Toxicity of Anionic Surfactants in a Primary Effluent: Identification, Characterization and Removal

by

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Abstract

This research project was undertaken to identify, characterize and remove anionic surfactant induced toxicity from a primary effluent. The anionic surfactants, present in the primary effluent, were first separated into low, medium and high molecular weight fractions using solid phase extraction columns and gradient methanol elution. By separating the anionic surfactants, on the basis of molecular weight, the relative toxicities of each fraction could be determined. A colourimetric method was developed that was used to measure the concentration of anionic surfactants as methylene blue active substances (MBAS). This method required less time and less reagents than the conventional method outlined in Standard Methods (APHA et al., 1992). Finally, the Microtox™ system was used to measure the toxicity of the whole sample and of the different molecular weight fractions.

Using the methods developed for the present study, the anionic surfactant concentration and associated toxicity in a primary effluent were determined. These determinations were performed on two sampling events at the Lions Gate Primary Wastewater Treatment Plant. In each sampling event primary effluent was collected in the morning, afternoon and night. MBAS concentrations in the primary effluent increased throughout the day and ranged from 1.20 mg/L MBAS to 9.34 mg/L MBAS. The anionic surfactant concentrations were highest in the medium molecular weight fraction and lowest in the high molecular weight fraction. The toxicity of the primary effluent was shown to increase as the concentration of anionic surfactants increased. The toxicity associated with anionic surfactants was highest in the high molecular weight fraction. While the high molecular weight fraction was the most toxic fraction, it contained the lowest anionic surfactant concentration.

A screening study was conducted to provide a preliminary indication of the feasibility of using either partitioning to abiotic bio-solids, biological treatment, alum coagulation/flocculation with gravity settling, ozonation, or air flotation to remove anionic surfactants and the associated toxicity from a primary effluent. Partitioning to abiotic bio-solids and biological treatment produced anionic surfactant removals of 64% and 96%, respectively. Alum coagulation/flocculation with gravity settling removed 46% of the anionic surfactants, while
ozone removed 95% of the anionic surfactants. Air flotation removed 77% of the anionic surfactants. Toxicity studies were conducted using the samples treated with ozone and air flotation only. The toxicity studies revealed that ozonation slightly increased the toxicity of the treated whole sample, possibly due to the formation of by-products from the oxidation process. On the other hand, air flotation reduced the toxicity of the whole sample significantly. In addition, air flotation removed all of the measurable toxicity, using the methods described in the present study, from the elution fraction corresponding to the high molecular weight fraction. These preliminary results indicate that air flotation may be an effective interim means of reducing both anionic surfactants and anionic surfactant induced toxicity from the Lions Gate Wastewater Treatment Plant primary effluent.
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Chapter 1

Introduction

The Lions Gate Wastewater Treatment Plant (WWTP) provides primary treatment to wastewater generated by approximately 160,000 people (Bailey and Elphick, 2001). Located in North Vancouver, the plant discharges effluent to the outer harbour of Burrard Inlet at First Narrows. Separate storm water and wastewater collection systems are in place. In 2001, the average daily flow to the Lions Gate WWTP was 92 MLD, and the dry-weather flow to the plant was 86 MLD (GVRD, 2001).

Monthly trout bioassays are conducted to monitor the acute toxicity of the primary effluent from the Lions Gate WWTP. In 2001, four of the bioassays were indicative of toxicity events (GVRD, 2001). Similar results were obtained in 1995, 1996 and 1999 (GVRD, 2001). A toxicity event was considered to have occurred when the LC$_{50}$ was below 100% (V/V). The LC$_{50}$ is that concentration of the effluent (percent by volume) resulting in 50% mortality of the species tested. It follows that the lower the percent concentration required for 50% mortality, the more toxic is the sample. The results of the bioassays prompted studies to be undertaken in 2000 and 2002 to further investigate the causes of toxicity. The 2000 study revealed that anionic surfactants, contained in the primary effluent, were the predominant cause of the toxicity to rainbow trout that were used in the bioassays (Bailey and Elphick, 2001). In a follow up study conducted in 2002, toxicity tests and toxicity identification and evaluation (TIE) studies were performed using Lions Gate primary effluent. Again, anionic surfactants were identified as the predominant cause of toxicity to rainbow trout that were used in the bioassays (EVS, 2003). In both the 2000 and 2002 studies, ammonia was found to contribute to the toxicity contained in the primary effluent from the Lions Gate WWTP. However, ammonia contributed significantly less to the toxicity than did the anionic surfactants.

Long-range plans indicate that secondary treatment will be implemented at the Lions Gate WWTP, and it is known that secondary treatment effectively removes the toxicity induced by anionic surfactants. However, an interim treatment method is sought that can be configured
within the existing primary plant until such time when secondary treatment can be provided. To address this need, the present study was undertaken.

The objectives of the present study were three-fold. The first objective was to develop analytical methods that could be used to quantify the concentration of anionic surfactants in the primary effluent, to fractionate the anionic surfactants on the basis of molecular weight, and to measure the toxicity of the whole and discrete molecular weight fractions. The second objective was to characterize the primary effluent from the Lions Gate WWTP. The parameters characterized included both the concentration of anionic surfactant and the toxicity contained in the whole samples as well as in the discreet molecular weight fractions. The third objective was to screen potential treatment methods to determine the effectiveness of the method at removing both anionic surfactants and toxicity. The treatability study was designed to screen the treatment methods, and it was a small portion of the overall work completed as part of the present study.

To achieve the stated objectives four main tasks were performed.

1. The literature pertaining to anionic surfactants was reviewed to first identify analytical methods that could be used to fractionate anionic surfactants on the basis of molecular weight and to measure the concentration of anionic surfactants. Secondly, the toxicity of anionic surfactants to fish was determined. Finally, potential treatment methods were identified.

2. Analytical procedures were developed and validated. The first analytical procedure was used to fractionate anionic surfactants on the basis of molecular weight. The second procedure was used to determine the concentration of anionic surfactants in each molecular weight fraction and in the whole sample. Finally the third analytical procedure was used to measure the toxicity of each molecular weight fraction and that of the whole sample.

3. Lions Gate primary effluent was characterized in terms of its anionic surfactant concentration and its associated toxicity.

4. A preliminary treatability study was conducted using four treatment methods for the removal of anionic surfactants and induced toxicity. The methods included biotreatment, alum coagulation/flocculation with gravity settling, ozonation, and air flotation.
This thesis is organized into eight sections. Chapter 2 consists of a literature review of materials relevant to the present study. Chapter 3 describes the experimental methods and procedures used in the present study. Chapter 4 contains a discussion of the development of the new analytical methods used in the present study. Chapter 5 consists of the results from the characterization of the primary effluent. Chapter 6 includes the results of the treatability study. Finally, chapter 7 offers conclusions arising from this research project, and chapter 8 provides recommendations for future research.
Chapter 2

Literature Review

2.1 Summary of Literature Review

Detergents are commonly used in residential, commercial and industrial cleaning products. Anionic surfactants are the main active components in household detergent formulations. Linear alkylbenzenesulfonate (LAS) is the most common type of anionic surfactant used in detergent formulations. LAS is highly biodegradable and is hydrophobic. LAS has previously been shown to be toxic to fish, and Microtox™ has previously been used as a surrogate for the standardized rainbow trout toxicity bioassay. Anionic surfactants can be measured as methylene blue active substances (MBAS) and can be partitioned into discrete molecular weight fractions following passage through a solid phase extraction column. Previous studies have identified anionic surfactants as a cause of toxicity in the effluent from the Lions Gate WWTP, and treatment studies were undertaken to evaluate methods of removing anionic surfactants from the Lions Gate effluent.

2.2 Anionic Surfactants

Linear alkylbenzenesulfonates (LAS) are synthetic anionic surfactants. A surface active agent, or surfactant for short, functions to alter the surface tension between two or more substances. LAS has a strongly hydrophobic linear carbon chain to which a hydrophilic, anionic group is attached. In the presence of anionic surfactants, the surface tension between two immiscible substances is lowered, and foams, bubbles, dispersions or emulsions are formed. For example, when anionic surfactants are used to wash clothes, the hydrophobic chains attach to dirt or grease while the hydrophilic groups remain in water. In this way, dirt and grease can be removed from the surface being cleaned, as the dirt and grease is suspended in and removed with the water. Figure 1 shows the structure of a LAS molecule.
Chapter 2. Literature Review

\[
\text{CH}_3-(\text{CH}_2)_n-\text{CH}-(\text{CH}_2)_m-\text{CH}_3 \quad \text{SO}_3\text{Na}
\]

where \( n + m = 7 \) to 11

Figure 1: Structure of linear alkylbenzenesulfonate.
From [Matthijs, E., and De Henau, H., 1987]

Approximately 2.5 million tonnes of LAS were consumed worldwide in 1995 (Ferrer et al., 1996). LAS was introduced in the early 1960's to replace the recalcitrant branched alkylbenzenesulfonates (ABS). This change brought about a reduction in foaming and persistence in the receiving environment caused by ABS. LAS solutions are typically discharged to wastewater collection systems and eventually to wastewater treatment facilities where influent concentrations of LAS typically range from 1-7 mg LAS/L (Rapaport et al., 1987 appearing in WHO, 1996).

The hydrophobic chain of LAS can be of varied length and structure. Typical commercial preparations of LAS have linear chains of 10 to 14 carbons in length (Painter and Zabel, 1989, appearing in WHO, 1996). The hydrophobicity of a LAS molecule increases as the length of the carbon chain increases. The structure of a LAS molecule differs by the placement of the phenyl group on the carbon chain. LAS molecules that have the same chain length, but have different placements of the phenyl group, are referred to as isomers. LAS molecules that have different chain lengths are referred to as homologues.

### 2.3 Toxicity of Anionic Surfactants

The following discussion on aquatic toxicity, induced by anionic surfactants, deals only with LAS, since LAS is the most prevalent surfactant in detergents and cleaning products for both industrial and residential use (WHO, 1996).
The aquatic toxicity of LAS depends on many factors, but generalities have been established which qualitatively describe trends in aquatic toxicity. In general, aquatic species are sensitive to surfactants and will avoid areas where surfactants are present. The toxicity of LAS depends on its carbon chain length (homologues). LAS molecules with longer chain lengths are typically more toxic than those with shorter chain lengths. When freshwater fish were exposed to LAS having carbon chain lengths ranging from 8 to 15 carbons, the LC$_{50}$ ranged from 125 to 0.1 mg/L LAS, respectively (WHO, 1996). The acute LC$_{50}$ (dose of LAS that kills 50% of the test organisms) is used to describe the toxicity of a substance. In another study, a two-fold decrease in fish and Daphnia toxicity resulted when the carbon chain length of LAS was decreased from 12 to 11 carbons. In the case of Rainbow Trout (Onchorynchus mykiss), the acute 96-hour LC$_{50}$ ranged from 0.36 mg/L LAS, for LAS with chain lengths of 12.6 carbons (Brown et al., 1978 appearing in WHO, 1996) to 2.1, 3.4, and 4.7 mg/L LAS, for LAS with chain lengths of 11.6 carbons (Wakabayshi et al., 1986; Wakabayshi et al., 1984 all appearing in WHO, 1996). On the other hand, saltwater organisms are generally more sensitive to LAS than are freshwater organisms. Fortunately, LAS concentrations are generally lower in marine environments, due to dilution (WHO, 1996). When marine fish were exposed to LAS that had carbon chain lengths of 11.7 carbons, the acute LC$_{50}$ ranged from 0.05 to 7 mg/L LAS (WHO, 1996). In addition, the toxicities of other substances can be increased when combined with LAS. Enhanced toxicities to aquatic organisms were observed in studies where LAS was combined with fuel oil, cadmium, copper or zinc (Hokanson and Smith, 1971; Pärt et al., 1985; Swedmark and Granmo, 1981; Tsai and McKee, 1978; all reported in WHO, 1996). LAS was shown to increase the transfer of cadmium or fuel oil across the gill membranes of fish resulting in increased toxicities.

The toxicity of LAS to aquatic organisms varies with species and is dependent upon the carbon chain length of LAS. Toxicity has been shown to increase as the attachment of the phenyl group is moved from the center of the carbon chain to the end, but the position of the phenyl group is less significant to toxicity than is increased carbon chain lengths (Swisher, 1987). Long-term and acute toxicity screening can be applied to test the effects of LAS on aquatic organisms. In the case of long-term tests, it is important to note that solids and/or food particles can adsorb LAS thereby lowering the amount of LAS in solution.
Significant physiological responses and impairment of normal gill physiology can lead to mortality when fish are exposed to various concentrations of LAS (WHO, 1996; Randall et al., 1996; Swisher, 1987). Decreased viability of the gills, separation of the gill structures, decreased oxygen transfers, and increased ventilation rates are all reported to occur when fish are exposed to LAS with doses ranging from 0.39 mg/L LAS to 36 mg/L LAS (Maki, 1979 as reported by WHO, 1996; Pärt et al., 1985 as reported by WHO, 1996; Zaccone et al., 1985).

### 2.3.1 Toxicity of Biodegradation By-Products

Biodegradation is an effective means of removing anionic surfactants from wastewaters as discussed in Section 2.4. Biodegradation of LAS using mixed bacterial cultures changes the three distinct structures making up a molecule of LAS: the chain, the ring and the sulfonate linkage (Swisher, 1987). As a result, many intermediate degradation byproducts are formed, as the parent LAS molecule undergoes biodegradation. Intermediate biodegradation by-products have been found to contain a carboxyl group at the end of the altered alkyl chain. This carboxylated by-product was found to be three to four times less toxic than the parent LAS compound (Kolbener et al., 1995; Swisher et al., 1978 as reported by WHO, 1996).

### 2.4 Methods for the Removal of Anionic Surfactants Contained in Wastewater

#### 2.4.1 Bio-treatment

Wastewater treatment plants provide varying degrees of removal of LAS during both primary and secondary treatments. Interestingly, it is reported that with favorable aerobic conditions up to 50% of the LAS present in wastewater can be biodegraded in wastewater collection networks before entering WWTPs (Moreno et al., 1990 appearing in WHO, 1996). A significant fraction (15-30%) of the LAS entering wastewater treatment plants can be removed via adsorption to primary sewage solids (Fauser et al., 2003; Giger et al., 1989 and Prats et al., 1993 both appearing in WHO, 1996; Swisher, 1987). During secondary treatment, the main mechanism for LAS removal is biodegradation where removals of 80-85% are typically reported (Fauser et al., 2003; Kolbener et al., 1995). As a result, complete removal of the parent LAS
molecule is possible in an aerobic biological treatment system (Kolbener et al., 1995). However, under anaerobic conditions LAS does not undergo biodegradation (Holt and Bernstein, 1992).

