht. Factors influencing this increase include tree weathering and lumber processing, dilution, microbial action and/or inhibition, "plug" flow regimes and river hydrology. Between Eichicht and Freienorla some reduction of resin acid concentrations may be achieved through dilution from tributaries. This effect is anticipated to be minor since tributaries to the River Saale

constitute minor water flow regimes and are more correctly referred to as creeks or streams. As previously noted, the DhA concentration was higher than that of the resin acid isomers. A single source of resin acids was not identified, although tree weathering and lumber processing does occur in the region. As well, the effects of discharges from a local cotton factory are unknown in terms of inhibiting the microbial action or other natural attenuation processes in the river. Furthermore, some resin acid concentration changes may be accounted for by river hydrology in that when a high concentration of resin acids is released, a "plug" of water will flow downstream and contain resin acid concentrations. In such instances, a difference between sampling locations on a given date may represent the recent history of variable discharge concentrations.

Natural diffusion and degradation processes appear to be sufficient for resin acids removal between Freienorla and Porstendorf. Although levels at Porstendorf remained higher than the levels measured at Joditz, total resin acid content was well below the level believed to be toxic to aquatic life. The total reduction of resin acids following the large input at Harra/Blankenstein indicates that the natural removal processes in the River Saale are capable of degrading resin acids to levels of ecological irrelevance with respect to acute toxicity. No chronic toxicity effects were evaluated in the present study.

5.4 Conclusions

With its headwaters in the densely forested regions of the northwest Czech Republic and the southern part of the German state of Thuringia, the River Saale is exposed to resin acids throughout its length. Resin acids derived from softwood bark and wood products may enter the river through natural tree weathering processes, pulp and paper manufacturing and other forestry operations. These observations of the resin acids throughout the southern reaches of the River Saale indicated that maximum observed concentrations in the river were approximately 600 μg/L immediately

downstream of a pulp and paper mill. The remainder of the river, whether in southern Thuringia or the northeastern state of Sachsen-Anhalt, contained only trace levels of resin acids well below those believed to be of environmental significance. Therefore, the observations noted here indicate that while there are site-specific regions of resin acids contamination near forestry processing facilities, concentrations of resin acids in the river and reservoirs decline due to natural attenuation, likely including microbial action and the addition of oxygen through aeration.

5.5 Acknowledgements

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6. PHOTOLYSIS AND BIODEGRADATION OF SELECTED RESIN ACIDS IN RIVER SAALE WATER, GERMANY

It is hypothesized that the application of a degradation scheme involving photolysis treatment prior to biodegradation will reduce the concentration of pulp and paper-associated resin acids and eliminate the associated acute toxicity from natural surface water. The facile photodegradation of the four resin acids under investigation was observed with pseudo-first-order kinetics when exposed to broadband and UV_{254}-radiation. Further, the microbial degradation of resin acids in the rotating annular biofilm reactors described in Chapter 1 indicated that photolysis is an effective pre-treatment method for resin acid biodegradation. To test the hypothesis that acute toxicity can be eliminated through the application of photolysis and/or biodegradation, the bacterial toxicity of the aqueous resin acids solutions was measured with Microtox® luminescence assays. The results of these analyses showed that acute toxicity decreased at rates commensurate with the removal of the parent resin acid compounds. Consequently, it was determined that photolysis and biodegradation of the resin acids did not generate any notable amounts of toxic intermediates and/or the intermediates formed were further degraded into compounds of lower toxicity than the parents. With photochemical and microbial treatment at pulp and paper mills, as well as in-situ degradation by solar radiation and natural biofilms within the River Saale, resin acid inputs can be reduced in both concentration and acute toxicity to near undetectable levels.

The related manuscript was published in 2003 and may be cited as follows:

McMartin, D.W., J.V. Headley, T.R. Neu and D.A. Friesen. 2003. Photolysis and biodegradation of resin acids in River Saale water, Germany. *Journal of Environmental Science and Health* **A38**(12): 2727-2747.

6.1 Introduction

The River Saale flows north from a region bordering the Czech Republic toward the highly industrialized city of Halle and joins the Elbe near the town of Calbe (Figure 5.1). Although the Saale has long been associated with pulp and paper processing and manufacturing, there is a scarcity of historical environmental information pertaining to water quality and industrial inputs. A few studies have been reported following the reunification of Germany addressing environmental assessments and degradation of toxins in aquatic environments (Stackpole, 1999; Rothkirch and Klinger, 1994). In the present investigation, the fate and transport of four prevalent pulp and paper processing-associated resin acids were evaluated via microbial and photochemical degradation processes.

6.2 Experimental

6.2.1 Water Samples and Chemicals

During the periods of April through August, 2001 and April through May, 2002 forty-six water samples were collected from the River Saale approximately 5 km from the confluence of the Saale and Elbe rivers near the town of Calbe (Figure 5.1). Water was collected in large-volume plastic containers every Tuesday and Friday throughout each study period. From these samples collected at Calbe, ambient resin acid concentrations in the River Saale were assessed simultaneously with samples collected from both the photolysis and biodegradation experiments.

Neat standards of the four resin acids were purchased from Helix Biotech (Vancouver, Canada) as previously described in Chapters 4 and 5. Reagents for Microtox® analyses were purchased from Azur Environmental (Newark, DE). All other required chemicals were previously described in Chapter 5.

6.2.2 Photolysis

Spiked water samples were exposed to ultraviolet radiation (broad band to simulate sunlight and UV_{254} used in wastewater disinfection) for periods of 10 hours in quartz photochemical reactors (Figure 6.1) with a delay of 15 minutes after spiking. The photochemical reactors were covered with aluminum foil to optimize UV exposure. A constant flow of cold water was maintained in the cooling chamber throughout the treatments to maintain a low reaction temperature of approximately 10 ± 2 °C. The magnetic stir block was used to maintain a consistent, well-mixed solution.

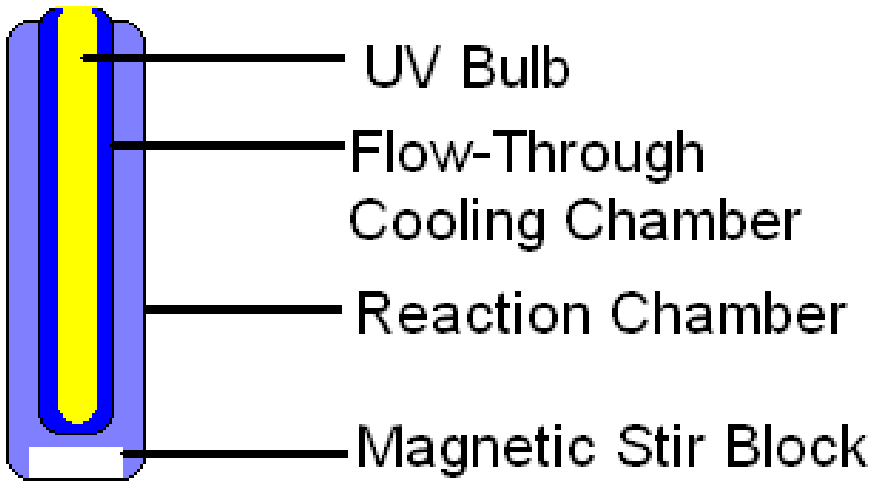

Figure 6.1. Quartz photochemical reactor apparatus.

Experiments at UV_{254} are indicative of the potential for development of effective water treatment processes, but not necessarily natural degradation processes. A Heraeus NK4/4 UV_{254}-radiation immersion lamp (Hanau, Germany) was employed for those experiments. The radiation flux of this Heraeus lamp was 500 mW inside the immersion tube of the photochemical reactor. Two broadband UV radiation sources were used to model environmental degradation potential. The first of these was a 15 W fluorescent blacklight (300-400 nm, max at 350 nm; Philips BLB). The spectra of this lamp includes the UV wavelengths of solar radiation that reach the earth's surface, is subsequently referred to as the UV-A/UV-B source. The second

broad band lamp was a Heraeus TQ 150 Z3 immersion lamp including wavelengths in both the UV and visible range (200 to 700 nm; Figure 6.2) and similar to that used by Corin *et al.* (2000). Since the Heraeus TQ 150 Z3 emits high energy UV-C radiation, it does not provide as accurate a simulation of natural solar UV radiation as does the Philips lamp. This lamp is subsequently referred to as the Heraeus broad band radiation source. The radiation flux of the Heraeus broad band immersion lamp was 34 W inside the immersion tube of the photochemical reactor.

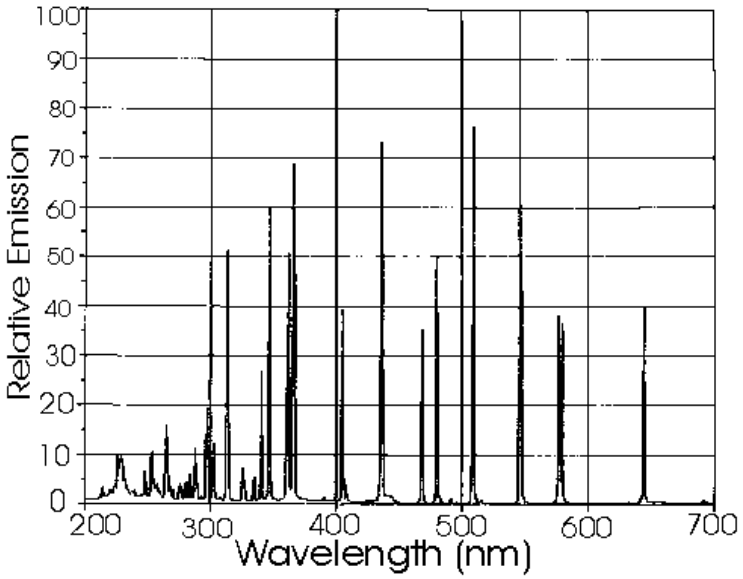

Figure 6.2. Heraeus TQ 150 Z3 immersion lamp relative emission spectra (reprinted from Heraeus product specifications).

Duplicate experiments with resin acids at 0.1, 0.5, 2 and 4 mg/L were conducted with River Saale water to assess the photolysis of each resin acid under natural water quality conditions. Single component and mixture systems of the resin acids were prepared and 1 mL aliquots collected at appropriate time intervals to assess photodegradation kinetics. High concentration experiments (2 and 4 mg/L) were completed over 24 hours; low concentration experiments (0.1 and 0.5 mg/L) were completed over 10 hours. All experimental work was conducted at room temperature (18 ± 2 °C). No commercial catalysts were added.

Photolysis pre-treatment prior to exposure to the mature River Saale biofilms was completed over 90 minutes under conditions identical to that outlined above using the Heraeus broad band and UV_{254}-radiation sources. The total effluent from the photochemical cells was then immediately transferred to the rotating annular biofilm reactors.

Control reactors were observed to determine the extent of resin acid adsorption to the quartz photochemical reactor and/or the Teflon-coated magnetic stir block. The controls were studied in the absence of UV light within aluminum foil-wrapped cells. The binding coefficient (K_p) for dehydroabietic acid in natural freshwater is approximately 500 L/kg indicating that associations between that resin acid and humic materials or particulates are unlikely (Kukkonen and Oikari, 1991). Binding coefficient values for the other three resin acids studied are not available in the literature. In general, resin acids are weak acids with pKa values in the range of 5.7 to 6.4 (Liss *et al.,* 1997), indicating that at ambient River Saale pH (approximately pH 8) most of the resin acids are in the ionized, more hydrophilic form that do not tend to accumulate well in sediments (Liss *et al.,* 1997; Kukkonen and Oikari, 1991).

Approximate quantum yields (Φ) at 254 nm for all four resin acids were calculated using equation (1.1).

6.2.3 Biodegradation

Native biofilm from the River Saale was developed in a reactor consisting of an outer vessel with a rotating inner cylinder (Figure 6.3). Throughout the development stage the reactor was flooded continuously with fresh water from the Saale (flow rate 105 mL/min; rotation rate 150 rpm). The water in the circulation vessel was exchanged with freshly collected water every Tuesday and Friday and circulated through the turbulently mixed system to maximize the nutrients available to the

growing biofilm. Re-circulating tubes in the inner cylinder ensured optimal mixing of the system and minimized nutrient gradients.

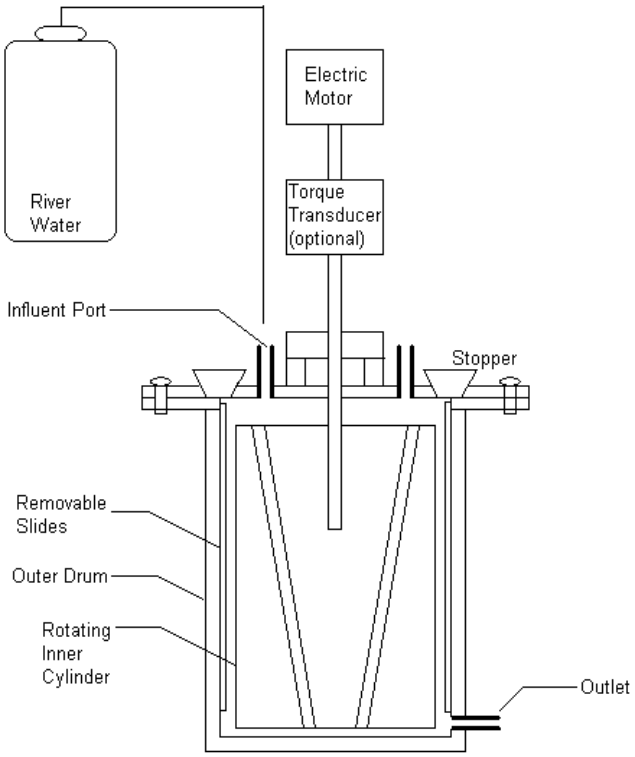

Figure 6.3. Rotating annular biofilm reactor (biofilm growth primarily occurs on both the removable slides and rotating inner cylinder).
Note: The optional torque transducer was used as part of the RARs utilized for resin acid biodegradation experiments.

At the start of biodegradation experiments (during which time the microbial communities take up resin acids from the aqueous solutions, otherwise noted as "uptake"), the flow of Saale water was discontinued and the water drained off through a bottom outlet. The outlet was then clamped and the reactor filled from the top with 700 mL of either spiked Saale water at 2 or 0.5 mg/L total resin acids or photo-treated effluent with the same initial resin acid concentrations. Following addition of the spiked River Saale solutions, the rotation rate of the reactor was again set to 150 rpm now under zero flow-through conditions. Eleven experiments spanning 3 to 5 days were completed. A time series of the effluent was obtained

from duplicate 1 mL samples collected in 2 mL amber glass vials stored at 4 °C until analysis. Quantitative analysis was completed within 24 hours of sample collection.

Along the inside of the reactor vessel were 12 removable slides (as noted in Figure 6.3). Portions of these slides were cut off, dyed and viewed under a confocal laser microscope to assess structural changes throughout each experiment. Other portions of the slides were freeze-dried to assess biofilm uptake (i.e., that portion of the resin acids no longer in aqueous solution but also not biodegraded). The freeze-dried biofilms were ultrasonically extracted with 5 mL of methanol for one hour at room temperature. The resulting extract was filtered using a 0.2 μm pore size surfactant-free cellulose acetate syringe filter (Nalgene, Rochester, NY). The extracts were reduced to 0.5 mL under a stream of dry nitrogen and analyzed by LC/MS (Headley *et al.,* 2001b). The biofilm extracts were used to calculate the extent of resin acid binding and uptake to the biofilm and served as a control system for evaluation of biodegradation versus loss to sorption.

Separate control biofilm reactors were implemented to determine the effect of methanol on mature biofilm communities using an equal volume of methanol as added to the treatment bioreactors in the form of resin acid spikes. The community structures of the control biofilms were compared to those of the active bioreactors using confocal laser microscopy.

6.2.4 Instrumental

The LC/MS analytical method used for resin acid concentrations changes due to the applied degradation processes is described in Chapter 4.