As discussed in Section 2.3, the toxicity of LAS tends to increase as the carbon chain length of the LAS molecule increases. Fortunately, as the carbon chain length increases, so too does the rate at which the LAS molecule is biodegraded (Divo, 1976 as reported by Swisher, 1987). Therefore, during biodegradation the more toxic, higher molecular weight fractions are removed from solution before the less toxic, lower molecular weight fractions. In addition, the more toxic, higher molecular weight fractions are more readily adsorbed to solids due to their greater hydrophobic characteristics.

2.4.2 Physical and Chemical Treatment

Alum coagulation/flocculation with gravity settling was considered as a treatment method for the removal of anionic surfactants from the effluent of the Lions Gate WWTP. The findings of a previous study in which alum coagulation/flocculation with gravity settling was used to treat the primary effluent from the Lions Gate WWTP are presented in Section 2.8. The effects of LAS on alum coagulation/flocculation of a synthetic wastewater has also been studied. Fettig and Ratnaweera (1993) reported that LAS had no significant affect on the coagulant dose needed to achieve maximum particle or turbidity removals (Fettig and Ratnaweera, 1993).

Beltrán et al., (2000) investigated the kinetics of LAS decomposition by ozonation. It was found that the pH and the amount of organic material initially present in the LAS solution influenced the removal of LAS (Beltrán et al., 2000). Beltrán et al., (2000) observed higher oxidation rates at higher pH's, and suggests that hydroxyl radicals were more abundant at higher pH's. LAS tends to react faster with hydroxyl radicals than it does with molecular ozone. At high organic concentrations, the oxidation rate of LAS tends to decrease. The oxidation of inorganic and organic compounds, other than LAS, present in wastewaters likely consumes the radicals thus reducing the amount of hydroxyl radicals that are available to oxidize the LAS molecules.
Chapter 2. Literature Review

2.5 Measurement of Anionic Surfactants

Anionic surfactants can be measured as methylene blue active substances (MBAS). In the MBAS test, aqueous anionic surfactants combine with aqueous methylene blue to form an ion pair which is then extracted into an immiscible chloroform liquid. The ion pair remains in the chloroform. Subsequently, an aqueous back-wash is used to remove excess methylene blue and positive interferences from the chloroform. Positive interferences, with poor extractability, are effectively removed by the aqueous backwash. Positive interferences include organic sulfonates, sulfates, carboxylates, phenols, inorganic cyanates, nitrates and chlorides. Negative interferences include cationic surfactants, cationic materials and particulate matter. Following back-wash, the volume of chloroform is made up to a standardized volume. Finally, the concentration of MBAS is determined spectrophotometrically at a wavelength of 652 nm. The conventional approach used to measure MBAS is presented in Standard Methods 5540 C (APHA et al., 1992). To overcome some of the limitations of Standard Method 5540 C, Chitikela, et al., (1995) developed another method (Chitikela, et al., 1995) (see Section 3.1.5).

2.5.1 Recovery of MBAS

Many researchers have presented methods to improve the recovery of MBAS during sample collection and analysis. Marcomini et al., (1987) added sodium chloride to samples before filtration to improve the recovery of LAS following filtration. Subsequently, the filter-cake was rinsed with methanol which was added to the filtrate (Marcomini et al., 1987). To preserve samples containing LAS, Matthijs and De Henau (1987) added formaldehyde to sample bottles which were subsequently filled with wastewater and then stored at 4°C. Both the formaldehyde and low temperature acted to slow or stop the biodegradation of LAS (Matthijs and De Henau, 1987).

2.6 Fractionation of Anionic Surfactants

Solid phase extraction (SPE) can be used to fractionate anionic surfactants on the basis of hydrophobicity. Hydrophobic surfactant molecules adsorb to the SPE column packing material.
Highly hydrophobic molecules, which have long carbon chains and higher molecular weights, are strongly adsorbed while the less hydrophobic molecules are more weakly adsorbed to the SPE column packing material. Solvents of decreasing polarity can then be used to elute surfactant molecules from the packing material. As the polarity of the solvents are decreased, the dissolving power increases, and the more hydrophobic surfactant molecules can be eluted. In this way, surfactants can be separated on the basis of hydrophobicity and therefore molecular weight.

A study was performed by EVS Environmental Consultants (2003) in which SPE columns were used to separate the anionic surfactants, contained in the Lions Gate primary effluent, into discrete fractions on the basis of hydrophobicity. Solutions containing methanol in water were used as solvents to elute the adsorbed anionic surfactants from the SPE packing material (EVS, 2003). A 65% solution of methanol in water was used to elute the less hydrophobic material. A 90% solution of methanol in water was used to elute the more hydrophobic material having longer carbon chains.

According to the directions supplied by the manufacturer of the SPE columns used in the present study, a wash step helps to eliminate interfering substances that are either weakly adsorbed to the packing material or are entrained in the packing material (Supelco, 1997).

2.6.1 Preservation of MBAS on SPE Columns

Molecules of LAS are highly biodegradable, particularly those having longer carbon chains. Therefore, proper sample storage is of the utmost importance to preserve the form and concentration of LAS. Petrović and Barceló (2000) observed a significant decrease in MBAS concentrations for wastewater samples when stored for 30 days at 4°C; these losses were greatest for the LAS molecules having longer carbon chains (Petrović and Barceló, 2000). On the other hand, minimal losses of LAS molecules were observed after SPE columns, containing LAS extracted from wastewater, were stored for 7 days at room temperature. No losses were observed when similar columns were stored at -20°C for 30 days.
2.7 Measurement of Toxicity

Monthly rainbow trout toxicity studies are used to monitor the toxicity of the Lions Gate effluent (GVRD, 2001). However, a number of other organisms can be used to evaluate the toxicity of anionic surfactants. The Microtox™ system uses *Vibrio fischeri* to evaluate the toxicity of liquid samples. The bioluminescence of *Vibrio fischeri* is indicative of their metabolic activity and provides an indication of the relative toxicity of a test sample. By comparing the bioluminescence of the test samples to that of a control, the IC$_{20}$ or IC$_{50}$ can be found. The IC$_{20}$ and IC$_{50}$ represent the concentrations of solution (% V/V) or of analyte (mg/L) that causes a 20% and 50% reduction in bioluminescence, respectively.

Two acute toxicity test protocols can be used with the Microtox™ system (Microbics Corp, 1992). The first type is the detailed basic test protocol in which the highest test sample concentration is 45% by volume. The second type is the 100% test protocol in which the highest test sample concentration is 90% by volume. The detailed basic test protocol provides more reliable results than does the 100% test protocol.

The Microtox™ system has been previously used to determine the toxicity of LAS. The IC$_{50}$ of LAS, having a chain length of 12 carbons, was found to be 14.29 mg/L LAS (Gutierrez *et al.*, 2002). Distributions of LAS carbon chain lengths and molecular weights and associated toxicities are presented in Tables 1 and 2.

<table>
<thead>
<tr>
<th>Product</th>
<th>Molecular Weight</th>
<th>C$_9$</th>
<th>C$_{10}$</th>
<th>C$_{11}$</th>
<th>C$_{12}$</th>
<th>C$_{13}$</th>
<th>C$_{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS-255</td>
<td>255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LAS-242</td>
<td>242</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS-240</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS-236</td>
<td>236</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAS-232</td>
<td>232</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d. not detected

[adapted from Vives-Rego *et al.*, 1991]
Table 2: Toxicity of some LAS formulations measured using the Microtox™ system.

<table>
<thead>
<tr>
<th>Product</th>
<th>IC$_{50}$ in mg/L</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
</tr>
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<tbody>
<tr>
<td>LAS-255</td>
<td>24.2</td>
<td>10.4</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>LAS-242</td>
<td>19.3</td>
<td>12.6</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>LAS-240</td>
<td>13.4</td>
<td>10.5</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>LAS-236</td>
<td>25.1</td>
<td>20.4</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>LAS-232</td>
<td>25.6</td>
<td>20.2</td>
<td>20.2</td>
<td></td>
</tr>
</tbody>
</table>

[adapted from Vives-Rego et al., 1991]

The range of 30-minute IC$_{50}$ values obtained above is 8.1 to 20.4 mg/L LAS, depending on the carbon chain length (Vives-Rego et al., 1991). As the carbon chain length and molecular weight increase, so too does the toxicity (see section 2.3).

2.7.1 Limitations in the Measurement of Toxicity Using Microtox™

The presence of methanol and specific ions, ions originally present in the sample or added during sample preparation, can both impact the measurement of toxicity using the Microtox™ system.

Ions, such as sodium, that are either intentionally added during sample preparation (see Section 2.5.1) or that are originally present in the sample, may affect the bioluminescence of Vibrio fischeri (Carlson-Ekwall and Morrison, 1995 as reported in Dizer et al., 2002). For example, the bioluminescence of Vibrio fischeri, measured using the Microtox™ system, increased in the presence of Na$^+$, K$^+$ or Mg$^+$ ions, and decreased in the presence of heavy metals (Bitton, 1983; Carlson-Ekwall and Morrison, 1995; Watanabe et al., 1991; all reported in Dizer et al., 2002). In addition, chloride ions may react with dissolved metal ions reducing both the inhibitory effect and the perceived toxicity of the metal ions.

Substances present in the matrix may induce or inhibit bioluminescence of the test organism (Dizer et al., 2002); therefore, appropriate sample preparation is of the utmost importance. Methanol added during sample preparation (see Section 3.1.4 F and G) may affect the bioluminescence of Vibrio fischeri.
Chapter 2. Literature Review

The results of short-term, acute toxicity tests are to be interpreted with care. Firstly, the Microtox™ test uses a single species of bacteria. Secondly, short-term, acute tests are unable to measure the effect of the toxicant over a longer period of time, such as the period of time required to degrade the toxicant in the aquatic environment (Vives-Rego et al., 1991). Despite these drawbacks, the Microtox™ test offers a number of advantages when compared to other test organisms. The Microtox™ test system is relatively inexpensive when compared to other toxicity tests. In addition, it rapidly produces results, and it is relatively easy to use.

2.8 Previous Characterization and Treatment Studies at Lions Gate

In 2000, Bailey and Elphick, (2001) undertook a study of four of the WWTPs in the GVRD, including the Lions Gate WWTP, to establish if the effluent from these WWTPs was toxic to the aquatic life in the receiving water bodies. Effluent samples were collected from the Lions Gate WWTP from August to November, 2000 during dry-weather conditions. Samples of the effluent were screened for acute toxicity using juvenile rainbow trout. Samples that caused 50% or greater mortality in the juvenile rainbow trout were subjected to further testing. Samples that were subjected to further testing underwent a Toxicity Identification and Evaluation (TIE) program. In the TIE program, the samples underwent physical and chemical manipulations to first determine the general properties of the toxicant and second to specifically identify the toxicant. The TIE study suggested that anionic surfactants in the effluent of the Lions Gate WWTP were the predominant cause of toxicity to rainbow trout. Ammonia was found to contribute to toxicity. However, ammonia contributed less to the toxicity than did anionic surfactants (Bailey and Elphick, 2001).

In 2002, a follow-up study was initiated to further investigate the sources of toxicity present in the effluent of the Lions Gate WWTP. This follow-up study was undertaken to investigate the impact of chemical substances of interest on the toxicity to three marine species. Samples of Lions Gate primary effluent were collected between April and November 2002 during dry-weather conditions. Half of the samples collected were chlorinated, as is normal plant practice during the summer season. The marine species that were considered in the study were the topsmelt (Atherinops affinis), blue mussel (Mytilus galloprovincialis) and giant kelp
Although the study by Bailey and Elphick (2001) indicated that anionic surfactants were responsible for the toxicity of the effluent from the Lions Gate WWTP, the contribution to toxicity from other possible contaminants/parameters was considered during the follow-up study. The contaminants/parameters that were investigated included metals, mercury, chlorine, weak acid dissociable (WAD) cyanide, sulphides, ammonia, phthalate esters, polychlorinated biphenyls (PCB), 4-nonylphenol, dichlorodiphenyltrichloroethane (p,p'-DDT), pH, dissolved oxygen, suspended solids, chemical oxygen demand (COD) and temperature. The follow-up study confirmed that anionic surfactants significantly contributed to the toxicity while the contributions from ammonia and the other parameters were of a much lesser extent (EVS, 2003).

In the follow-up study conducted in 2002 by EVS Environmental Consultants, the anionic organic toxicant was retained on hydrophobic SPE columns. Gradient methanol elution was used to separate the anionic surfactants into three separate molecular weight fractions. The anionic organic toxicant was predominantly recovered in the high molecular weight fractions. Intermediate molecular weight fractions were determined to be non-toxic, although they contained a high concentration of anionic surfactants measured as MBAS. It was shown that the high molecular weight fractions had a lower MBAS concentration but a higher toxicity. It was suggested that the anionic surfactants in the high molecular weight fractions are less polar and more hydrophobic, and, therefore, more likely to be toxic (EVS, 2003).

A bench-scale treatability study of the primary effluent from the Lions Gate WWTP was undertaken from late August until early November 2002. The goal of the treatability study was to determine the feasibility of removing MBAS from the primary effluent using chemical precipitation. Lime and calcium chloride were initially evaluated to determine MBAS removal by precipitation from both the influent and effluent at the Lions Gate WWTP. High dosages of lime and calcium chloride were necessary to remove MBAS. In addition, adsorption and or coagulation of surfactant molecules with suspended solids, and not chemical precipitation, was found to be the mechanism responsible for the MBAS removals. For these two reasons, lime and calcium chloride were abandoned in favor of examining treatment using alum and ferric chloride coagulation/flocculation with gravity settling. Jar testing revealed that the optimal dose of alum
(as Al), or ferric chloride (as Fe) for the removal of MBAS using coagulation/flocculation with gravity settling was 40 mg/L. Using alum, a 50% reduction in MBAS concentrations was achieved in both the influent and effluent. 96-hour trout toxicities were reduced from an average 96-hour LC$_{50}$ of 45% (V/V) to 91% (V/V) and 77% (V/V) following treatments using alum and ferric chloride, respectively (CH2MHILL, 2002).
Chapter 3

Analytical Procedures and Experimental Setups

Analytical procedures were developed and four treatment methods were used to fulfill the objectives of the present study. First, analytical procedures were developed to preserve and fractionate the anionic surfactants present in a primary effluent, to measure the concentration of the anionic surfactants and to measure the anionic surfactant induced toxicity. These analytical procedures are presented in Sections 3.1.1 to 3.1.8. In the second part of the present study, four treatment methods were used to remove anionic surfactant induced toxicity from a primary effluent. The treatment methods that were used included bio-treatment, alum coagulation/flocculation with gravity settling, ozonation, and air flotation. The experimental set-ups used during the treatability study are presented in Sections 3.2.I to 3.2.IV.