6.2.5 Confocal Laser Microscopy

Biofilm samples were visualized by confocal laser scanning microscopy prior to and following each biodegradation experiment to determine how the addition of contaminants affected the microbial community. Biofilm samples from both methanol controls and bioreactors were compared using a TCS SP (Leica, Germany) controlled by Leica Confocal Software Version 2.00 Build 0477. Biofilms were observed using an upright microscope with water-immersible 20 x 0.5 NA and 63 x 0.9 NA lenses. The system was equipped with Ar (488 nm), Kr (568 nm) and HeNe (633 nm) lasers.

Biofilms were stained with nucleic acid specific SYTO9 green fluorescent dye for evaluation of bacterial cell distribution. Autofluorescence of cyanobacteria was recorded in the red and far-red channel; algae were recorded in the far-red channel only. Image series were projected employing the microscope software and printed from Photoshop 7.0 (Adobe, Edinburgh, UK). Quantification of image data was done with ScionImage (Scion Corp., Frederick, MD) using an automated macro written at the UFZ,Germany.

6.2.6 Toxicity Test

Acute toxicity was measured prior to and following experiments using the Microtox® luminescence assay. Water samples from experiments conducted at 0.5 mg/L resin acid spike concentrations exposed to UV radiation and/or treated in the biofilm reactors were withdrawn in sterilized test tubes (autoclaved at 121°C for 20 minutes) prior to, during and following experiments. Toxicity of the resin acid samples was completed using the 90% strength serial dilution basic test. The light emissions from each cuvette were read at 5, 15 and 30 minutes of incubation using a model 500 Microtox® Analyzer (Azur Environmental, Newark, DE; equipment belonging to the Toxicology Centre, University of Saskatchewan, Saskatoon, Canada was used for this analysis). Duplicate tests of the serial dilutions were completed for each sample. Adjustments to pH were not required since sample pH

fell within the 6.0 to 8.0 range required for the method (Environment Canada, 1992). The instrument was calibrated using phenol as a reference toxicant according to the Environment Canada method (1992).

6.3 Results and Discussion

Based on a five-point calibration, a linear response was observed from 0.01 ng to 3.5 ng of resin acid on column using 10 μL injections. Method detection limits based on a signal:noise ratio of 3:1 in river water samples were 0.40, 0.40, 0.30 and 0.25 μg/L for abietic, dehydroabietic, isopimaric and pimaric acids, respectively. The reproducibility of the method was ≥ 97% based on measurements of laboratory standards and duplicate analysis taken throughout a five-month period, in which more than 1000 resin acids determinations were performed. There was little or no matrix interference based on the observed recovery of matrix spikes.

6.3.1 Photolysis

To the authors' knowledge, no previously published research has investigated multiple concentrations of resin acid mixtures in photolysis experiments. All four of the resin acids studied were observed to undergo facile photodegradation in River Saale water samples with pseudo-first-order kinetics when exposed to the range of radiation investigated. Half-life values varied with UV radiation source, but were not concentration-dependent. No statistical difference in the persistence of dehydroabietic acid and the two pimaranes investigated was observed.

The UV_{254}-radiation source was effective for removal of resin acids. Half-life values ranged between 18 and 100 minutes when the resin acid solutions in River Saale water were exposed to the monochromatic immersion lamp (Tables 6.1 and 6.2). With the addition of further UV and visible wavelengths provided by the Heraeus broad band UV lamp, the resin acids were most effectively removed from River

Saale water. Exposure to this lamp provided further indication of the efficacy of sunlight supplemented with UV_{254}-radiation for pre-treatment to microbial applications followed by release to the environment. Experiments with the Philips UV-A/UV-B lamp also resulted in rapid removal of the resin acids from natural waters. However, the half-life values were, in general, approximately twice as long as those observed using the Heraeus broadband and UV_{254} lamps (Tables 6.1 and 6.2).

Table 6.1. Half-life values in aqueous solutions of individual resin acids exposed to UV_{254}-radiation or simulated sunlight sources.

Resin Acid	Radiation Source	Half-Life (minutes) 0.1 mg/L	0.5 mg/L	2 mg/L	4 mg/L
AbA	UV_{254}	18.6 ± 1.7	20.3 ± 0.1	20.9 ± 3.0	19.8 ± 0.6
	Heraeus solar	13.03 ± 0.3	14.26 ± 0.7	N/A	N/A
	Philips solar	45.2 ± 3.0	43.3 ± 1.6	N/A	N/A
DhA	UV_{254}	93.6 ± 5.6	92.9 ± 4.9	94.3 ± 4.4	95.6 ± 3.2
	Heraeus solar	65.7 ± 4.8	56.5 ± 8.1	N/A	N/A
	Philips solar	189 ± 2.4	194 ± 5.9	N/A	N/A
IpA	UV_{254}	95.6 ± 0.9	90.1 ± 4.1	90.6 ± 9.1	95.7 ± 2.0
	Heraeus solar	64.5 ± 6.6	63.5 ± 2.5	N/A	N/A
	Philips solar	156 ± 6.5	159 ± 4.2	N/A	N/A
PA	UV_{254}	98.3 ± 3.3	92.1 ± 1.8	101 ± 4.2	97.7 ± 3.7
	Heraeus solar	67.6 ± 3.0	59.6 ± 2.0	N/A	N/A
	Philips solar	178 ± 8.7	199 ± 5.5	N/A	N/A

$n \geq 8$; based on duplicate samples for both one- and two-component systems; error limits are 95 % confidence levels of the mean of analytical results.

Half-life values for each of the four resin acids were independent of initial concentration between 0.1 and 4 mg/L (Tables 6.1 and 6.2). No residual resin acids were detected following 24 hours of UV_{254} exposure in the high concentration experiments (2 and 4 mg/L), nor after 10 hours of exposure in the low concentration experiments (0.1 and 0.5 mg/L).

Table 6.2. Half-life values in aqueous solutions of resin acids in combination with DhA exposed to UV_{254}-radiation or simulated sunlight sources.

Resin Acid	Radiation Source	Half-Life (minutes) 0.1 mg/L	0.5 mg/L	2 mg/L	4 mg/L
AbA	UV_{254}	22.6 ± 0.8	21.8 ± 2.7	22.7 ± 2.4	20.6 ± 1.6
	Heraeus solar	17.8 ± 3.3	16.9 ± 1.6		
	Philips solar	59.9 ± 2.7	57.1 ± 1.6		
DhA*	UV_{254}	93.7 ± 1.6	92.8 ± 1.3	102 ± 5.6	98.4 ± 3.7
	Heraeus solar	62.5 ± 0.9	53.6 ± 0.9		
	Philips solar	188 ± 11.8	172 ± 16.0		
IpA	UV_{254}	90.1 ± 5.6	97.2 ± 14.9	94.4 ± 13.4	93.0 ± 0.9
	Heraeus solar	59.3 ± 1.6	56.1 ± 8.5		
	Philips solar	178 ± 1.8	179 ± 2.2		
PA	UV_{254}	92.1 ± 6.2	97.7 ± 4.9	91.0 ± 1.7	98.7 ± 2.8
	Heraeus solar	68.9 ± 3.0	64.1 ± 1.2		
	Philips solar	159 ± 2.5	169 ± 3.1		

*Values for DhA indicate the half-life for that compound when in aqueous mixture with one of the other three resin acids investigated.
$n \geq 8$; based on duplicate samples for both one- and two-component systems; error limits are 95 % confidence levels of the mean of analytical results.

Abietic acid was the resin acid most susceptible to photolysis under all applied sources of radiation. Generally, more than 95 % of the AbA parent compound was photolyzed in the first 90 minutes, depending on UV radiation source. At low AbA concentrations (0.1 and 0.5 mg/L), no residual parent compound was detected following 60 minutes of exposure to the Heraeus broadband source. Exposure to the UV-A/UV-B radiation source induced degradation of abietic acid at a reduced rate compared to those observed with either the Heraeus broadband or UV_{254}-radiation sources. At the higher initial concentrations (2 and 4 mg/L) virtually all AbA was removed following 90 minutes of exposure to the UV_{254}-radiation source. Initial concentration of abietic acid did not affect the degradation rate (Figure 6.4). It is generally accepted that the first step in AbA degradation is to DhA, monitored here using the negative ion *m/z* 299.3 (Patoine *et al.*, 1996; Zender *et al.,* 1994).

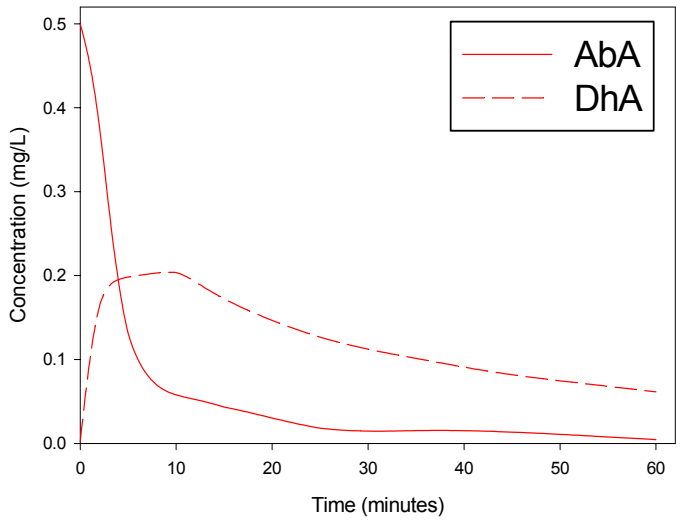

Figure 6.4. Degradation of 0.5 mg/L abietic acid and production of 299.3 ions (assumed to be DhA) in River Saale water when exposed to the Heraeus artificial solar radiation source.

The pimarane resin acids, IpA and PA, were degraded at similar rates to each other regardless of which radiation source was used. The degradation rates for IpA and PA were dependent on UV radiation source, but independent of initial concentration and the presence of DhA in solution (Tables 6.1 and 6.2). Although the pimaranes were less susceptible to photolysis than abietic acid, each pimarane had similar half-life values to those calculated for DhA. As noted by previous researchers, the aromatic ring of DhA is likely responsible for the increased environmental persistence of that compound relative to other abietane resin acids, such as AbA (Patoine *et al.,* 1996; Liver and Hall, 1996; Wang *et al.,* 1995; Zender *et al.,* 1994; Volkman *et al.,* 1993).

Degradation of DhA in River Saale water proceeded at rates similar to those previously observed by Corin *et al.* (2000). The half-life value for DhA indicated that it was approximately 5 times less susceptible to the photolysis processes investigated than AbA (Table 6.1). Dehydroabietic acid was also exposed to each of the radiation sources investigated in aqueous solution with each of the resin acid isomers (abietic, isopimaric and pimaric). Even in this slightly more complex aqueous mixture (i.e., two resin acids were spiked instead of only one as shown in

Table 6.1), the four resin acids were degraded rapidly (Table 6.2). There was no statistical difference between the half-life values shown in Table 6.1 versus Table 6.2. No dependence on concentration or isomer used in the mixture was noted in the kinetics of DhA photolysis.

Quantum yields for abietic, dehydroabietic, isopimaric and pimaric acids at UV_{254} were calculated to be 0.278, 0.057, 0.058 and 0.056, respectively. As anticipated, the quantum yield indicates that abietic acid is the most susceptible to photodegradation with a quantum yield approximately five times higher than those calculated for dehydroabietic, isopimaric and pimaric acids.

6.3.2 Biodegradation

A series of eleven biodegradation experiments was completed with mature River Saale biofilm, including pre-treatment with UV_{254}-radiation, pre-treatment with the Heraeus broadband radiation source, and no pre-treatment of resin acid spiked solutions. Assessments of degradation kinetics were conducted during photolysis pre-treatment and during microbial degradation. Calculations of the photolysis pre-treatment kinetics indicated statistically similar degradation rates as noted in the 10-hour exposure experiments. Concentrations of the resin acids were also measured at the conclusion of photolysis pre-treatment to ascertain initial conditions in the rotating annular biofilm reactors.

Each microbial system was spiked with abietic and dehydroabietic acid and duplicate experiments were completed in each bioreactor to investigate differences in reaction rate in non-acclimated and acclimated systems. Without prior exposure to the resin acids the biofilms required acclimation periods of less than one day before the onset of rapid biodegradation of both resin acids. In acclimated bioreactors (i.e., those in which a previous biodegradation experiment had been investigated), both resin acids were degraded at rates approximately double those observed in non-acclimated reactors. All biodegradation proceeded by pseudo-first

order kinetics regardless of conditioning and acclimation, although acclimated bioreactors displayed significantly more rapid biodegradation of each resin acid (Table 6.3). In acclimated bioreactors, half-life values for AbA and DhA were reduced to approximately half those observed in non-acclimated bioreactors. The kinetics calculated account for only the degradation due to microbial exposure, not photolysis pre-treatment. The degradation rates of both AbA and DhA were significantly increased following UV treatment with either UV radiation source. Using pre-UV treatment, both resin acids were biodegraded even more rapidly in acclimated reactors, presumably due to increased bioavailability achieved during photolysis. The Heraeus broadband radiation source including UV-C radiation (ARS) was the more efficient pre-treatment method, as each resin acid was photodegraded faster than using UV_{254}-radiation. However, independent of pre-treatment, both AbA and DhA were degraded to levels below the analytical detection limit of 0.40 μg/L within the experimental time frame. Initial resin acids concentration did not affect the degradation rate.

Table 6.3. Half-life values for abietic and dehydroabietic acids exposed to natural River Saale biofilms.

Pre-Condition	Acclimation	Half-Life (hours)	
		Abietic Acid	Dehydroabietic Acid
None	non-acclimated	15.94 ± 0.17	40.40 ± 0.77
	acclimated	8.56 ± 0.39	26.27 ± 3.05
Pre-UV_{254}	non-acclimated	10.21 ± 1.44	26.96 ± 5.29
	acclimated	6.38 ± 0.15	13.68 ± 0.40
Pre-ASR	non-acclimated	8.48 ± 0.01	19.06 ± 0.27
	acclimated	3.41 ± 0.14	6.54 ± 0.13

n = 5; ASR = Heraeus artificial solar radiation

Systems in which pre-treatment was not included experienced half-life values that were significantly higher. Dehydroabietic acid in non-acclimated reactors was degraded with a mean half-life of approximately 40 hours, while in an acclimated reactor under identical conditions the half-life was reduced to approximately 26 hours when not UV-exposed prior to biodegradation. Without pre-treatment, AbA

was degraded at a slightly slower rate than when first exposed to UV radiation. Although DhA was noted to degrade more slowly than AbA, its prolonged existence in the bioreactors may have been at least partially due to the metabolism of AbA to DhA. Since this metabolite was not distinguishable from the DhA parent resin acid, it was not possible to determine conclusively the microbial degradation rate of DhA in the rotating annular biofilm reactors (Patoine *et al.,* 1996; Zender *et al.,* 1994; Grimalt *et al.,* 1989).

In the biofilm control reactors to which only methanol was added in equal portions as that added to the active bioreactors, the microbial population was somewhat different from that observed in the resin acid reactors. Those control populations did not experience a shock load, or toxicity, in acclimation to the presence of foreign contaminants. Therefore, the population was more stable and able to grow throughout the experimental time frame. As a further control, biofilm uptake of the two resin acids was measured using extracted freeze-dried biofilm on removable bioreactor slides. Adsorption of the resin acids was minimal, as demonstrated from the freeze-dried biofilm extracts of the active reactors. Similar results were noted in the photolysis control reactors that clearly indicated static systems. No resin acid loss was noted in the absence of UV radiation in the photochemical cells. These results support previous findings in which no strong association between resin acids and natural particulate matter in the water column was observed (Corin *et al.*, 2000; Kukkonen and Oikari, 1991).

In terms of developing treatment strategies for removal of organics in pulp and paper mill effluent streams and downstream receiving waters, rather than pursue complete mineralization in a single stage, it may be more effective to use photolysis as a viable pre-treatment in conjunction with biofilms in the overall attenuation of aquatic organic contaminants. Short UV exposure periods were illustrated in this work as very effective for significantly reducing resin acids entering the microbial system, thus eliminating potential shock effects and enhancing the biodegradation rate.