3.1 Analytical Procedures

The analytical procedures used in the present study are detailed in the following sections: (Section 3.1.1 - sample collection), (Section 3.1.2 - filtration), (Section 3.1.3 - sample storage), (Section 3.1.4 - solid phase extraction), (Section 3.1.5 - MBAS), (Section 3.1.6 - Microtox™). Figure 2 illustrates the order in which the analytical procedures were performed. It also provides the names of samples and procedures to which reference is made in this thesis.
Figure 2: Flowchart of analytical procedures.
3.1.1 Sample Collection

A) Collection from WWTP

Primary effluent samples were collected from the Lions Gate WWTP and from the UBC Pilot Plant. Waste activated sludge was also collected from the UBC Pilot Plant. Primary effluent collected from the UBC Pilot Plant was used for method development while the waste activated sludge collected from the UBC Pilot Plant was used in the treatability study.

Samples of Lions Gate primary effluent were collected downstream of the dechlorination mixers. A steel bucket was used to draw samples from the final effluent weir. The bucket was rinsed three times with primary effluent before samples were collected.

Samples of UBC Pilot Plant primary effluent were collected from a sampling port located on the primary clarifier. The sampling port and associated pipes were flushed with primary effluent prior to sampling. A steel bucket was used to collect samples. The bucket was rinsed three times with primary effluent before samples were collected.

As discussed in Section 3.2.1, UBC Pilot Plant waste activated sludge was used in the treatability study (see Chapter 6). The sludge samples were collected from a sampling port located on the membrane activated sludge tank. The sampling port and associated pipes were first flushed with waste activated sludge. A graduated plastic container was used to collect samples. The container was rinsed three times with waste activated sludge before the sample was collected.

B) Collection Following Treatment

Following treatment as outlined in Chapter 6, samples were immediately collected and preserved in accordance with the procedural steps illustrated in Figure 2. Further details regarding sample collection following treatment are provided in Sections 3.2.1 through to 3.2.4 and in Section 3.2.7.
3.1.2 Filtration

All samples were filtered prior to storage or SPE column loading as illustrated in Figure 2. Samples were filtered using VWR #413 (VWR International, West Chester, PA.) qualitative filter papers. The filtrates were used in MBAS and toxicity tests. The filter cake was discarded, as only soluble anionic surfactants were of interest in the present study. The filter papers were prewashed to remove any adventitious anionic surfactants (see Section 4.1.5.1.A). The washing procedure consisted of soaking the filter papers for approximately 10 minutes in a beaker containing de-ionized water, obtained from a Milli-Q water system (Molsheim, France). The de-ionized water was then drained and replaced with fresh de-ionized water. The beaker and filter papers were then sonicated for 10 minutes. The de-ionized water was once again drained and replaced with fresh de-ionized water a total of three times. The wet filter papers were then wrapped in aluminum foil until used.

3.1.3 Sample Storage

A) Whole Sample Storage

Filtered whole samples were stored in brown glass jars with Teflon lined lids. In all cases, the brown glass jars were rinsed twice with sample. When transported, the glass jars were stored along with ice packs in coolers. At the lab, glass jars containing sample were stored at 4 °C until needed. In every instance, attempts were made to minimize the storage time particularly for unfiltered whole samples.

B) SPE Sample Storage

SPE columns have been previously shown to preserve LAS molecules (see Section 2.6.1). For this reason, SPE columns were used to store anionic surfactants during the characterization and treatability studies presented in Chapters 5 and 6. In the case of the characterization study, SPE columns were loaded on-site at the Lions Gate WWTP in an attempt to minimize the biodegradation of analyte. The SPE columns were activated, loaded and washed.
according to the procedures detailed in section 3.1.4. The loaded columns were wrapped in aluminum foil, transported in a cooler containing ice packs and stored at 4 °C until needed.

C) Waste Activated Sludge Storage

Waste activated sludge samples collected from the UBC Pilot Plant were stored as described in Section A above, for whole sample storage.

3.1.4 Solid Phase Extraction

Solid phase extraction consists of five steps: activation, analyte loading, column washing, analyte elution, and reconstitution.

A) Solid Phase Extraction Apparatus

A standard 10-port vacuum box (J.T. Baker company, Phillipsburg, N.J.) solid phase extraction system was used. Figure 3 illustrates the 10-port vacuum box solid phase extraction system with all of the supporting apparatus used in the present study. Stopcocks were used to control the flow through each column. 75 mL reservoirs were used to increase the volumetric capacity of each SPE column. Standard laboratory 4 mL test tubes were used to collect the eluants from the SPE column. The 4 mL test tubes are subsequently referred to as elution vials. A gas flow meter was used to monitor and control the flow induced by the vacuum that was applied to the 10-port vacuum box (see Section C below). Supelclean® LC-18 SPE columns (Supelco, Bellefonte Pennsylvania) were used in the present study. Each column had 0.5 g of reversed phase packing material and a volume of 6 mL. The reversed phase packing material retains non-polar to moderately polar analytes from aqueous solutions. HPLC grade methanol was obtained from Fisher Scientific (Hampton, NH).
B) Reagent Preparation

Four methanol solutions were prepared for use in the SPE procedures. The following solutions of methanol in de-ionized water, obtained from a Milli-Q water system (Molsheim, France), were prepared: 40%, 65%, 75% and 90% (V/V). A solution containing 40% methanol in de-ionized water (V/V) was used to wash the loaded column. The three other solutions (65%, 75% and 90% methanol in water) were used to sequentially extract the analyte from the column. The purpose and rationale for selecting these solutions is discussed in Sections 2.6 and 4.1.5.4.
C) Activation

The SPE column packing material was activated before a sample was loaded. Activation leaves a thin film of water-miscible solvent on the packing material which promotes better contact between the aqueous sample matrix and the hydrophobic solid phase packing material thereby increasing the recovery of analyte (Supelco, 1997). A SPE column was positioned in the 10-port vacuum box, as shown in Figure 3. A vacuum was applied to the 10-port vacuum box and the resulting gas flow rate was adjusted to provide a liquid flow rate through the SPE column of 5 mL per minute or less. A gas flow meter was used to monitor and control the liquid flow rate. (The gas flow meter settings were determined in previous trials, and the settings are dependent upon the number of SPE columns being used in the 10-port vacuum box at one time.) To this SPE column, 1 mL of 100% methanol was added. Once half of the methanol added was drawn through the packing material, the vacuum was stopped for approximately 1 minute by closing the stopcock. This enabled the methanol to contact all of the packing material for a given amount of time. The vacuum was then resumed and the remaining methanol was drawn through the packing material. Another 1 mL aliquot of 100% methanol was then added to the column and drawn through the packing material without stopping the vacuum. Following methanol addition, 1 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was added to the column. Once half of the de-ionized water was drawn through the packing material, the vacuum was once again stopped for approximately 1 minute by closing the stopcock. This enabled the de-ionized water to contact all of the packing material for a given amount of time. The vacuum was resumed and the remaining water was drawn through the packing material. Another 1 mL aliquot of de-ionized water was then added to the column. When 1 to 2 mm of de-ionized water remained on top of the packing material, the vacuum was stopped by closing the stopcock. This thin layer of de-ionized water was left on top of the SPE packing material to prevent the SPE packing material from drying. If the packing material dried before the sample was added, it was necessary to repeat the activation procedure, as the analyte would not be effectively retained by the hydrophobic packing material.
D) Analyte Loading

The SPE columns were loaded by passing filtered samples (see Section 3.1.2) through an activated SPE column. Since only the material that can be retained on the SPE column packing material was of interest, the SPE column underflow was discarded.

A total of 50 mL of filtered sample was loaded onto a SPE column. This volume was selected since MBAS losses in the SPE column underflow were shown to be negligible at MBAS concentrations encountered in the primary effluent from the Lions Gate WWTP (see Section 4.1.5.3 A). Initially, 3 mL of filtered sample was added directly to the SPE column. A 75 mL reservoir was then fitted to the SPE column and the remaining 47 mL of sample was added to the reservoir. The vacuum was controlled to achieve a liquid flow rate of 5 mL per minute or less through the SPE column. Had all of the filtered sample been added to the 75 mL reservoir, the SPE column packing material may have gone to dryness. When the vacuum was first applied, the SPE column packing material could have gone to dryness as the 6 mL of air, contained above the SPE column packing material, was first drawn through the SPE column packing material before the filtered sample was drawn from the 75 mL reservoir into the SPE column.

After the sample was drawn through the packing material, the packing material was dried. The packing material was dried by continuing the vacuum for approximately 30 seconds after all of the sample had been drawn through the packing material.

E) Column Washing

Washing was done to remove weakly retained and entrained materials from the packing material. Six-1 mL aliquots of a solution having 40% methanol in water (V/V) was used to wash the SPE columns. A 1 mL aliquot of the wash solution was washed over the sides of the 75 mL reservoir and drained into the SPE column. The SPE packing material was dried by continuing the vacuum for approximately 30 seconds before the next 1 mL aliquot of solution was added.
The SPE column wash underflow was discarded since weakly retained and entrained materials, present in the SPE column packing material, were not of interest in the present study.

F) Elution

The anionic surfactant components adsorbed to the SPE column packing material can be eluted into discrete fractions based on the hydrophobic nature of the components (see Section 2.6). Using gradient methanol elution, the anionic surfactants were separated into low, medium and high molecular weight fractions.

The loaded SPE column was positioned above an elution vial in the sample rack (see Figure 3). The vacuum induced liquid flow rate through the SPE column was adjusted using a flow meter to achieve a liquid flow rate not exceeding 5 mL per minute. To the SPE column, a total of 2-1 mL aliquots of solution containing 65% methanol in water (V/V) were added. Initially, 1 mL of eluting solution (65% methanol in water) was added to the SPE column, and as soon as half of the eluting solution was drawn through the packing material, the vacuum was stopped for approximately 1 minute by closing the stopcock. This enabled the eluting solution to contact all of the packing material for a given amount of time. The vacuum was resumed and the remaining eluting solution was drawn through the packing material. The packing material was dried by continuing the vacuum for approximately 30 seconds before the next 1 mL aliquot of eluting solution was added. The second aliquot of eluting solution was passed through the packing material without stopping the vacuum. Following the removal of all traces of eluting solution from the packing material, the packing material was then dried by continuing the vacuum for approximately 30 seconds. Next, the vacuum was stopped by closing the stopcock. The SPE column was then moved above an unused elution vial in the sample rack.

The above procedure was repeated with a solution having 75% methanol in water. When applied after the solution having 65% methanol in water, a 75% methanol in water eluting solution can be used to elute the anionic surfactant components that exhibit intermediate hydrophobic characteristics (i.e., intermediate molecular weights). The above procedure was once again repeated with a solution having 90% methanol in water. When applied after the
eluting solution having 75% methanol in water, the 90% eluting solution can be used to elute the anionic surfactants that exhibit high hydrophobic characteristics (i.e., high molecular weights).

G) Reconstitution

*Methanol Evaporation from the SPE Column Extracts*

The methanol used in the elution process needed to be evaporated from the SPE column extracts to eliminate interferences with the Microtox™ toxicity test results (see Section 2.7.1). To do this, the SPE column extracts were transferred from the elution vials into 50 mL beakers. Two-1 mL aliquots of 100% methanol were used to rinse each elution vial ensuring complete transfer of the analyte from the elution vials to the 50 mL beakers. The addition of 100% methanol also acted to lower the boiling point of the methanol contained in the SPE column extract (Stecher, 1968). The 50 mL beakers were placed in a water bath at 88-90 °C for 4 minutes at which time 5 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was added. This solution remained in the water bath for an additional 20 minutes. The purpose of this evaporation procedure was to remove as much of the methanol as possible without allowing the contents of the beakers to go to dryness. The SPE column extracts must not go to dryness during the evaporation step; otherwise, the analyte may be baked onto the 50 mL beakers resulting in poor analyte recovery. Therefore, the SPE column extracts were watched closely during the evaporation procedure to ensure that the beakers did not go to dryness.

*Standard Volume of the SPE Extracts*

Reconstitution of the SPE column extract to a known standard volume is required so that subsequent toxicity and MBAS analyses can be conducted. The evaporated SPE column extracts were transferred from the beakers into 25 mL volumetric flasks. Each beaker was rinsed three times with de-ionized water, obtained from a Milli-Q water system (Molsheim, France), to ensure complete transfer of MBAS from each beaker to each volumetric flask. The volume in the volumetric flask was made up to 25 mL by adding de-ionized water. The solution contained
in the volumetric flask was subsequently referred to as the reconstituted SPE extract. It should be noted that a concentration factor of 2x was achieved through the extraction procedure. Recall that 50 mL of sample was passed through a SPE column. A 10 mL screw top test tube was rinsed three times with the reconstituted SPE extract before it was filled and the contents stored for subsequent toxicity analysis (see Section 3.1.6). The remaining reconstituted SPE extract was stored in a 25 mL volumetric flask for MBAS analysis (see Section 3.1.5).

A summary of the procedures used during the SPE method is presented in Table 3.
Table 3: Summary of the procedures used during the SPE method.

<table>
<thead>
<tr>
<th>Step 1 -- Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add 1 mL of 100% methanol to the SPE column and apply the vacuum to produce a flow rate of 5 mL per minute through the column (subsequently, use this flow rate).</td>
</tr>
<tr>
<td>Stop the vacuum for 1 minute when half of the methanol has been drawn through the SPE column. Continue the vacuum and allow the remaining methanol to be drawn through the packing material.</td>
</tr>
<tr>
<td>Add 1 mL of 100% methanol to the column and apply the vacuum until the methanol is drawn through the packing material.</td>
</tr>
<tr>
<td>Repeat the above procedure using de-ionized water, obtained from a Milli-Q water system (Molsheim, France), in place of 100% methanol, and, for the second-1 mL aliquot of water, stop the vacuum when 1 to 2 mm of water remain on the top of the packing material.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2 -- Analyte Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add approximately 3 mL of filtered sample to the SPE column.</td>
</tr>
<tr>
<td>Place the 75 mL reservoir above the column and add the remaining filtered sample (47 mL) to the reservoir.</td>
</tr>
<tr>
<td>Apply the vacuum at a rate of 5 mL per minute or less until the packing material goes to dryness.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 3 -- Column Washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apply vacuum, and in succession, wash 6-1 mL aliquots of 40% methanol in water over the sides of the reservoir allowing the solution to drain into the SPE column.</td>
</tr>
<tr>
<td>Allow the packing material to go to dryness before adding the next aliquot.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 4 -- Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position the SPE column above an elution vial.</td>
</tr>
<tr>
<td>Apply the vacuum, and add 1 mL of 65% methanol in water. Stop the vacuum for 1 minute when half of the 65% methanol in water has been drawn through the packing material.</td>
</tr>
<tr>
<td>Allow SPE packing material to go to dryness before adding another 1 mL aliquot of 65% methanol in water.</td>
</tr>
<tr>
<td>Let column go to dryness. Stop vacuum and move SPE column above a new elution vial.</td>
</tr>
<tr>
<td>Repeat above procedure using solutions of 75% and 90% methanol in water.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 5 -- Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add contents of elution vial to a beaker.</td>
</tr>
<tr>
<td>Rinse elution vial with 2-1 mL aliquots of 100% methanol adding the rinse to the beaker.</td>
</tr>
<tr>
<td>Heat the contents of the beaker in a 88-90°C water bath.</td>
</tr>
<tr>
<td>After 4 minutes, add 5 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), to the beakers and continue to heat for 20 minutes.</td>
</tr>
<tr>
<td>Do not allow the contents of the beakers to go to dryness</td>
</tr>
<tr>
<td>Transfer the contents of the beaker into a 25 mL volumetric flask.</td>
</tr>
<tr>
<td>Rinse the beaker 3 times with de-ionized water adding the rinse to the volumetric flask.</td>
</tr>
<tr>
<td>Rinse a 10 mL screw top test tube 3 times with reconstituted SPE extract.</td>
</tr>
<tr>
<td>Fill the rinsed test tube with reconstituted SPE extract and store the test tube for toxicity analysis.</td>
</tr>
<tr>
<td>Store the remaining SPE extract in the 25 mL volumetric flask for MBAS analysis.</td>
</tr>
</tbody>
</table>
H) Additional Notes on Solid Phase Extraction

Often an air bubble in the frit of the SPE column prevented the passage of liquid through the packing material. By tapping the column, the bubble could often be freed. Failing this, it was sometimes necessary to close the stopcock to allow the vacuum to build-up within the vacuum box. When the stopcock was opened, the air bubble was often freed due to the greater downward force exerted by the increased vacuum.