6.3.3 Confocal Laser Microscopy

Confocal laser microscopy imaging indicated a considerable microbial response to the resin acids. At the start of each experiment the microbial communities were complex and diverse, including bacteria, cyanobacteria and algae. In addition, the biofilm developed into a complex, 3-dimensional structure showing ridges in flow direction (Figure 6.5a). Bacteria and algae comprised the highest biovolume in the mature River Saale biofilm. T-tests were used to statistically determine the overall biovolume and loss throughout the biodegradation experiments. In general, the biovolume of the cyanobacteria was one order of magnitude lower than that of either bacteria or algae.

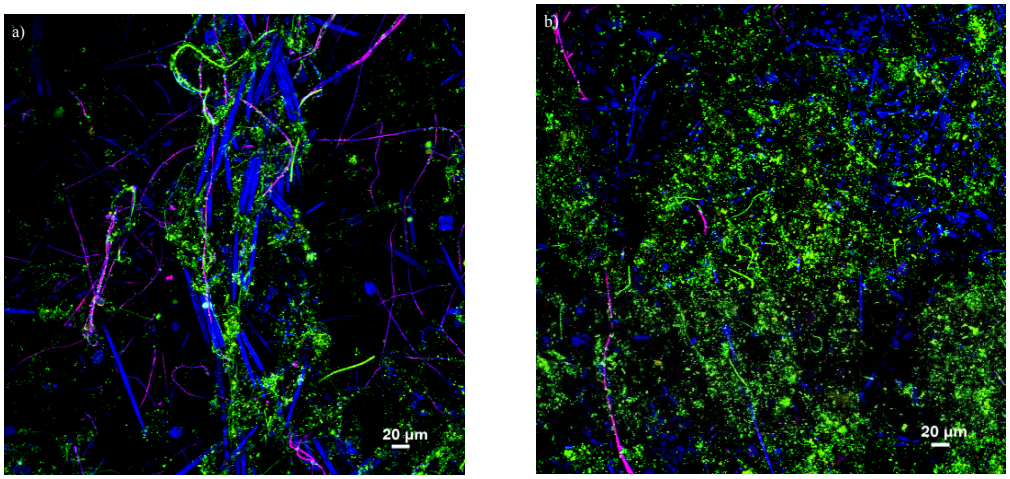

Figure 6.5. Confocal laser scanning micrograph of mature microbial biofilm communities from the River Saale (a) prior to resin acids addition and (b) following five days of exposure to abietic and dehydroabietic resin acids at initial concentration of 0.5 mg/L. Colour allocation: bacteria (green), cyanobacteria (pink), algae (blue)

The addition of resin acids in methanol solution to the rotating annular biofilm reactors resulted in changes to the proportions and volumes of the micro-organisms present. Imaging of the control biofilms indicated both growth and loss responses to methanol addition. The total biovolume of bacteria and cyanobacteria populations in

the control reactors increased by approximately 30 % overall, while the algae population was observed to decrease by approximately 30 %.

Overall changes in the biovolume of resin acid bioreactors indicated growth of the microbial populations, although confocal laser microscopy results revealed some death in biofilm and changes in striations from the initial observations. From visual inspection, neither the microbial diversity nor characteristic growth patterns were evident subsequent to resin acid exposure. Images taken at the end of each experiment revealed that the striated growth pattern along the flow direction in the bioreactors was no longer apparent (Figure 6.5b). However, the images also indicated a second stage of microbial growth capable of degrading the target resin acids.

While biovolumes were observed to increase in reactors to which resin acid solution was added, the biovolume change was often not as high as that observed in the methanol control reactors. This suggests that the presence of the resin acids, whether pre-treated or not, had an initial negative impact on the biofilm communities, as compared to the methanol. Overall growth percentages for the bacteria and cyanobacteria populations were lower than those observed in the methanol control reactors. The effect on algal growth was less pronounced for the untreated and Heraeus broad band-treated resin acid solutions. However, algal growth of more than 15 % was observed in the bioreactors to which UV_{254}-treated resin acid solutions were added. This is evidence that the metabolites produced by photolysis with the UV_{254}-radiation source are significantly less toxic and seemingly more bioavailable to the algal communities present than the parent molecules. This was shown by the drastic algae biovolume loss experienced in the untreated and Heraeus broad band-treated solutions where as much as 30 % of the initial biovolume was lost over the duration of the experiment.

However, this trend did not hold for the cyanobacteria population in which UV_{254}-treated resin acid solutions produced a significantly lower growth rate than that

observed in the methanol control reactor or the bioreactors containing the two other pre-treatment options (i.e., Heraeus broadband and untreated). In both non-acclimated and acclimated bioreactors, the cyanobacteria population was not significantly affected by the addition of resin acids that were pre-treated by exposure to Heraeus broadband radiation or not pre-treated. In those instances, the biovolume change was statistically indistinguishable from the methanol control reactors. This indicates that the parent molecules and metabolites produced from Heraeus broadband photolysis are not overtly toxic to the cyanobacteria species present in the River Saale biofilm. However, from the UV_{254} photolysis results, it was noted that much smaller growth of the cyanobacteria population was achieved over the experimental period. This is likely due to a required acclimation period for the cyanobacteria population to the resin acid parents and metabolites. In fact, the cyanobacteria growth in acclimated bioreactors was similar to that noted in the methanol controls, where in non-acclimated reactors the growth was much lower.

The results of bacterial biovolume analysis indicate that the populations were capable of readily degrading the resin acids, regardless of the pre-treatment condition. Resin acids pre-treated with UV_{254} appeared to be the most bioavailable or least toxic to bacteria. Solutions exposed to artificial radiation indicated that the resin acids were slightly less degradable than those exposed to UV_{254}-radiation, but were slightly more degradable than those not pre-treated. It is likely that the metabolites produced by UV_{254}-radiation were more bioavailable than those produced by Heraeus broadband exposure. However, the inclusion of pre-treatment resulted in better bioavailability or reduced toxicity as evidenced by the increased bacterial biovolume growth noted in both UV-treated samples. In general, bacterial biovolume increase in the bioreactors to which untreated resin acids were added was approximately half that observed in the methanol control reactors. Bacterial biovolume increase in the UV_{254}-treated bioreactors was approximately 65 % that of the control. Although there was a slight increase in bacterial biovolume growth in the acclimated bioreactors, level of acclimation did not have a statistically

significant effect on the biovolume growth or loss of bacterial species in the River Saale biofilm.

6.3.4 Toxicity Test

The toxicity changes during irradiation and biodegradation of the resin acids were monitored with the Microtox® luminescence assay. The bacterial light emission in unspiked natural water samples was determined as a background control and indicated no measurable toxicity. The 5-minute IC_{50} for the reference toxicant phenol was in the range of 13 to 26 mg/L at 15°C as suggested by Environment Canada (1992).

Bacterial light emission of untreated, spiked resin acid solutions indicated significant toxicity. Following exposure in either the photochemical reactor or bioreactor, the relative toxicity of each solution was reduced commensurate with removal of the resin acids. The decreased toxicity might therefore be explained by either decreased concentration and/or by formation of metabolite compounds of lower toxicity than the parent compounds.

In photolysis experiments, the relative toxicity of each solution was significantly reduced following 4 hours of exposure to UV/vis radiation and in all cases was undetectable following 10 hours regardless of radiation source (Table 6.4). Since AbA was removed from solution within the 4-hour sampling period, toxicity was correspondingly absent from the 4-hour aliquots. In biodegradation experiments, toxicity tests indicated similar results. Measurements taken at the approximate mid-point (Day 2 of microbial exposure) of biodegradation experiments indicated toxicity reduction, while those taken at the conclusion of biodegradation (i.e., after 5 days) indicated no measurable toxicity. Un-treated bioreactor samples showed less reduction in toxicity at Day 2 than those in which UV pre-treatment was utilized. However, none of the acclimated or non-acclimated systems, and UV-treated or un-treated systems displayed toxicity at the end of the experiments (Day 5). Toxicity

reduction was influenced by the level of acclimation in the bioreactors, but not significantly impeded.

Table 6.4. Relative toxicity values in aqueous solutions of resin acids exposed to UV_{254}-radiation or simulated solar radiation.

Resin Acid	Radiation Source	Relative Toxicity Over Time*	
		0 hours	4 hours
AbA	UV_{254}	1.0	N/A
	Heraeus solar	1.0	N/A
	Philips solar	1.0	N/A
DhA	UV_{254}	1.0	0.45
	Heraeus solar	1.0	0.41
	Philips solar	1.0	0.53
IpA	UV_{254}	1.0	0.48
	Heraeus solar	1.0	0.45
	Philips solar	1.0	0.55
PA	UV_{254}	1.0	0.44
	Heraeus solar	1.0	0.40
	Philips solar	1.0	0.51
AbA + DhA	UV_{254}	1.0	0.52
	Heraeus solar	1.0	0.47
	Philips solar	1.0	0.58
IpA + DhA	UV_{254}	1.0	0.56
	Heraeus solar	1.0	0.51
	Philips solar	1.0	0.64
PA + DhA	UV_{254}	1.0	0.55
	Heraeus solar	1.0	0.53
	Philips solar	1.0	0.62

*mean values of duplicate serial dilution tests

Table 6.5. Relative toxicity values in aqueous solutions of resin acids exposed to microbial degradation processes (using toxicity reduction noted in pre-UV radiation exposure).

Bioreactor Conditions	Relative Toxicity Over Time*	
	0 hours	2 days
non-acclimated; no UV	1.0	0.68
non-acclimated; UV_{254}	0.59	0.30
non-acclimated; ASR	0.53	0.25
acclimated; no UV	1.0	0.49
acclimated; UV_{254}	0.59	0.21
acclimated; ASR	0.53	0.18

*mean values of duplicate serial dilution tests; ASR = artificial solar radiation

6.4 Conclusions

The River Saale receives pulp and paper-processing discharges in densely forested regions along that river. Despite effluent treatment systems at pulp and paper mill facilities, some resin acids persist and enter the river. The results demonstrate that light-induced degradation is an important removal pathway of resin acids from aquatic environments. The reaction rates indicate that the efficacy of applied photolytic removal for these target compounds is significant. When exposed to radiation sources including high energy UV radiation (such as the UV_{254} single wavelength bulb and Heraeus broad band immersion lamp) all four resin acids were rapidly degraded from River Saale water. Degradation using the lower energy UV wavelengths such as those emitted by the Philips broad band lamp was rapid, but notably slower that that observed with exposure to the higher energy radiation.

Confocal laser microscope images indicated significant changes in the microflora of biofilms exposed to the two resin acids, particularly in untreated spikes of water from the River Saale. Photolysis pre-treatment of resin acid spiked water samples from the River Saale was shown to be effective for reducing resin acid concentrations and eliminating potential shock in microbial treatment systems. Microbial degradation was successful in reducing both resin acid concentrations and toxicity. Both degradation systems were also effective for toxicity removal from the water samples, as indicated by the results of Microtox® testing. The application of treatment using photolysis and microbial systems for pulp and paper mill effluent and exploitation of *in-situ* degradation processes using solar radiation and natural biofilms within the River Saale has great potential for reducing resin acid inputs in both concentration and toxicity to near undetectable levels with little or no ecological significance.

6.5 Acknowledgements

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7. GENERAL DISCUSSION AND CONCLUSION

7.1 Synthesis and Significance of Results

In this study, two hydrocarbon acid groups were examined for their aquatic environmental fate and persistence. For each, an analytical method was developed, field studies and sample collection were completed and laboratory degradation studies were conducted. Naphthenic acids and resin acids are hydrocarbon acids with similar pK_a values and relatively high acute toxicity (Lee *et al.,* 2000; CEATAG, 1998; Liver and Hall, 1996; Werker and Hall, 1998; Brient *et al.,* 1995; Herman *et al.,* 1994; Lee *et al.,* 1990; Zanella, 1983). Due in part to concerns related to this toxicity and the environmental persistence of each compound group, it was determined that the persistence and fate of naphthenic acids and resin acids are of environmental relevance and require further investigation.

7.1.1 Naphthenic Acids

Naphthenic acids are indigenous to hydrocarbon deposits, such as the Athabasca Oil Sands in northern Alberta, Canada. The natural concentration of naphthenic acids in the Athabasca River at and north of Fort McMurray, AB is generally quite low (in the μg/L range or lower). However, tailings pond water may contain naphthenic acids at levels greater than 100 mg/L and may negatively impact on the surrounding aquatic environments (water column and sediments) and terrestrial environment, as well as wildlife and aquatic biota stocks. There is also concern about the potential for accidental discharge and reclamation of the region as tailings ponds fill. Oil production companies in the Athabasca Oil Sands have lease agreements

requiring that landscape reclamation occur to the point of environmental stability at the conclusion of mining operations in the area (Leung *et al.,* 2003). Currently at bitumen extraction facilities the primary tailings pond water treatment objective is related to the removal and settling of fines, not organics, in the tailings and process waters. The lack of active aeration (which would limit the primary goal of fines settling) translates into a lost opportunity for the aerobic biodegradation of organic contaminants, such as naphthenic acids, in the tailings pond water. There are also limitations related to nutrient availability, sunlight and adequate temperature. This deficiency of optimal growing conditions for bacterial, fungal and algal populations, in addition to a potential lack of biodiversity, limits the potential for bioremediation *in situ*. Even with these poor water quality and cold climate conditions, naphthenic acids in the extraction effluents are likely biodegraded at slow rates. However, naphthenic acids remain in the tailings ponds at significant concentrations (in the 100 mg/L range). Therefore, a study of photolysis to ascertain whether or not overall concentration can be reduced and bioavailability improved was completed. In many cases, hydrocarbon compounds that are resistant to biodegradation may be effectively treated by UV radiation (Goslich *et al.,* 1997; Christman and Collins, 1990; U.S.EPA, 1990). *It was hypothesized that photolysis will affect the concentration and/or composition of naphthenic acid mixtures and model compounds, rendering the overall solutions more bioavailable (defined here as being more easily degraded by micro-organisms).*

Several milestones were met with respect to increasing the scientific body of knowledge related to naphthenic acids in aquatic environments. First, the occurrence and fate of naphthenic acids in the aquatic environment was reviewed, a manuscript of which is in press in the *Journal of Environmental Science and Health Part A*. Second, a new and innovative analytical method was developed and the findings published in the *Journal of the AOAC International*. Third, the degradation of naphthenic acids was investigated with respect to photolysis in water from the Athabasca River north of Fort McMurray where Canada's largest oil sands deposit lies. The results of photodegradation kinetic studies were published in the *Journal of*

Environmental Science and Health Part A.

A novel method employing negative ion electrospray ionization mass spectrometry (LC/ESI/MS) was developed. The development of a novel analytical method for reliable and timely detection of aqueous naphthenic acids dealt with challenges related to the dependence of solubility on ambient water pH, low environmental concentrations, structural similarities between the target analytes and dissolved organic carbons (DOC) and the significant complexity of naphthenic acids which is dependent upon individual oil source and geological factors. In most cases, the application of the chosen LC/MS procedure was limited to natural waters with relatively low to moderate concentrations of DOC or with masses beyond the naphthenic acid mass envelope range. Despite these challenges, the analytical technique developed here was shown amenable for the detection of naphthenic acid compounds in aqueous solutions. In particular, the results indicated that the chosen LC/MS application is an excellent method for detection and separation of the three model naphthenic acids investigated.

The photochemical degradation of model naphthenic acid compounds and naphthenic acid mixtures was evaluated using the LC/MS/MS method developed. Included in this study were natural and simulated solar radiation, as well as highly energetic, near monochromatic UV_{254}-radiation. Of these, UV_{254}-radiation was deemed the most effective in all cases, although no radiation source investigated was truly efficient for degrading the overall concentrations of naphthenic acids in natural surface water.