To ensure an airtight seal between the stainless steel body of the 10-port vacuum box and the white diaphragm attached to the lid of the 10-port vacuum box, the white diaphragm was wetted with water. Downward force on the lid, held for approximately 20 seconds, helped to establish an airtight seal as the vacuum in the 10-port vacuum box was built-up.

3.1.5 MBAS Analysis

The procedure developed by Chitikela et al., (1995) to measure the concentration of MBAS was used as the basis for developing a suitable method for use in the present study. The method that was developed as part of the present study requires less sample volume, less chloroform, less glassware and less labour than does the Standard Method 5540 C (APHA et al., 1992). In addition, fewer organic extractions are required than are required by the method developed by Chitikela et al., (1995).

In the present study, the working MBAS concentration for the analytical procedure described below ranged from 0 mg/L LAS to 4 mg/L LAS. Within this range, the relationship between absorbance at a wavelength of 652 nm and MBAS (measured as mg/L LAS) was linear.

The MBAS procedure consists of three steps: organic extraction, aqueous back-wash, and measurement. The theoretical basis of the method is discussed in Section 2.5. In the following discussion, sample refers to any aqueous phase intended for MBAS analysis. In the context of the present study, the term, sample, corresponds to filtered whole sample, stored whole sample or any one of 65%, 75%, or 90% reconstituted SPE extracts (see Figure 2).
Fresh chloroform refers to uncontaminated, reagent chloroform. American Chemical Society (ACS) chloroform was obtained from Fisher Scientific (Hampton, NH). It should be pointed out that High Performance Liquid Chromatography (HPLC) grade chloroform should not be used in the MBAS test as preservatives, used to preserve the chloroform, may interfere with the colourimetric development of the methylene blue. Chloroform, on the other hand, refers to reagent grade chloroform containing extracted methylene blue-anionic surfactant ion pairs.

A) Organic Extraction

The organic extraction step was performed in 50 mL Pyrex round bottomed vials. The vials were capped with Teflon coated lids. The vials were first rinsed 6 times with tap water and twice with de-ionized water obtained from a Milli-Q water system (Molsheim, France). The vials were then baked in a muffle oven for one hour at 450 – 500 °C. Fresh chloroform was used to rinse the vials before samples were added.

To a vial, 5 mL of sample was added. Dilutions were necessary when the expected MBAS concentration exceeded 4 mg/L. To the vial, 1 drop of the alcoholic phenolphthalein indicator (Standard Method 5540 C (APHA et al., 1992)) and one drop of 1 N NaOH were added. The vial was swirled until the solution turned a uniform pink colour. 1 N H₂SO₄ was then added drop wise until the pink colour of the solution disappeared and the solution became colourless. The vial was swirled after each drop of 1 N H₂SO₄ was added. Two mL of fresh chloroform and 2 mL of methylene blue solution were then added to the vial. Methylene blue solution was prepared as outlined in Standard Methods 5540 C (APHA et al., 1992) using methylene blue (basic blue 9) obtained from Matheson, Coleman and Bell (Norwood, OH). The vial was capped and mixed using a vortex mixer for 30 seconds. Mixing caused the organic and aqueous phases to emulsify.

The vial was allowed to sit for 2 to 3 minutes. During this time, the two phases would usually separate except for small droplets adhered to the vial wall. At this stage, complete phase separation would be ideal but not necessary. If complete phase separation occurred, a sharp separation between organic and aqueous phase could be made. However, if complete phase
Chapter 3. Analytical Procedures and Experimental Setups

separation did not occur, an emulsion was evident. The vial was then gently swirled to ensure a more complete separation of the phases. This was necessary as droplets of aqueous phase and organic phase, adhered to the walls of the vial, would be present in the opposite phase. The chloroform, containing the surfactant-methylene blue ion pairs, was extracted from the bottom of the vial using a Pasteur pipette. The extracted chloroform was transferred to a second vial. A small residual amount of chloroform was left in the bottom of the first vial to prevent the transfer of any of the aqueous phase to the second vial.

During the first extraction step, the aqueous phase may contain anionic surfactant molecules that have not yet formed ion pairs with methylene blue. Transferring these unpaired surfactant molecules would result in a reduction of the analyte recovery. When complete phase separation did not occur, less chloroform was present in a separate phase. Therefore, less chloroform was transferred to the second vial. Invariably, when complete phase separation did not occur, some aqueous phase, emulsified with the chloroform, was likely transferred to the second vial. However, every attempt was made to minimize the transfer of aqueous phase during the first step of the extraction.

The organic extraction step was repeated by adding 2 mL of fresh chloroform to the first vial. The vial was capped and mixed for 30 seconds, and then allowed to sit for 2 to 3 minutes. The vial was then gently swirled to produce a sharper separation of organic and aqueous phases. Emulsions were, generally, less problematic, as greater phase separation usually occurred following the second extraction. In the event that good phase separation occurred, all of the chloroform was extracted and transferred to the second centrifuge tube. As this is the final organic extraction, a small amount of aqueous phase may be extracted to ensure complete recovery of all the chloroform. The potential transfer of some of the aqueous phase, during the second extraction, is not problematic since complete recovery of chloroform is more likely. Also, the aqueous back-wash step, described below, removes the residual unpaired aqueous methylene blue that may be present. In the event that good phase separation did not occur, all of the separated chloroform and all of the emulsion was extracted to the second vial. This ensured that all of the chloroform containing anionic surfactant-methylene blue ion pairs was recovered.
B) Aqueous Back-wash

To the 4 mL of chloroform contained in the second centrifuge tube, 10 mL of aqueous wash solution was added. The aqueous wash solution was prepared in accordance with Standard Method 5540 C (APHA et al., 1992) using sodium phosphate, monobasic monohydrate obtained from EM Science (Gibbstown NJ). The second vial was then capped and mixed using the vortex mixer for 30 seconds. Ideally, the two phases would separate after the vial was allowed to sit for 2 to 3 minutes. The vial was then gently swirled. If a sharp phase separation occurred, a new Pasteur pipette was used to extract the chloroform to a third vial. It is of the utmost importance that none of the aqueous phase be transferred with the chloroform to the third vial. The presence of any aqueous phase in the third vial would interfere with the absorbance reading obtained during the measurement step described below.

If persistent emulsions were present after the vial was allowed to sit for 2 to 3 minutes, glass wool plugs and sodium sulfate anhydrous were used together to remove aqueous phase from the chloroform. Sodium sulfate anhydrous is often used to remove aqueous phase from organic extracts. Glass wool was obtained from Ohio Valley Specialty Chemical (Marieatta, Ohio). Small glass wool plugs were inserted into the stem of a glass funnel. The glass wool plugs were rinsed with fresh chloroform to remove adventitious surfactants. Sodium sulfate anhydrous was obtained from EM Science (Gibbstown, NJ.) and enough reagent was placed on top of the glass wool plug to just cover the top surface. Together, the glass wool plugs and sodium sulfate anhydrous functioned to remove aqueous phase from the chloroform as it was being transferred from the second vial to the third vial.

Following aqueous backwash, one organic back-extraction was performed to ensure complete recovery of all methylene blue anionic surfactant ions pairs to the organic phase. To the second vial, 2 mL of fresh chloroform was added, the solution was mixed for 30 seconds, then allowed to sit for 2 to 3 minutes and then gently swirled. The chloroform was transferred to the third vial as described above. The third vial contained a total of 6 mL of chloroform following completion of the aqueous backwash procedural steps.
C) Measurement

Fresh chloroform was added to the third vial to increase the volume to 20 mL. If a glass wool plug was used in the aqueous backwash procedural steps, the fresh chloroform was filtered through the plug to ensure that all the anionic surfactant-methylene blue ion pairs were transferred to the third vial. The 20 mL of chloroform was then mixed to ensure both uniform colour distribution and reliable absorbance measurements.

A new Pasteur pipette was used to rinse and partially fill a 1 cm square cuvette with the chloroform contained in the third vial. A square cuvette minimizes interferences due to differences in alignment of round and possibly scratched cuvettes. The outer surface of the cuvette was wiped and dried with a methanol soaked lab glassware wipe. High performance liquid chromatography grade methanol was obtained from Fisher Scientific (Hampton, NH). A Turner 690 spectrophotometer (Dubuque, IA.) was zeroed on a process blank that had gone through the above MBAS analytical procedure. Using a Turner 690 spectrophotometer (Dubuque, IA.) the absorbance of each sample was measured at a wavelength of 652 nm and compared to that of a standard curve to determine the MBAS concentration. MBAS Standards were prepared using Dodecylbenzenesulfonic acid sodium salt (MW = 348.48) obtained from Fluka (Buchs SG Switzerland).

It should be noted that it is best if the third vial is graduated, as the volume of chloroform can be accurately increased to 20 mL. If graduated vials are unavailable, a strip of lab marking tape placed at the level equivalent to a volume of 20 mL is sufficient. A mark placed on the tape where the readings should be sighted will help increase reproducibility. The tape can be baked onto the vial by placing the marked centrifuge tube in a 40 °C drying oven for 20 minutes.

Table 4 summarizes the procedures used in the MBAS method.
Table 4: Summary of the MBAS method procedures.

**Step 1 -- Organic Extraction**

Place 5 mL of sample in the first 50 mL vial.
Add 1 drop phenolphthalein to the sample and 1 N NaOH drop wise until solution turns pink.
Add 1 N H₂SO₄ drop wise until solution turns colourless.
Add 2 mL of fresh chloroform.
Add 2 mL of methylene blue.
Mix for 30 seconds.
Let sit 2 to 3 minutes to separate phases and gently swirl to ensure complete separation.
Extract chloroform and transfer into a second 50 mL vial using a Pasteur pipette. Repeat the extraction in the first centrifuge tube once using 2 mL of fresh chloroform.

**Step 2 -- Aqueous Backwash**

To second vial, containing approximately 4 mL of extracted chloroform from step 1, add 10 mL of wash solution to the second vial.
Mix for 30 seconds, allow phases to separate and swirl.
Extract chloroform and transfer it to a third 50 mL vial using a new Pasteur pipette. To second vial, add 2 mL of fresh chloroform, mix, allow the phases to separate and extract to third vial.
If persistent emulsions are present, use a plug of glass wool to filter the chloroform extract as it is being transferred into the third vial.

**Step 3 -- Measurement**

To the third vial, containing approximately 6 mL of extracted chloroform from step 2, make up the volume in the third vial to 20 mL by adding fresh chloroform.
Measure the absorbance at 652 nm using a standard curve.
3.1.6 Microtox™ Analysis

A detailed description of the procedures and the materials used in the Microtox™ test system are outlined in the owners manual (Microbics Corporation, 1992). The detailed basic test protocol, recommended by the equipment manufacturer, was used (see Section 2.7). Figure 4 illustrates the Microtox™ apparatus used in the present study.

The bioluminescence of the bacteria contained in the reconstituted acute reagent decreases with time (Microbics Corporation, 1992). For this reason, once a vial of acute reagent is reconstituted, it has a shelf life of 2 hours. During the present study, six detailed basic test protocols could be performed within approximately 1.5 hours. Any acute reagent remaining after this time was discarded to ensure that the shelf life was not exceeded during a test run.

Figure 4: Microtox™ apparatus.

When following the detailed basic test protocol, outlined in the Microtox™ users manual, many test configurations are possible. In the present study, the detailed basic test protocol was
followed with three samples being run in parallel. Each of the three samples were analyzed with one control and four serial dilutions of the sample. As a result, the whole filtered sample concentrations that were examined included 5.625%, 11.250%, 22.500%, and 45.000%. Similarly, in the reconstituted SPE column extracts, where a concentration factor of 2x was used, the sample concentrations examined were 11.250%, 22.500%, 45.000% and 90.000%.

The data reduction formulae, used to calculate the toxicity of a sample, are presented in the Microtox™ users manual (Microbics Corporation, 1992). The IC$_{20}$ represents the sample concentration or analyte concentration which inhibits the bioluminescence of the Vibrio fischeri by 20%. The IC$_{20}$'s were calculated and presented in the present study. When reconstituted SPE extracts were tested, correction factors were determined as part of the present study and applied to the bioluminescent values measured using the Microtox™ instrument (see Section 4.2.4). These correction factors accounted for the influence of foreign substances introduced from the SPE column packing material.

Although the Microtox™ users manual provides a detailed description of the test protocols, it does not adequately emphasize the importance of proper reagent reconstitution. To achieve reproducible results, it is of the utmost importance that the acute reagent be reconstituted properly. Specifically, the reconstitution solution must be poured - as quickly as is possible - from the cuvette containing the reconstitution solution into the reagent vial. In the present study, this was accomplished by holding both the cuvette and reagent vial at an angle to vertical, and inserting the cuvette into the reagent vial - so that the open end of the cuvette was lined up over the open end of the reagent vial (see Figure 5). The reconstitution solution was rapidly transferred to the reagent vial by inverting the cuvette over the reagent vial. The reagent vial was then swirled 3 to 4 times. Next, the activated reagent was transferred from the reagent vial into the cuvette that previously contained the reconstitution solution. Finally, the cuvette was immediately placed in the reagent well of the Microtox™ instrument (see Figure 4).
The Microtox™ users manual (Microbics Corporation, 1992) does not adequately emphasize the sensitivity of *Vibrio fischeri* to temperature. An increase in the temperature will increase the bioluminescence. Therefore, the cuvettes should be transferred to the read well of the instrument as quickly as possible without excessive handling. During the present study, physical contact with the bottom of the cuvette (e.g. wiping the cuvettes with laboratory wipes or handling the cuvettes for more than just a few seconds) was shown to increase the bioluminescence.