The results show that while photolysis is not an efficient means for degrading the overall concentration of naphthenic acids in natural surface waters, it does effect compositional changes. The results of research using an Athabasca Oil Sands extract of naphthenic acids and two commercial naphthenic acids mixtures indicate that higher molecular weight compounds are degraded to lower molecular weight

compounds. That is, as the naphthenic acid mixtures were degraded, the composition shifted toward the C10 to C12 groups from the higher C groups.

Care must be taken in the interpretation of these results, however, since with the limitations of the current analytical chemistry techniques it can not be claimed with certainty that these lower molecular weight compounds are indeed naphthenic acids. It is possible that during photolysis, the higher molecular weight naphthenic acids are transformed to harmless dissolved organic carbons or other non-naphthenic acid compounds. Therefore, further development of analytical methods is required, as is the use of appropriate toxicological measures to determine conclusively whether or not photolysis of naphthenic acid mixtures results in higher or lower acute toxicity. The application of the aryl hydrocarbon receptor (Ah) assay for assessing acute toxicity changes in the naphthenic acid mixtures is not an especially meaningful one since it has not yet been established whether or not receptor binding capability indicates a trend toward higher or lower toxicity. That is, without knowing the mechanism of receptor binding, the enzymes involved and the potential metabolites formed with naphthenic acids, this assay does not currently provide meaningful toxicological data. In further assessments of the acute toxicity of naphthenic acids it is recommended that standardized tests such as those involving rainbow trout and *Daphnia magna* be completed.

Finally, research related to the process of naphthenic acids photolysis (i.e., direct vs. indirect) was also completed in natural surface water. Spectrophotometer absorbance scans at relevant concentrations were included to determine overall UV/vis absorbance between the wavelengths of 200 and 800 nm. These results indicate that the model naphthenic acid compounds and naphthenic acid mixtures do not readily absorb UV/vis radiation between 230 and 800 nm. Therefore, it is likely that any observed photolysis of the naphthenic acids investigated occurred via indirect means. To further prove this point, a study of hydroxyl radical formation and scavenging was completed. Hydroxyl radicals were produced in Athabasca River water at a predictable rate that was measured using the benzoic acid chemical

probe. Although hydroxyl radicals were not produced in great concentrations, the majority of naphthenic acid photodegradation observed was most likely due to indirect photolysis involving hydroxyl radicals.

In terms of overall reduction of naphthenic acid concentrations in the water column, photolysis was largely unsuccessful. Further, issues concerning the potential for increased toxicity as lower molecular weight compounds are formed as a result of the photolysis of higher molecular weight naphthenic acids must be considered in developing any remediation technology. Previous researchers have noted that the lower molecular weight compounds are responsible for the majority of toxicity attributed to naphthenic acids (Rogers *et al.,* 2002b; MacKinnon and Boerger). The results of the various photodegradation processes, coupled with their low affinity for adsorption to soils, reveal that naphthenic acids are likely to persist in the water column. The key finding from the results of naphthenic acid photolysis is that UV/vis radiation is capable of significantly changing the composition of mixtures in the aquatic ecosystem, but not necessarily reducing overall naphthenic acid concentrations. However, this changing of composition may not be beneficial due to the potential for increased toxicity of lower molecular weight naphthenic acids relative to higher molecular weight compounds.

7.1.2 Resin Acids

The vast majority of Canadian and European pulp and paper mills have incorporated secondary (microbial) treatment of process-affected waters at their operations (Lowell *et al.,* 2003). However, despite this intensified application of microbial methods, resin acids remain in effluents and are discharged to aquatic environments. Therefore, the application of pre-photolysis to remove both resin acid parent compounds and the related toxicity was investigated as a method for further reducing the concentrations of resin acids entering surface waters at pulp and paper mills. *It is hypothesized that the application of a degradation scheme involving photolysis treatment prior to biodegradation will reduce the concentration of pulp*

and paper-associated resin acids and eliminate the associated acute toxicity from natural surface water.

Like the naphthenic acids, the first step taken for investigating the fate and persistence of resin acids involved the development of a fast, rugged analytical method using LC/ESI/MS. The negative ion electrospray ionization LC/MS method was not capable of adequately resolving the three structural resin acid isomers (abietic, isopimaric and pimaric acids) under any of the conditions evaluated, including attempts for collision induced dissociation for MS/MS analyses. The four resin acids studied produced intense $[M - H]^-$ ions with no collision induced dissociation ions being observed. Despite this, LC/MS was deemed a sensitive quantification method well suited for trace analysis of resin acids in natural waters where isomeric speciation is not required. Although the isomers were not sufficiently separated for absolute identification in ambient samples, the method is a rapid and highly sensitive procedure for environmental screening.

Samples from photochemical and microbial degradation experiments were collected and analyzed to determine the degradation kinetics of each degradative process for the four resin acids investigated. Exposure of the four chosen resin acids was accomplished using two types of radiation sources including a UV_{254}-radiation immersion lamp and UV/vis lamps to simulate solar radiation. The results indicate that the four resin acids investigated are highly susceptible to UV/vis radiation, and especially UV_{254}-radiation. At UV254, all four resin acids were degraded rapidly, with half-life values between 18 and 100 minutes. Using the UV/vis sources previously identified, half-life values were slightly higher ranging between 40 and 200 minutes. In all instances, abietic acid was the least resistant to photolysis likely due to its conjugated double bonds that absorb UV/vis radiation more effectively than the other three resin acids investigated. Absorbance scans between 200 and 800 nm indicated that abietic acid is the most UV/vis absorbent resin acid of the four investigated and the most likely to be photochemically degraded via direct photolysis. While no experiments were conducted to determine the photochemical

process responsible for resin acid photolysis (i.e., direct versus indirect), it is likely that all four resin acids are degraded primarily via direct photolysis with indirect methods playing a minor role in overall concentration reduction.

Since the photochemical reaction responsible for resin acid degradation is highly efficient, as evident from the quantum yields, there is potential for industrial wastewater treatment applications. In industrial photolysis treatment systems, UV exposure of contaminated solutions is optimized and may be an effective and economical pre-treatment option prior to biodegradation. Pre-treatment photolysis is a practical means for decreasing toxicity and shock loading to microbial systems, while increasing the bioavailability of the target compounds. In the natural environment, it must be anticipated that photolysis in river water has minimal effect on both concentration and toxicity of resin acids since light penetration beyond the upper 5 mm is attenuated.

Microbial degradation studies completed with both untreated resin acid solutions and pre-photo-treated resin acid solutions indicated that the pre-treated solutions were much more readily biodegraded. As anticipated, the untreated resin acids were well degraded by microbial communities indigenous to the River Saale on which several pulp and paper mills are located. Biodegradation is a standard removal process used by pulp and paper mills for the removal of effluent pollutants in most countries. It has been shown previously that biodegradation methods are adequate for degrading resin acids to concentrations in the μg/L range. However, the rate of biodegradation and, therefore, the time and space requirements of biodegradation are prohibitive for reducing concentrations further. The rate of biodegradation observed in this study was significantly less than that observed for photolysis, but was significantly increased (by a factor of 2) through the use of pre-treatment photolysis. The level of microbial acclimation to the presence of the target resin acids was also a significant factor. Biodegradation of the resin acids occurred at nearly double the rate in acclimated versus non-acclimated biofilm reactors. Confocal laser microscopic analyses of the biovolume changes with respect to

bacteria, algae and cyanobacteria populations revealed changes in both the biofilm populations and in flow-induced striations from the initial observations due to exposure to the target resin acids in aqueous solution. These results also indicated that the microbial communities were capable of acclimating to the presence of the aqueous resin acid solutions through a second stage of microbial growth.

The acute toxicity of aqueous resin acid solutions was measured using Microtox® luminescence assays prior to and following photochemical, microbial and combination photochemical/microbial degradation. In all instances, acute toxicity decreased commensurate with the loss of parent resin acids. It was concluded that each degradation process generated metabolites of lower toxicity than the parents and/or removed both the parents and metabolites to levels below that detected using the Microtox® method. From these results it was determined, therefore, that as a pre-treatment to microbial systems at pulp and paper mills, implementation of photolysis may provide several benefits including reduced resin acid concentrations and potential toxicological shock to existing microbial treatment systems.