### 3.1.7 Methanol Concentration

An HP 6890 series gas chromatogram (GC) with a flame ionization detector (Richmond, BC) was used to determine the methanol concentration present in the reconstituted SPE extracts. The GC was operated with a split ratio of 10:1, an injection volume of 1.0 μL, an inlet heater temperature of 150 °C, and a flame ionization detector operated at 250 °C. An Agilent J&W
Chapter 3. Analytical Procedures and Experimental Setups

DB624 capillary column (Palo Alto, CA) with dimensions of 28.0 m x 530 μm x 3.0 μm was used. The column was operated in the constant flow mode having a flow of 35 cm/sec. The oven set point was 35 °C for 4 minutes after which a 20 °C/minute ramp occurred until the oven temperature reached 100 °C. The total run time was 7.25 minutes.

3.1.8 Total Suspended Solids Analysis

The total suspended solids concentrations were measured for the bio-treatment methods used in the treatability study presented in Chapter 6. The procedure outlined in Standard Methods, 2540 D (APHA et al., 1992) was followed to determine the total suspended solids.

3.2 Experimental Set-up and Analytical Procedures for Treatment Studies

Bench-scale treatment methods were considered as part of the present study. The methods were selected based on their documented or expected abilities to remove anionic surfactants from wastewater (see Sections 2.4 and Chapter 6). The flowchart presented in Figure 6 illustrates the treatment and analytical procedures followed.
Figure 6: Flowchart of treatment and analytical procedures.

*Microtox™ only applied to ozone and air flotation treatments
1 Bio-Treatment

A) Biological Degradation

Five hundred mL of waste activated sludge from the UBC Pilot Plant was aerated for 10 minutes using a coarse bubble diffuser. The aerated waste activated sludge was added to a 2 L beaker. Next, 500 mL of Lions Gate effluent was added to the beaker containing the activated sludge. Aeration was applied for 30 minutes. After 30 minutes of aeration, the total suspended solids concentration was measured (see Section 3.1.8). Approximately 400 mL of the mixed liquor was transferred from the beaker into two-200 mL centrifuge tubes. The mixed liquor was centrifuged for 5 minutes at 3000 RPM. The supernatant present at the top of the centrifuge tubes was defined as the treated effluent from the biological treatment test. The treated effluent was subject to further procedural steps and analyses as discussed in section 3.2.7.

B) Partitioning to Abiotic Bio-Solids

Five hundred mL of waste activated sludge was aerated for 10 minutes using a coarse bubble diffuser. The aerated waste activated sludge was added to a 2 L beaker. Five grams (1% by weight) of sodium azide was added to the 500 mL of waste activated sludge to inactivate the biological solids (Bérubé, 2000). This mixture was then mixed for five or ten minutes to ensure that inactivation of the biological solids was complete.

Five hundred mL of primary effluent from the Lions Gate WWTP was added to the beaker containing the inactivated sludge. The mixture was aerated for 30 minutes. After 30 minutes of aeration, total suspended solids concentration was measured (see Section 3.1.8). Approximately 400 mL of the mixed liquor was transferred from the beaker into two-200 mL centrifuge tubes. The mixed liquor was centrifuged for 5 minutes at 3000 RPM. The supernatant present at the top of the centrifuge tubes was defined as the treated effluent from the partitioning to abiotic bio-solids test. The treated effluent was subject to further procedural steps and analyses as discussed in Section 3.2.7.
II Alum Coagulation/Flocculation with Gravity Settling

Alum coagulation/flocculation with gravity settling tests were performed using a jar testing apparatus (Phipps and Bird, Richmond Virginia). Eight hundred mL of primary effluent from the Lions Gate WWTP was added to a 1 L beaker, to which alum was added. A stock 100 mg/mL alum solution was prepared in advance, and 4 mL of the stock solution was added. This resulted in an alum concentration of 40 mg/L (as Al) present in the beaker. A previous study showed that this dose was the most effective at removing MBAS (see Section 2.8).

Immediately following the addition of alum, the pH of the solution was adjusted to target a pH of 7.0 by adding six drops of 6N NaOH. The solution was rapidly stirred at 300 RPM for 60 seconds followed by a slow mix at 50 RPM for five minutes. Following mixing, the solution was settled for 30 minutes.

Following settling, the supernatant present at the top of the 1 L beaker was defined as the treated effluent from the alum settling test. The treated effluent was subject to further procedural steps and analyses as discussed in Section 3.2.7.

III Ozonation

2 L of primary effluent from the Lions Gate WWTP was added to a 2 L Erlenmeyer flask. The flask was fitted with a bung having an inlet and an exit port. Ozone was added to the system through the inlet port. The off gas exited the system through the exit port. Residual ozone in the off gas was captured using potassium iodide (KI) traps. The sample was ozonated for ten minutes at the highest capacity of the ozone generator (Azco Industries, Surrey, BC.). Figure 7 depicts the experimental set-up used.

The whole ozonated sample was defined as the treated effluent. The treated effluent was subject to further procedural steps and analyses as discussed in section 3.2.7.
IV Air Flotation

400 mL of primary effluent from the Lions Gate WWTP was added to a 500 mL glass graduated cylinder. A fine bubble diffuser was placed at the bottom of the cylinder. Figure 8 depicts the experimental set-up used. Air was introduced at such a rate that a stable froth layer formed on the liquid surface. Sufficient air flow was required to generate a stable froth. Insufficient or excessive air flow rates would result in a froth that collapsed making froth removal impossible. The froth layer was removed by applying vacuum to a fine tipped Pasteur pipette. The Pasteur pipette was positioned in the froth layer just above the liquid/froth interface. Air flotation and froth removal were applied for five minutes. Both the initial and final volumes of the liquid were noted. The froth product removed with the vacuum was discarded.
After air flotation, the sample remaining in the graduated cylinder was defined as the treated effluent. The treated effluent was subject to further procedural steps and analyses as discussed in Section 3.2.7.

Figure 8: Air flotation experimental set-up.

3.2.7 Analytical Procedures

The MBAS concentrations in the whole filtered raw Lions Gate primary effluent and in the 65%, 75% and 90% reconstituted SPE extracts were determined in triplicate (see Figure 6). The toxicities contained in the whole filtered raw Lions Gate primary effluent and in the 90% reconstituted SPE extract were determined in triplicate using the Microtox™ system.

The initial MBAS concentration contained in the bio-treatment systems was determined using a mass balance approach. The contributions of MBAS in the whole and 65%, 75% and
90% elution fractions from both the waste activated sludge and the Lions Gate primary effluent were considered.

The treated effluents from the partitioning to abiotic bio-solids, biological treatment, alum coagulation/flocculation with gravity settling, ozonation and air flotation treated effluent samples were analyzed in triplicate for whole filtered MBAS and MBAS in the reconstituted SPE extracts (65%, 75% and 90% elution fractions) as illustrated in Figure 6.

The toxicity contained in the treated effluents from the ozonation and air flotation treatment methods was determined using the Microtox™ system. The whole filtered samples and the 90% reconstituted SPE extracts were analyzed in triplicate. The toxicity contained in the treated effluents from the partitioning to abiotic bio-solids, the biological, and the alum coagulation/flocculation with gravity settling tests were not measured. When conducting these treatment tests, additional material (i.e., abiotic sludge containing sodium azide (see Section 3.2.I.B), waste activated sludge (section 3.2.I.A) or alum (section 3.2.II)) was introduced into the primary effluent matrix. As a result, the characteristics of the primary effluent matrix was changed making it impossible to establish the impact of the treatment on the original primary effluent matrix. Therefore, toxicity measurements were not performed on these modified matrices.
Chapter 4

Development of Analytical Methods

To fulfill the objectives of the present study, analytical methods were developed to quantify the concentration of anionic surfactants in a primary effluent, to fractionate the anionic surfactants on the basis of molecular weight and to measure the toxicity of the whole and discrete molecular weight fractions. The analytical methods needed to be reliable and compatible with each other. Determining the best set of sample preparation procedures and operating conditions, for both the MBAS and the Microtox™ tests, required a significant amount of work.

4.1 Methylene Blue Active Substances Method Development

A new analytical method was developed to measure MBAS concentrations in the present study. A number of tasks were performed as part of the method development. First, sample storage procedures, working MBAS concentration ranges and the precision of the method were investigated, to establish the conditions within which meaningful and reproducible MBAS data could be obtained. Second, a series of quality control tests were performed to ensure that the new method was reproducible while producing high analyte recoveries. Finally, the effect of sample preparation procedures on MBAS recovery was investigated. Sample preparation procedures consisted of the preliminary steps required to prepare a sample for analysis.

4.1.1 Short-Term Sample Storage

MBAS concentrations in the whole filtered samples were measured as soon as was possible following sample collection. The time interval between sample collection and sample analysis was due to the time spent preparing the sample for analysis and to the time spent transporting the sample from the collection site to the Environmental Engineering Laboratories at UBC. However, as discussed in Section 2.6.1, LAS has been shown to degrade rapidly if not stored properly. To investigate the impact of short-term sample storage, the concentration of MBAS in a preserved sample was compared to that of an unpreserved sample, after a one-hour period had elapsed. Short-term preservation procedures were considered to minimize the
degradation of MBAS over a one-hour period. The unpreserved samples were simply stored at 5 °C. Formaldehyde was added to the preserved samples, and the mixture was stored at 5 °C (see Section 2.5.1).

Following collection, one primary effluent sample from the UBC Pilot Plant was immediately preserved on site with 1% (W/W) formalin (37% formaldehyde in water) (Fisher Scientific, Fair Lawn, NJ). A second sample, collected at the same time, was not preserved with formaldehyde. Both samples were filtered on-site and stored for 1 hour at 5 °C before whole filtered MBAS tests were conducted in the Environmental Engineering Laboratory at UBC. The sample preserved with formaldehyde was found to have an MBAS concentration of 5.9 mg/L, while the control sample had an MBAS concentration of 6.3 mg/L. The raw data is presented in Table D.3 in Appendix D. The results of this investigation indicate that MBAS biodegradation did not occur during the time interval between sample collection and analysis. Therefore, when the sample was immediately filtered and stored at 5 °C, formaldehyde addition was not required to preserve MBAS.

Greater MBAS stabilities can be achieved using SPE columns and refrigeration as a preservation method. As presented in Section 2.6.1, MBAS biodegradation does not occur when a sample is loaded onto a SPE column and stored at 4 °C (Petrović and Barceló, 2000). Therefore, samples loaded onto a SPE column can be stored for a few days prior to analysis.

4.1.2 Working MBAS Concentration Range

As presented in Section 2.5, the concentration of MBAS in a sample is determined spectrophotometrically by measuring the absorbance at a wavelength of 652 nm. MBAS standards were prepared using a 1 000 mg/L LAS (Fluka, Buchs SG Switzerland) stock solution prepared with de-ionized water, obtained from a Milli-Q water system (Molsheim, France). A linear relationship was observed between the MBAS concentration and the absorbance over an MBAS concentration range from 0 to 4 mg/L. Figure 9 shows this linear relationship.
Throughout the present study, samples were diluted with de-ionized water, obtained from a Milli-Q water system (Molsheim, France), prior to analysis when the MBAS concentration of samples were expected to exceed 4 mg/L.

**4.1.3 Precision of the MBAS Analytical Method**

The precision of the MBAS analytical method was determined. Precision provides an indication of the quality of the data by considering the variability of the results obtained from multiple analyses of a given sample. The standard deviation associated with multiple analyses was used as a measurement of the precision. A precise analytical procedure is characterized by a small standard deviation. The type of sample (i.e., whole sample versus LAS standard) and the nature of the sample preparation procedures (i.e., SPE) used may affect the precision of the analytical method. The precision associated with the MBAS analytical method was determined by considering the standard deviation of the results obtained when developing the standard calibration curve, when analyzing the reconstituted SPE extracts and when analyzing the whole sample. This enabled the impact of sample preparation on the precision of the MBAS analytical method to be quantified.
A) Precision of the MBAS Analysis for the Standards

The precision of the MBAS analytical method was determined using standards containing 0.5, 1, 2 and 4 mg/L LAS. These standards were prepared on December 16th, 2003, and February 3rd and 26th, 2004. The raw data is presented in Tables C.1, C.2 and C.3 (Appendix C). Outliers were not included in the determination of precision. Outliers included all the MBAS values determined on March 1st presented in Table C.3 (Appendix C). A standard curve was prepared from the data presented in Tables C.1, C.2 and C.3 (except the outliers of March 1st). Table 5 below contains the number of samples considered, the average MBAS concentration measured, and the standard deviation.

Table 5: Precision of the MBAS analytical method found using MBAS standards.

<table>
<thead>
<tr>
<th>Concentration of Standard (mg/L LAS)</th>
<th>Number of Replicates</th>
<th>Mean Concentration (mg/L MBAS)</th>
<th>Standard Deviation (mg/L MBAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4</td>
<td>0.48</td>
<td>0.04</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>0.92</td>
<td>0.19</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>1.85</td>
<td>0.02</td>
</tr>
<tr>
<td>4.0</td>
<td>3</td>
<td>4.13</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The highest standard deviation is approximately 0.2 mg/L MBAS as found in the 1 mg/L LAS standard. This means that, for the analysis of standards prepared using various concentrations of LAS, 68.3% of the measured values will be within 0.2 mg/L MBAS of each other assuming the measured values are normally distributed. For the purposes of the present study, which focuses on relative trends rather than absolute values, the variability is considered to be acceptable.

B) Precision Following Sample Preparation Procedures

As part of the Lions Gate WWTP effluent characterization study presented in Chapter 5, the MBAS concentrations were measured in triplicate in the whole sample and in the 65%, 75% and 90% reconstituted SPE extracts. Standard Method 1030 C was followed to determine the precision of the MBAS measurements that had been performed in triplicate (APHA et al., 1992). (Note, the whole sample analysed on February 3, 2004 was not done in triplicate, and it was, therefore, not included in the determination of precision following sample preparation procedures). Table 6 below contains the number of replicates, the average range of measured
MBAS concentrations and the calculated standard deviations for both the whole samples and the reconstituted SPE column extracts.

Table 6: Precision of MBAS method following sample preparation procedures.

<table>
<thead>
<tr>
<th></th>
<th>MBAS Analysis Following SPE</th>
<th>Whole Sample MBAS Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Average Range (mg/L MBAS)</td>
<td>0.44</td>
<td>0.65</td>
</tr>
<tr>
<td>Standard Deviation (mg/L MBAS)</td>
<td>0.26</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Sample preparation procedures, such as filtration and SPE and reconstitution induces some variation in the measured MBAS values i.e., increases the standard deviation. The standard deviations of the results obtained following sample preparation procedures are greater than those found following the analysis of standards as presented above. The decrease in the precision is likely due to the greater number of procedural steps involved in these sample preparation procedures.