Photolysis of select resin acids was more efficient than the microbial removal process applied. However, biodegradation is likely to play the more significant role in both industrial treatment systems and the natural aquatic environment.

7.2 Assessment of Research Approach

Integrated approaches to the study of environmental fate and persistence of contaminants are required to address critical gaps in knowledge. This research project integrated an assessment of microbial and photochemical effects as well as environmental monitoring and modelling of resin acids. Although the use of combination treatments (such as settling and filtration prior to biodegradation) is not new, the application of combination photochemical-microbial degradation remediation technologies for removal of persistent organic compounds in natural

waters is novel.

The conditions under which laboratory experiments were conducted were designed to best represent both industrial situations and environmental conditions anticipated at locations where contamination related to naphthenic acids and resin acids is of concern. Several design configurations were applied for the evaluation of photolysis for degradation of each compound group – some of which were meant to represent aquatic environment conditions and others meant to simulate industrial technology applications. Those experiments conducted in natural and artificial sunlight (rooftop of the National Hydrology Research Centre in Saskatoon and phytotron growth chambers at the University of Saskatchewan, respectively) enabled observations for the evaluation of environmental degradation of the naphthenic acids and resin acids in aquatic systems. The design of laboratory photolysis experiments utilized thin layer solutions exposed to commercial UV lamps. This design mimics shallow, open-channel beds. Mixing within the photochemical reactors simulated both the turbulent flow of wastewater streams between processing areas and microbial treatment systems, as well as riverine shear.

The process used to evaluate the microbial degradation of the resin acids (rotating annular biofilm reactors, RARs) is widely accepted as the best laboratory simulation of natural river biofilms and shear due to riverine flow regimes. In the RARs it was possible to somewhat control and reproduce generation of thin biofilms under controlled fluid shear stress, independent of bulk residence time. These reactors do not prevent the formation of gradients, creating yet another similarity to that experienced in natural river courses. The RAR is considered as one of the very few available test systems with the ability to control a number of physical, chemical and biological parameters under laboratory and field conditions (Bryers, 2000; Griebe and Flemming, 2000; Madsen, 1998). Industrial application of biodegradation was not specifically examined, although the biodegradation kinetics noted in the acclimated RAR systems are anticipated to be of similar magnitude as expected in biodegradation treatment cells at industrial facilities. This similarity in kinetic

behaviour is anticipated since specialized microbial communities from affected regions are cultured in each instance and mixing, aeration and acclimation are vital to each process.

7.3 Engineering Significance

Clean fresh water is essential to life. However, most of the world's rivers have traditionally been used for diluting and transporting waste away from the source that has resulted in reduced biodiversity, harm to human health and pollution of coastal waters. The development, design and implementation of new remediation technologies and configurations for the removal of target pollutants from wastewater effluents requires knowledge of the chemical and physical parameters of those pollutants as well as their laboratory responses to the proposed technologies. The laboratory experiments conducted herein describe the potential for photochemical and microbial degradation of two hydrocarbon acid groups of differing complexity. While the resin acids investigated were susceptible to both photochemical and microbial degradation processes, the naphthenic acids were not. Good quality data is a pre-requisite for the design, development and operation of industrial wastewater treatment systems, for modelling the fate and persistence of organic compounds in the aquatic environment and for establishing limits for organics discharge to the aquatic environment.

Naphthenic acids photolysis was observed to be an inefficient degradation process under the conditions investigated. However, this process may still be viable for bitumen extraction facilities with the addition of photo-catalysts and the enhanced production of reactive oxygen species within both effluent treatment systems and tailings ponds. Due to the potential for increased acute toxicity as a result of the photochemical degradation process, sufficient safety factors must be incorporated into the design of a photochemical treatment system for the northern Alberta bitumen extraction industry. The optimization of photochemical degradation

processes requires a multi-disciplinary approach involving engineers, photochemists, analytical chemists, biologists and microbiologists, toxicologists, geologists, soil scientists and others.

For the design and operation of effluent treatment systems for the removal of resin acids at pulp and paper mills, further engineering considerations are required. At UV_{254}, removal of the majority of resin acids from wastewater streams at pulp and paper mills may be possible depending on the effluent quality (colour, interfering compounds and other chemical and physical parameters). The addition of pre-treatment photolysis at pulp and paper mills prior to biodegradation treatment cells is likely to have a significant impact on the efficiency of resin acids removal. In addition, it is likely that this added treatment will also enhance the removal of other pulp and paper mill effluent contaminants such as lignins, chlorinated resin acids and fatty acids prior to effluent discharge. However, since pulp and paper mills will continue to rely on microbial degradation as a finishing step, the addition of photo-catalysts may not be viable in all cases due to concerns related to the health of the microbial populations responsible for biodegradation subsequent to photolysis, as well as the additional expense involved with the addition of photo-catalysts. Pulp and paper mills tend to operate on a slim profit margin as compared to bitumen extraction facilities and, therefore, the use of photochemical treatment more may not be economically viable in these instances. However, if we consider that photochemical degradation systems are becoming more common, and the cost thereof is declining as the technology's applications and acceptance grow, it is advisable that pulp and paper mills consider the addition of pre-treatment photolysis to their existing biodegradation treatment systems. Again, a multi-disciplinary team is required to develop and implement an optimized combination photochemical-microbial degradation treatment process.

7.4 Conclusion and Recommendations

This research project was undertaken to further the knowledge base related to the environmental fate and persistence of naphthenic acids and resin acids in aquatic environments. Study sites were chosen to reflect regions of high risk relative to each industrially related compound group and a variety of microbial and photolytic degradation processes assessed in laboratory and field.

An evaluation of the photochemical degradation of various naphthenic acids in the Athabasca Oil Sands region was completed to determine whether or not this technology is viable. However, the results of this research indicated that photochemical degradation is not an efficient removal process and may, in fact, increase the acute toxicity of naphthenic acid mixtures. In studies using UV_{254}-radiation, higher molecular weight naphthenic acids appear to be more susceptible to UV/vis radiation, as evidenced by a reduction in relative concentrations of those compounds versus the lower molecular weight naphthenic acids. Further research related to the photochemical-microbial degradation kinetics, and photo-catalysts, for the reduction of overall naphthenic acid concentrations may provide more insight to the persistence and fate of these complex mixtures in aquatic environments. In addition, further research and development is essential in the areas of analytical chemistry and toxicology. Without adequate resolution of naphthenic acid compounds in mixtures, it is not possible to state with certainty that photolysis has a significant impact on either the overall naphthenic acid concentrations or the overall naphthenic acids composition. Also, with improved analytical methods, it may be possible to identify those naphthenic acids that are primarily responsible for the acute toxicity of naphthenic acid mixtures and thus target those compounds using appropriate degradation technology. Care must be taken for such application and extrapolation to ensure that refined naphthenic acids (such as those in the AOS extract) are used for these investigations since the chemistry of these differ from natural Athabasca Oil Sands naphthenic acids.

The second phase of this research project involved an evaluation of resin acid

concentrations in the River Saale, Germany and two applied methods of degradation for four selected resin acids. Effluents from some modernized pulp and paper mills with advanced effluent treatment may negatively impact aquatic environments and aquatic biota. This research identified two successful degradation processes – photochemical and microbial degradation – for efficiently reducing the concentrations and associated acute toxicity of the four resin acids investigated in River Saale water. An extension of this work to Canadian water sources downstream of representative pulp and paper mills may provide further insight to the extrapolation of these results from one water source to another. Inclusion of all eight identified resin acids and their chlorinated counterparts, along with the development of an analytical method capable of distinguishing each in aqueous samples would be prudent for future work. Further, an investigation of resin acid photodegradation in effluent samples versus receiving water would be valuable, along with an evaluation of the use of photo-catalysts for improved photochemical degradation reaction rates.

The evaluation of anticipated aquatic environmental persistence and fate of naphthenic acids and resin acids described herein addresses critical gaps in knowledge for aquatic environments downstream of industrial activities and in other affected regions. It is predictable that implementation of these findings to effluent streams and/or tailings water treatment may serve to reduce the level of these substances in the environment.

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