The whole sample analysis exhibited a substantially higher variation. This was likely due to the persistent emulsions which occurred during the analysis of whole samples. Glass wool plugs were used to break these emulsions. As discussed in Section 4.1.5.2.A, glass wool plugs impact the results from the MBAS analysis.

The highest standard deviation following sample preparation procedures was approximately 0.4 mg/L MBAS. This means that approximately 68.3% of the measured MBAS values will be within 0.4 mg/L MBAS assuming the measured values are normally distributed. For the purposes of the present study, which focuses on relative trends rather than absolute values, this variability is considered to be acceptable.

4.1.4 Recovery of Analyte During The MBAS Analytical Procedure

Both Standard Methods 5540 C (APHA et al., 1992) and the method developed by Chitikela et al., (1995) recommend the use of three organic extraction steps, followed by one aqueous backwash and two additional organic back-extraction steps. Organic extraction and
organic back-extraction steps are used to recover analyte to the organic phase while aqueous backwash steps are used to remove excess methylene blue and some interferences from the organic phase (see Section 2.5). It is well known that greater analyte recoveries and more reproducible results are achieved as the number of organic extraction steps increases. However, more time is spent completing the analysis as the number of extractions increases. The feasibility of using only two organic extractions and two aqueous backwashes was considered.

A test was designed to determine the MBAS recovery following just two organic extractions, one aqueous backwash and one organic back-extraction. A 1 mg/L standard LAS (Fluka, Buchs SG Switzerland) solution was prepared. Two analyses were performed on this standard LAS solution. The first analysis used 2 organic extractions followed by one aqueous backwash and 1 organic back-extraction. The second analysis used 3 organic extractions followed by one aqueous backwash and 2 organic back-extractions. Each analysis was conducted four times. MBAS recoveries obtained using the two test conditions are presented in Table 7.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Two extractions followed by one back-extraction Recovery (%)</th>
<th>Three extractions followed by two back-extractions Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>102</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>111</td>
</tr>
<tr>
<td>Average</td>
<td>97 ± 8</td>
<td>100 ± 13</td>
</tr>
</tbody>
</table>

± corresponds to the 95% confidence interval

The recoveries were 97 ± 8% and 100 ± 13 % following 2 or 3 organic extractions, respectively. In addition, the 95% confidence interval for two organic extractions was 8%. This narrow confidence interval indicates that the use of two extractions and one organic back-extraction generates reproducible results. Given the high recovery of analyte (97%) and the narrow confidence interval (± 8%) obtained, the procedure which consists of using two organic extractions, one aqueous backwash and one organic back-extraction was deemed suitable for use in the present study.
4.1.5 Impacts of Sample Preparation Procedural Steps on MBAS Recovery

Sample preparation procedural steps, shown in Figure 2, were used to ready samples for analysis using the MBAS method. As discussed below, some sample preparation procedural steps have been identified that increase or decrease the recovery of MBAS in the sample being prepared for analysis using the MBAS method.

4.1.5.1 Positive Interferences of Sample Preparation Procedural Steps on MBAS Recovery

Standard Methods (APHA et al., 1992) cautions the reader of potential contamination from adventitious surfactants adsorbed to filter media. Adventitious surfactants are those that are adsorbed to unwashed filter media, for example. Adventitious surfactants act as a positive interference. Positive interferences were found in the filter papers and in the SPE column packing material used in the present study.

A) Filtration

Preliminary investigations, undertaken as part of the present study, indicated that the filter papers used to filter raw effluent could act to increase the MBAS concentration of a filtered sample. Initially, 60 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was used to rinse a VWR #413 (VWR International, West Chester, PA.) filter paper. Next, approximately 60 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was filtered through the pre-rinsed filter paper. The filtrate was analyzed for MBAS. This analysis was performed in duplicate. The concentration of MBAS in the filtrate was 0.06 and 0.07 mg/L MBAS. Although the MBAS concentrations are very low, they are consistently greater than 0 mg/L MBAS. These results suggest that the filtration process can impact the results.

These preliminary results prompted an additional test to be conducted which was designed to investigate the effectiveness of a more thorough filter paper wash procedure used to
remove adventitious surfactants from filter papers. The procedure consisted of pre-washing and sonicating the filter papers as outlined in section 3.1.2. By following this procedure, no MBAS was detected in the filtrate.

### B) SPE

SPE columns and gradient methanol elution were used in the present study to separate anionic surfactants on the basis of hydrophobicity, which for anionic surfactants is an indication of the molecular weight. Since filtered sample is passed through the SPE column packing material, any adventitious surfactants adsorbed to the SPE column packing material could influence MBAS recoveries in the reconstituted SPE extracts.

The potential MBAS contamination from a new SPE column was investigated. This was done by passing 50 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), through an activated SPE column according to the procedure outlined in Section 3.1.4. Following the SPE wash procedure, elution was carried out using solutions containing 65%, 75%, and 90% methanol in de-ionized water (V/V) as described in Section 3.1.4.F. The elution extracts were evaporated and reconstituted using de-ionized water to give a final volume of 25 mL as described in Section 3.1.4.G. Trace concentrations of MBAS were detected in the blank reconstituted SPE column extracts as presented in Table 8.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>MBAS Measured in Reconstituted SPE Column Extracts (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65% Reconstituted SPE Extract</td>
<td>0.09*</td>
</tr>
<tr>
<td>75% Reconstituted SPE Extract</td>
<td>0.07*</td>
</tr>
<tr>
<td>90% Reconstituted SPE Extract</td>
<td>0.08*</td>
</tr>
</tbody>
</table>

* value obtained by interpolation

A more thorough SPE column wash/activation procedure to eliminate the presence of adventitious surfactants was not developed. The MBAS measured in the reconstituted SPE column extracts was very low, and no correction factors were applied. For the purposes of the present study, which focuses on relative trends rather than absolute values, this MBAS
concentration is considered to be acceptable. In addition, effects of a more rigorous wash/activation procedure on the surface properties of the SPE packing material were unknown. The standard procedure outlined by the SPE column manufacturer was used (Supelco, 1997) to activate the reverse phase packing material.

4.1.5.2 Negative MBAS Interferences

MBAS recoveries decreased when either glass wool or sodium chloride was used during sample preparation procedures.

**A) Glass Wool**

Glass wool filter plugs were used to break persistent emulsions by removing aqueous phase from organic phase as discussed in Section 3.1.5.B. Persistent emulsions are commonly encountered when biologically treated wastewaters are analysed using the MBAS method. The effect of glass wool plugs on MBAS recovery was investigated by filtering a 1 mg/L LAS Fluka (Buchs SG Switzerland) solution through a glass wool plug and measuring the MBAS concentration in the filtrate. Method 5540 C (APHA et al., 1992) was followed to measure the concentration of anionic surfactants, as this series of investigations preceded the implementation of the modified MBAS method used in the present study (Section 3.1.5). The concentration of MBAS in the filtrate was then compared to that of a 1 mg/L LAS Fluka (Buchs SG Switzerland) control solution that had not been filtered. The results are presented in Table 9.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Set 1 Glass Wool Recovery (%)</th>
<th>Set 2 No Glass Wool Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>102</td>
</tr>
<tr>
<td>Average</td>
<td>86 ± 15</td>
<td>100 ± 3</td>
</tr>
</tbody>
</table>

(± 95% confidence interval)
Chapter 4. Development of Analytical Methods

Based on a 95% confidence interval, the use of glass wool plugs did not significantly affect the recovery of MBAS. However, the recovery of MBAS was consistently lower (approximately 14% lower) in those samples that had been passed through glass wool plugs. In addition, a narrower confidence interval was achieved for the samples that had not been filtered through glass wool plugs. Therefore, glass wool plugs should only be used when necessary to break-up persistent emulsions. Persistent emulsions would otherwise interfere with the accurate determination of absorbance measured using a Turner 690 spectrophotometer (Dubuque, IA.) with a wavelength of 652 μm.

**B) Sodium Chloride**

Marcomini *et al.*, (1987) reported that the recovery of MBAS during filtration and SPE could be increased by adding sodium chloride to the sample prior to filtration or extraction. After filtering the sample, Marcomini *et al.*, (1987) rinsed the filter paper and the SPE column packing material with a solvent capable of eluting adsorbed MBAS. Therefore, Marcomini *et al.* (1987) measured both the dissolved and adsorbed fractions of MBAS simultaneously.

The effects of sodium chloride addition on MBAS recovery following filtration and SPE were investigated. In the present study, 8% NaCl (W/W) was added to a 1 mg/L LAS Fluka (Buchs SG Switzerland) solution. This solution was then filtered through a washed filter paper (see Section 3.1.2). MBAS recoveries were compared to those of a control solution consisting of a 1 mg/L LAS Fluka (Buchs SG Switzerland) solution to which sodium chloride had not been added. The results are presented in Table 10.

<table>
<thead>
<tr>
<th>Trial</th>
<th>8% NaCl (w/w) + 1 mg/L LAS Recovery (%)</th>
<th>1 mg/L LAS Control Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67.5</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>95.5</td>
</tr>
<tr>
<td>Average</td>
<td>69 ± 22</td>
<td>94 ± 22</td>
</tr>
</tbody>
</table>

Based on a 95% confidence interval, the addition of sodium chloride did not significantly affect the recovery of MBAS after the sample had been filtered. However, the recovery of MBAS was consistently lower (approximately 25% lower) in those samples to which sodium
chloride had been added prior to filtration. These results are somewhat contradictory to those reported by Marcomini et al., (1987). However, in the present study, only soluble MBAS was considered. Marcomini et al., (1987) considered both soluble and particulate (i.e., adsorbed) MBAS. These adsorbed MBAS species were subsequently eluted from the filter paper and added to the soluble MBAS contained in the filtrate.

The effect of sodium chloride addition on the recovery of MBAS following SPE elution was also investigated. In the present study, 8% NaCl (W/W) was added to a 1 mg/L LAS Fluka (Buchs SG Switzerland) solution. This solution was then loaded onto an activated SPE column as described in Section 3.1.4. Elution was carried out using solutions containing 80%, 85%, and 90% methanol in water (V/V). The cumulative MBAS recoveries from all three fractions were compared to that of a control solution consisting of a 1 mg/L LAS (Fluka, Buchs SG Switzerland) solution to which sodium chloride had not been added. The results are presented in Table 11.

Table 11: The effect of sodium chloride on MBAS recovery following SPE

<table>
<thead>
<tr>
<th>Trial</th>
<th>NaCl (8% W/W) + 1 mg/L LAS</th>
<th>Cum. Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>71.5</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>52 ± 114</td>
<td></td>
</tr>
</tbody>
</table>

While not based on a 95% confidence interval, a trend was identified in which lower recoveries of MBAS were obtained for the samples to which sodium chloride had been added. The use of a preliminary MBAS analytical method accounts for the low MBAS recoveries presented in Table 11.

Consistently, the addition of sodium chloride to sample solutions before filtration or SPE decreased the recovery of MBAS. In addition, sodium ions are a known interference with the bioluminescence of Vibrio fischeri an organism that is used in the Microtox™ analytical method. For these two reasons, sodium chloride was not used in the present study.
4.1.5.3 Potential Loss of MBAS

The potential loss of analyte in the SPE underflow and wash underflow streams was investigated. The potential loss of analyte during the SPE extract reconstitution procedure was also investigated.

A) SPE Underflow and Wash Underflow

During SPE column loading, sample is drawn through the SPE column packing material, and anionic surfactants are retained on the hydrophobic packing material. The remainder of the sample leaves the column through the bottom of the column. This stream is named the SPE underflow (see Section 3.1.4.D). A wash step is then performed to remove weakly retained and entrained matter from the SPE column packing material. The wash solution, containing 40% methanol in water (V/V), passes through the packing material and leaves the column through the bottom of the column. This stream is named the SPE wash underflow (see Section 3.1.4.E). The SPE underflow and the SPE wash underflow streams both have the potential to contain MBAS that should otherwise be retained by the SPE column packing material and recovered following gradient methanol elution. To establish if MBAS is lost to these streams, primary effluent from the UBC Pilot Plant was loaded onto a SPE column, and the MBAS concentrations of the SPE underflow and wash underflow streams were measured. The results are presented in Table 12. Raw data is presented in Tables D.4 and D.5 (Appendix D).

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Recovery (%) of MBAS to SPE Underflow Streams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPE Underflow</td>
</tr>
<tr>
<td>UBC Pilot Plant - Nov 26, 2003</td>
<td>4</td>
</tr>
<tr>
<td>UBC Pilot Plant - Dec 11, 2003</td>
<td>3.4</td>
</tr>
</tbody>
</table>

The MBAS contained in the SPE underflow streams represented a potential loss in analyte recovery of approximately 3.7%. However, the potential loss of MBAS in the SPE wash underflow stream was much larger. It is likely that a portion of the MBAS lost to the underflow streams consisted of either weak hydrophobic anionic surfactant molecules or of positive interferences (see Section 2.5). However, further testing would be necessary to address this
hypothesis. Weak hydrophobic anionic surfactant molecules were not of interest in the present study, as they have not been identified as being highly toxic (see Section 2.3).

**B) SPE Extract Reconstitution**

The potential loss of analyte during the procedure used to reconstitute the SPE column extracts was investigated (see Section 3.1.4.G). The reconstitution process consisted of evaporating the methanol contained in the SPE extracts. While the removal of methanol is important for accurate determination of MBAS toxicity using the Microtox™ system, heating the extracts may bake the MBAS onto the glassware. It is of the utmost importance that the extracts do not go to dryness during the evaporation step. Otherwise, low recoveries of MBAS will occur.

The effects of the methanol evaporation procedure (see Section 3.1.4.G), used in the present study, on the recovery of MBAS in the reconstituted SPE column extracts was investigated. Fifty mL of a solution containing 1 mg/L LAS Fluka (Buchs SG Switzerland) was loaded onto an activated SPE column. Gradient methanol elution was carried out using 2 mL of 70% and 90% solutions of methanol in water (V/V). These elution solutions were arbitrarily chosen to limit the number of samples requiring analysis. Next, 2 mL of 100% methanol was used to rinse any residual analyte from the elution vial into a beaker containing the SPE column extract. The 100% methanol was used to rinse the SPE elution vial ensuring complete MBAS transfer, and to lower the boiling point of the methanol contained in the SPE column extract (see Section 3.1.4.G). The SPE column extract was then evaporated for 4 minutes at which time 5 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was added to the beaker. The de-ionized water was added to ensure that the SPE column extract did not go to dryness while being evaporated for an additional 20 minutes. These tests were done in duplicate. MBAS recoveries and the concentration of methanol present in each of the reconstituted SPE column extracts are presented in Table 13.
Table 13: MBAS recovery following methanol evaporation.

<table>
<thead>
<tr>
<th>Elution Fraction</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cum. Recovery (%)</td>
<td>Methanol Conc. (mg/L)</td>
</tr>
<tr>
<td>70%</td>
<td>44</td>
<td>79</td>
</tr>
<tr>
<td>90%</td>
<td>100</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. = Non-detect

Using the evaporation method outlined in Section 3.1.4.G, high MBAS recoveries were achieved. The concentration of methanol in the reconstituted samples was relatively low. At these concentrations, methanol does not produce a toxic response in the bioluminescence of *Vibrio fischeri* measured with the Microtox™ system (see Sections 2.7.1 and 4.2.5.A).

**4.1.5.4 Recovery of MBAS to Elution Fractions**

As discussed in Section 3.1.4.F, gradient methanol elution solutions containing 65%, 75% and 90% methanol in water (V/V) were used in the present study. These elution fractions were chosen based on the recovery of MBAS to each fraction following SPE column gradient methanol elution. The 65%, 75% and 90% methanol elution solutions represent cumulative analyte recoveries of approximately 35%, 75% and 95%, respectively as illustrated in Figure 10. Primary effluent from the UBC Pilot Plant was used in this investigation. Similar recoveries of MBAS to each elution fraction were expected to be found when the Lions Gate primary effluent was analyzed.
4.2 Microtox™ Method Development

The results obtained from the Microtox™ system can be impacted by the effects of the sample preparation procedures. To identify and eliminate (or minimize) the effect of the sample preparation procedures, a number of investigations were undertaken.

4.2.1 Microtox™ Acute Reagent Reconstitution

Proper acute reagent reconstitution is of the utmost importance in achieving reproducible results. A method for acute reagent reconstitution is outlined in Section 3.1.6. During the course of the present study, it was observed that the bioluminescence of the controls would decrease between 30 to 40% when improper acute reagent reconstitution procedures were used. A significant decrease in the bioluminescence of the controls can mask the effect of a toxicant contained in a sample.
When the acute reagent is properly reconstituted, the light levels of the controls should not decrease substantially during a 15-minute acute toxicity test. A substantial decrease in the bioluminescence of the controls during a 15-minute acute toxicity test may indicate that the acute reagent was not reconstituted as described in Section 3.1.6.

4.2.2 Standard Zinc Toxicant

As recommended by the Microtox™ system manufacturer, a zinc sulphate solution can be used as a standard toxicant to verify that the Microtox™ system is performing as expected. The toxicity of a solution containing 50.8 mg/L of zinc sulphate was assessed. The resulting IC$_{50}$ was 7.0 ± 4.1 mg/L ZnSO$_4$ (± corresponds to the 95% confidence interval). The normal IC$_{50}$ range is between 5 and 12 mg/L ZnSO$_4$ (Microbics Corporation, 1992). The results indicated that the Microtox™ system was performing as expected. Figure A.1 (APPENDIX A) contains a plot of the data obtained from the standard zinc toxicant investigation.

4.2.3 SPE Reconstitution Water

Preliminary tests suggested that the type of water used to reconstitute the SPE column extracts could decrease the bioluminescence of the *Vibrio fischeri* used in the Microtox™ toxicity test system. The effects of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), and distilled de-ionized water on the bioluminescence of *Vibrio fischeri* was investigated further.

The impact of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), and distilled de-ionized water, on the bioluminescence of *Vibrio fischeri* were tested using the detailed basic test protocol (Microbics Corporation, 1992). The results are presented in Table 14, and the raw data is found in Table A.1 (APPENDIX A).
As presented in Table 14, de-ionized water, obtained from a Milli-Q water system (Molsheim, France), had the least affect on the bioluminescence of *Vibrio fischeri* measured using the Microtox™ system. Therefore, de-ionized water, from a Milli-Q water system (Molsheim, France), was used in all subsequent sample preparation and analytical procedures used in the present study.

### 4.2.4 SPE Correction Factors

SPE columns were used to retain and fractionate anionic surfactants on the basis of hydrophobicity and molecular weight (see Section 2.6). The SPE columns used in the present study contained 0.5 g of reverse-phase packing material. Preliminary trials indicated that substances eluted from blank packing materials induced a toxic response measured using the Microtox™ system. Attempts were made to remove the substances inducing toxicity by thoroughly rinsing the SPE column during the activation procedure. However, the blank SPE column extract continued to induce toxicity in the Microtox™ test, despite thorough rinsing. The technical support personnel at Microbics Corporation recommended that a series of correction factors could be used to adjust the results from the Microtox™ test to account for the toxic responses induced by the blank SPE columns.

The first step in determining the magnitude of the correction factors was to establish the percent reduction in the bioluminescence of *Vibrio fischeri*, measured using the Microtox™ system, when blank reconstituted SPE column extracts were analyzed. An activated SPE column was loaded with 50 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France). Extraction was carried out using solutions having 65%, 75% and 90% methanol in.
water (V/V). The extracts were evaporated and made up to a volume of 25 mL. These procedures followed those outlined in Section 3.1.4.

Table 15 lists the magnitude of the reduction in the bioluminescence of *Vibrio fischeri*, measured using the Microtox™ system for different dilutions. Values are reported as the percent decrease in bioluminescence resulting from the blank SPE column extract. Table B.1 (APPENDIX B) contains the percent reductions in bioluminescence, measured using the Microtox™ system, for all three trials.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Light decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.625(%)</td>
</tr>
<tr>
<td></td>
<td>11.25(%)</td>
</tr>
<tr>
<td></td>
<td>22.5(%)</td>
</tr>
<tr>
<td></td>
<td>45(%)</td>
</tr>
<tr>
<td>65% Fraction</td>
<td>0.77</td>
</tr>
<tr>
<td>75% Fraction</td>
<td>0.36</td>
</tr>
<tr>
<td>90% Fraction</td>
<td>0</td>
</tr>
</tbody>
</table>

The 45% dilution exhibited the greatest decrease in bioluminescence for all three elution fractions. Of the three elution fractions, the 90% elution fraction had the greatest induced toxicity.

In subsequent tests using samples of reconstituted SPE column extracts, correction factors, corresponding to the respective dilution and elution fraction, were used to correct the measured 15-minute bioluminescence values.

### 4.2.5 Residual Methanol in the Reconstituted SPE Extracts

Methanol was used in the present study to separate anionic surfactants into discrete fractions, and to rinse analyte from the elution vials into beakers containing the SPE extracts (see Section 3.1.4.F and G). However, methanol is known to inhibit the bioluminescent bacteria used in the Microtox™ system (see Section 2.7.1). Therefore, the methanol in the SPE extracts must be removed before toxicity analyses can be performed.
A) Methanol Evaporation

A methanol evaporation procedure was developed to reduce the methanol concentration in the reconstituted SPE column extracts without decreasing the recovery of analyte. A series of tests were performed using solutions containing methanol in water at concentrations equivalent to those used in the SPE elution procedures (see Section 3.1.4.F). As the concentration of methanol in water increases, the boiling point of methanol decreases (Stecher, 1968). For this reason, a second series of tests were performed in which the concentration of methanol in water was increased by adding 100% methanol (see Section 3.1.4.G).

Solutions 1 and 2 contained 4 mL of 70% and 90% methanol in water (V/V), respectively. Solutions 3 and 4 contained 2 mL of 100% methanol plus 2 mL of 70% and 90% methanol in water (V/V), respectively. These solutions were heated in a water bath having a temperature of approximately 90 °C. After 4 minutes of heating, 5 mL of de-ionized water, obtained from a Milli-Q water system (Molsheim, France), was added to the solutions. The solutions were heated for an additional 15 to 30 minutes. The methanol concentrations in the solutions were determined at the end of the different heating periods. The results are presented in Figure 11.

Figure 11: Residual methanol concentrations versus heating time

![Figure 11: Residual methanol concentrations versus heating time](image-url)
Following 20 minutes of heating, solutions 3 and 4 had the lowest residual concentrations of methanol. The residual methanol concentrations after 20 minutes of heating were 316 mg/L, 79 mg/L, not detected, and 8 mg/L, respectively for solutions 1, 2, 3, and 4, respectively.

Following 20 minutes of heating, the estimated decrease in the bioluminescence of *Vibrio fischeri*, corresponding to a methanol concentration of 8 mg/L found in solution 4, is 0.2% (see Section 4.2.5.A). This decrease is very low and considered acceptable given the purpose of the present study which is to identify trends rather than obtain absolute values. Therefore, the addition of 2 mL of 100% methanol to the SPE extracts followed by a heating time of 20 minutes was used in the present study as outlined in Sections 3.1.4.F and G.

**B) Toxicity of Methanol**

The toxicity of methanol to the bioluminescent bacteria used in the Microtox™ system was investigated. Three methanol in water solutions were prepared. Following the 100% test procedure (see Section 2.7), the toxicity of methanol to *Vibrio fischeri* was determined using the Microtox™ toxicity test system. Table 16 contains the results of the investigations, and the raw data is presented in Figures A.2, A.3, A.4 (for methanol concentrations of 791, 1582 and 3955 mg/L, respectively) (APPENDIX A).

<table>
<thead>
<tr>
<th>Residual amount of methanol in solution</th>
<th>15 min IC$_{50}$ (mg/L methanol)</th>
<th>15 min IC$_{50}$ (mg/L methanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>791 mg/L methanol</td>
<td>2 300*</td>
<td>11 700*</td>
</tr>
<tr>
<td>1582 mg/L methanol</td>
<td>1 200</td>
<td>12 000*</td>
</tr>
<tr>
<td>3955 mg/L methanol</td>
<td>1 700</td>
<td>3 800*</td>
</tr>
</tbody>
</table>

* Values obtained by extrapolation.
Chapter 5

Sampling Program & Characterization of Primary Effluent

Lions Gate primary effluent was characterized on three occasions. Grab samples were collected in the morning, evening and night on two sampling events, while a morning grab sample was collected on the third occasion. This third sample was used in treatment studies presented in Chapter Six.

The primary effluent was characterized in terms of the concentration of anionic surfactants (measured as MBAS) and the anionic surfactant induced toxicity (measured using the Microtox™ system). These parameters were measured in the whole filtered samples, and in the 65%, 75% and 90% reconstituted SPE extracts.

UBC Pilot Plant primary effluent was also characterized in terms of the concentration of anionic surfactants and the anionic surfactant induced toxicity. These parameters were measured in whole filtered samples, and in the 65%, 75% and 90% reconstituted SPE extracts. The results of the UBC Pilot Plant primary effluent characterization study are presented in Appendix D.

5.1 Sampling at The Lions Gate WWTP

Characterization studies were undertaken using the primary effluent from the Lions Gate WWTP. The primary effluent was sampled on December 16,\textsuperscript{th} 2003 at 9:30 AM, 6:30 PM, and 11:30 PM and on February 3,\textsuperscript{rd} 2004 at 9:45 AM, 6:45 PM and 11:30 PM. Both sampling events were performed during periods of rain. A grab sample was taken on the morning of February 26,\textsuperscript{th} 2004 for use in the characterization study (current Chapter) and in the treatability study that is presented in Chapter Six.

For the grab samples collected on December 16,\textsuperscript{th} 2003 and February 3,\textsuperscript{rd} 2004, preliminary sample preparation was done on-site at the Lions Gate WWTP to minimize the biodegradation of MBAS. Approximately 250 mL of primary effluent was filtered (see Section 3.1.2). Of the filtered sample, 150 mL was withdrawn and loaded onto three SPE columns as
Chapter 5. Sampling Program & Characterization of Primary Effluent

outlined in Section 3.1.4.D. The SPE columns were wrapped in aluminum foil and stored along with the remaining whole filtered sample (see Section 3.1.3). All subsequent sample preparation procedures and analyses were performed at UBC in the Environmental Engineering Laboratory. Sample handling procedures, for the grab sample collected on February 26,\textsuperscript{th} 2004, are outlined in Section 6.1.A. Table 17 contains an accounting of sample storage and analysis times.

Table 17: Sample storage and analysis times - characterization.

<table>
<thead>
<tr>
<th>Sample Collection</th>
<th>MBAS Analysis</th>
<th>Microtox\textsuperscript{TM} Analysis</th>
<th>Methanol Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:30 AM Dec 16</td>
<td>afternoon Dec 16</td>
<td>afternoon Dec 16</td>
<td>night Dec 18</td>
</tr>
<tr>
<td>6:30 PM Dec 16</td>
<td>evening/night Dec 16</td>
<td>morning Dec 18</td>
<td>night Dec 18</td>
</tr>
<tr>
<td>11:30 PM Dec 16</td>
<td>after night Dec 16</td>
<td>afternoon Dec 18</td>
<td>night Dec 18</td>
</tr>
<tr>
<td>9:45 AM Feb 3</td>
<td>afternoon Feb 3</td>
<td>afternoon Feb 4</td>
<td>night Feb 5</td>
</tr>
<tr>
<td>6:45 PM Feb 3</td>
<td>night/morning Feb 3/4</td>
<td>morning Feb 5</td>
<td>night Feb 5</td>
</tr>
<tr>
<td>11:45 PM Feb 3</td>
<td>after night Feb 4</td>
<td>afternoon Feb 5</td>
<td>night Feb 5</td>
</tr>
<tr>
<td>8:00 AM Feb 26</td>
<td>Feb 27</td>
<td>Feb 27</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

5.2 Characterization of the Lions Gate WWTP primary effluent

The results from the characterization study of the Lions Gate WWTP primary effluent are presented in the sections below. The results are presented in terms of: MBAS concentration and the induced toxicity.

5.2.1 MBAS

MBAS was measured in the whole filtered samples and in the 65%, 75% and 90% reconstituted SPE elution fractions. Cumulative recoveries to each of the three elution fractions were calculated based on the MBAS concentration measured in the whole filtered sample.

In general, cumulative recoveries were high with the exception of the morning sample taken on February 3,\textsuperscript{rd} 2004. The poor recoveries observed for the reconstituted SPE column extracts prepared from the sample collected on the morning of February 3,\textsuperscript{rd} 2004 are likely due to an analytical error. These results are considered to be outliers and have not been included in subsequent discussions or in plots of the data presented in this Chapter. The results are
summarized in Table 18 and illustrated in Figure 12. (Detailed results can be found in Tables C.1, C.2 and C.3 (APPENDIX C).

Table 18: MBAS concentration measured in the Lions Gate WWTP primary effluent.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Morning</th>
<th>Evening</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MBAS (mg/L)</td>
<td>Cum. Rec. (%)</td>
<td>MBAS (mg/L)</td>
</tr>
<tr>
<td>Dec 16</td>
<td>Whole</td>
<td>1.20 ± 0.35</td>
<td>4.30 ± 0.60</td>
<td>6.40 ± 1.32</td>
</tr>
<tr>
<td>Dec 16</td>
<td>65%</td>
<td>0.42 ± 0.22</td>
<td>35</td>
<td>1.10 ± 0.24</td>
</tr>
<tr>
<td>Dec 16</td>
<td>75%</td>
<td>0.76 ± 0.10</td>
<td>98</td>
<td>2.80 ± 1.30</td>
</tr>
<tr>
<td>Dec 16</td>
<td>90%</td>
<td>0.10 ± 0.10</td>
<td>106</td>
<td>0.38 ± 0.21</td>
</tr>
<tr>
<td>Feb 3</td>
<td>Whole</td>
<td>4.39</td>
<td>8.63</td>
<td>9.34</td>
</tr>
<tr>
<td>Feb 3</td>
<td>65%</td>
<td>outlier</td>
<td>outlier</td>
<td>2.28 ± 1.85</td>
</tr>
<tr>
<td>Feb 3</td>
<td>75%</td>
<td>outlier</td>
<td>outlier</td>
<td>5.89 ± 1.94</td>
</tr>
<tr>
<td>Feb 3</td>
<td>90%</td>
<td>outlier</td>
<td>outlier</td>
<td>1.60 ± 0.32</td>
</tr>
<tr>
<td>Feb 26</td>
<td>Whole</td>
<td>3.71 ± 1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb 26</td>
<td>65%</td>
<td>1.70 ± 0.35</td>
<td>45.8</td>
<td></td>
</tr>
<tr>
<td>Feb 26</td>
<td>75%</td>
<td>2.39 ± 0.82</td>
<td>110.2</td>
<td></td>
</tr>
<tr>
<td>Feb 26</td>
<td>90%</td>
<td>0.39 ± 0.21</td>
<td>120.8</td>
<td></td>
</tr>
</tbody>
</table>

± corresponds to the 95% confidence interval of the measurements made, n=3

Figure 12: MBAS versus time - whole filtered primary effluent.
The MBAS concentration ranges in the morning between 1.20 mg/L and 4.39 mg/L, and increases throughout the day ranging between 6.4 mg/L and 9.34 mg/L late at night. The sampling events, which occurred on December 16,\textsuperscript{th} 2003 and February 3,\textsuperscript{rd} 2004, both took place during periods of rain. Storm water and seawater infiltration is known to significantly increase the wet-weather flows to the Lions Gate WWTP. As a result, the characteristics of the Lions Gate WWTP effluent is impacted by rain events. It can be expected that the dilution effect, caused by rain events, can reduce the MBAS concentration in the wastewater. The difference in the MBAS concentrations observed between the two sampling events can potentially be explained by the difference in the magnitude of the rain intensity on both of these days which causes storm water and seawater infiltration. Despite the difference in the MBAS concentrations between the two days, there is a trend exhibited in which MBAS concentration increases throughout the day and into the night.

The MBAS content of the 65\%, 75\% and 90\% elution fractions, reported as a percentage of the whole filtered mass of MBAS, was determined for the morning, evening and night sampling events. The percent of the whole filtered mass of MBAS, in each elution fraction, was averaged for the two sampling events and results are presented in Figure 13. Since the morning sample collected on February 3,\textsuperscript{rd} 2004 contained outliers in the reconstituted SPE extracts, the data obtained from the morning sample collected on February 26,\textsuperscript{th} 2004 was used to replace the outliers.

**Figure 13:** Average MBAS content in each of the elution fractions.
As presented in Figure 13, the percentage of the whole filtered mass of MBAS in each of the elution fractions was similar for each sampling time (i.e., morning, evening, and night) (based on a 95% confidence interval). The distribution of MBAS observed in the three elution fractions likely reflects the original surfactant formulations discharged to the wastewater collection system. Recall from Table 1 that many anionic surfactant formulations have somewhat normal distributions, centered around a particular molecular weight as is also the case in Figure 13. The observed MBAS distribution confirms the work done by EVS (2003) in which the concentration of MBAS, found in the high molecular weight fraction, was lower than the concentration found in either the low or medium molecular weight fractions (see Section 2.8).

The results of the MBAS analysis are further discussed in Section 5.2.3.

### 5.2.2 Toxicity

Methanol concentrations were measured in the samples prepared for toxicity tests, and the concentrations are presented in Tables C.4 and C.5 (APPENDIX C). The highest methanol concentrations measured in the reconstituted SPE extracts was 478 mg/L. As presented in section 4.2.5.B, at this methanol concentration, the bioluminescence of *Vibrio fischeri* is estimated to decrease by 6.2%. This value is not expected to significantly impact the toxicity measurements, as it is just one of three replicates.

15-minute acute toxicities were determined for the whole filtered sample and for the 65%, 75% and 90% reconstituted SPE extracts on both December 16,\(^{th}\) 2003 and February 3,\(^{rd}\) 2004 for the morning, evening and night sampling events. On February 26,\(^{th}\) 2004 only the whole filtered sample and the 90% reconstituted SPE extract toxicities were determined. The results are summarized in Table 19. Raw data is presented in Figures C. 3 to C.28 (Appendix C).

The toxicities are presented in terms of the percent of sample volume and in terms of the concentration of MBAS required to produce a 20% reduction in the bioluminescence of *Vibrio fischeri* measured using the Microtox\textsuperscript{TM} system. With this nomenclature, a lower percentage, or a lower concentration, is indicative of a sample exhibiting greater toxicity.
Table 19: Toxicity contained in the Lions Gate WWTP primary effluent.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Morning</th>
<th>Evening</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC₂₀ % (Vol)</td>
<td>IC₂₀ mg/L MBAS</td>
<td>IC₂₀ % (Vol)</td>
</tr>
<tr>
<td>Dec 16</td>
<td>Whole</td>
<td>18.93±5.62</td>
<td>13.59±5.58</td>
<td>9.08±0.71</td>
</tr>
<tr>
<td>Dec 16</td>
<td>65%</td>
<td>100*±212</td>
<td>109*±61</td>
<td>52*±36.87</td>
</tr>
<tr>
<td>Dec 16</td>
<td>75%</td>
<td>36.26±37.94</td>
<td>33.36±9.05</td>
<td>17.64±23.19</td>
</tr>
<tr>
<td>Dec 16</td>
<td>90%</td>
<td>18.02±27.18</td>
<td>34.44±17.06</td>
<td>17.82±7.97</td>
</tr>
<tr>
<td>Feb 3</td>
<td>Whole</td>
<td>26.61±7.37</td>
<td>13.17±2.53</td>
<td>11.81±1.60</td>
</tr>
<tr>
<td>Feb 3</td>
<td>65%</td>
<td>outlier</td>
<td>43.08±16.04</td>
<td>44.56±25.51</td>
</tr>
<tr>
<td>Feb 3</td>
<td>75%</td>
<td>outlier</td>
<td>21.99±17.83</td>
<td>22.80±26.90</td>
</tr>
<tr>
<td>Feb 3</td>
<td>90%</td>
<td>outlier</td>
<td>10.27±6.22</td>
<td>8.42±7.42</td>
</tr>
<tr>
<td>Feb 26</td>
<td>Whole</td>
<td>16.94±5.48</td>
<td>0.63±0.27</td>
<td></td>
</tr>
<tr>
<td>Feb 26</td>
<td>90%</td>
<td>68.15*±35.39</td>
<td>0.27*±0.20</td>
<td></td>
</tr>
</tbody>
</table>

± corresponds to the 95% confidence interval of Microtox™ measurements, n=3
* value obtained by extrapolation

The toxicity contained in the whole filtered primary effluent from the Lions Gate WWTP versus time is presented in Figure 14.

The percent of sample volume needed to cause an IC₂₀, measured using the Microtox™ system, ranges from 17% to 27% (V/V) in the morning, and decreases throughout the day ranging from 9% to 12% late at night. As presented in Figure 14, the whole filtered primary effluent became more toxic throughout the day and into the night. Storm water and seawater
infiltration is known to significantly increase the wet-weather flows to the Lions Gate WWTP. As a result, the characteristics of the Lions Gate WWTP effluent is impacted by rain events. It can be expected that the dilution effect, caused by rain events, can reduce the MBAS concentration in the wastewater. Therefore, the difference in the anionic surfactant induced toxicities contained in the whole filtered samples on the two sampling events can potentially be explained by the difference in the magnitude of the rain intensity on both of these days which causes storm water and seawater infiltration. Despite the difference in the toxicities between the two days, there is a trend exhibited in which toxicity increases throughout the day and into the night.

As presented in Figure 15, a linear relationship was observed between the IC_{20} values (expressed as percent of sample) and MBAS concentrations in the whole filtered sample. The slopes of the lines, for the two days, are relatively similar. While wet-weather conditions may influence the magnitudes of the MBAS concentrations and IC_{20} values, the toxicological response (slope of the lines) to an increasing MBAS concentration is similar for both sampling events.

Figure 15: Toxicity of whole filtered primary effluent versus MBAS concentration.
Figures 16 and 17 are plots of IC$_{20}$'s, expressed as percent volume and as mg/L MBAS versus elution fractions for December 16$^{th}$, 2003. Similarly, Figures 18 and 19 are plots of IC$_{20}$'s, expressed as percent volume and as mg/L MBAS versus elution fractions for February 3$^{rd}$ and February 26$^{th}$, 2004. The results from February 3$^{rd}$, 2004 are outliers and have not been included in the plots (see Section 5.2.1).


The IC$_{20}$'s expressed in terms of mg/L MBAS (Figure 17) were calculated based on the IC$_{20}$, as percent volume, for a given sample and the corresponding MBAS concentration for that sample. However, the relationship between the magnitude of the values for the IC$_{20}$ expressed as percent volume (Figure 16) and as mg/L MBAS (Figure 17) is not consistently similar. This discrepancy is likely due to the error associated with the measured MBAS concentrations and IC$_{20}$ values determined using the Microtox™ system. Tables 18 and 19 contain the experimental uncertainty, at the 95% confidence interval, associated with the measured MBAS concentrations and toxicities. Despite the discrepancies that are likely a result of experimental uncertainties, overall trends can be observed.
Figures 18 & 19: IC\textsubscript{20}'s versus elution fractions - February 3 and 26, 2004.

Figure: 18. Figure: 19.

The trends exhibited in Figures 16 and 18 show that the toxicity of the reconstituted SPE extracts, measured using the Microtox\textsuperscript{TM} system, increases as the eluting solvent strength increases. Similarly, the trends exhibited in Figures 17 and 19 show that the toxicity of the reconstituted SPE extracts is greatest for the 90\% elution fraction. These results indicate that the 90\% elution fraction is consistently the most toxic component of the whole filtered primary effluent. These findings are consistent with the discussion presented in Section 2.3 which indicates that hydrophobic anionic surfactants are more toxic to aquatic organisms. In addition, these findings support the results obtained from a study conducted by EVS (2003) (see Section 2.8). In the study conducted by EVS (2003), toxicity tests were conducted using three marine species as the test organisms. The more hydrophobic (i.e., higher molecular weight) fractions were found to be more toxic to the test organisms.

The results from the present study and those from the EVS (2003) study suggests that the *Vibrio fischeri* used in the Microtox\textsuperscript{TM} test system respond in a similar manner to other aquatic organisms (i.e., *A. affinis*, *M. galloprovincialis* and *M. pyrifera*) when exposed to anionic surfactants. The toxicity analysis results are further discussed in Section 5.2.3.
5.2.3 Toxicological Impact

The average toxicities and the average MBAS concentrations, for the sampling events of December 16, 2003 February 3, and 26, 2004, are presented in Figures 20 and 21.

Figures 20 & 21: Average toxicity and average MBAS measured during all sampling events.

The 90% elution fraction was identified as being the most toxic of the three elution fractions studied (see Section 5.2.2). In addition, it was found that the 90% elution fraction had the lowest amount of MBAS (see Section 5.2.1). These results indicate that a relatively large proportion of the toxicity that is associated with the primary effluent is due to a relatively small proportion of the total MBAS concentration. These findings are consistent with the discussion presented in Section 2.3 that indicates that the high molecular weight anionic surfactants are more toxic to aquatic organisms. These findings also confirm the previous work of EVS (2003) (see Section 2.8).
The toxicological impact of the different elution fractions is defined as the percent of MBAS in excess of that amount of MBAS which corresponds with the IC$_{20}$. The calculated values for the toxicological impact of the different elution fractions are presented in Figure 22.

Figure 22: Toxicological impact of elution fractions.

While not based on a 95% confidence interval, the 90% elution fraction appears to have the greatest toxicological impact of the three elution fractions. Even though the relative abundance of MBAS contained in the 90% elution fraction is low (see Figure 21), the toxicity of the 90% elution fraction is the highest of the three elution fractions (see Figure 20). Therefore, the 90% elution fraction has the greatest toxicological impact of the elution fractions studied. The molecular structure of the anionic surfactant molecules contained in the 90% elution fraction likely accounts for this observation. In the 90% elution fraction, high molecular weight anionic surfactant molecules are likely present (see Section 2.3). For this reason, the 90% elution fraction was the subject of further investigations as reported in Chapter Six.
Chapter 6

Treatability Studies

A treatability study was conducted in which various physical/chemical and bio-treatment methods were considered to remove both anionic surfactants and anionic surfactant induced toxicity from a primary effluent. The treatability study was designed to screen the treatment methods, and it was a minor aspect in the overall work completed as part of the present study. The four treatment methods considered were bio-treatment, alum coagulation/flocculation with gravity settling, ozonation and air flotation. The experimental procedures used as part of the treatability study are presented in Section 3.2. The basis for selecting these four methods is discussed below and in Section 2.4. Table 20 contains a summary of the rationale for selecting each of the treatment methods considered.

<table>
<thead>
<tr>
<th>Treatment Method</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Bio-Treatment</td>
<td></td>
</tr>
<tr>
<td>A) Partitioning to Abiotic Bio-Solids</td>
<td>Documented ability of solids to adsorb and remove LAS from solution (see Section 2.4.1).</td>
</tr>
<tr>
<td>B) Biological Degradation</td>
<td>Documented ability of bio-solids to degrade up to 85% of LAS while producing less toxic by-products (see Sections 2.3.1 and 2.4.1).</td>
</tr>
<tr>
<td>II Alum Coagulation/Flocculation with Gravity Settling</td>
<td>Findings from a previous study indicated that a 50% reduction in MBAS concentration was possible (see Section 2.8).</td>
</tr>
<tr>
<td>III Ozonation</td>
<td>Documented ability to oxidize LAS (see Section 2.4.2).</td>
</tr>
<tr>
<td>IV Air Flotation</td>
<td>Favourable surface chemistry. Pre-concentrates anionic surfactants.</td>
</tr>
</tbody>
</table>

A literature search did not find any studies that investigated the removal of anionic surfactants from wastewater using air flotation. However, because of their surface characteristics, anionic surfactants are expected to adsorb to the surface of air bubbles (Rubio et al., 2002). Subsequently, the air bubbles and adsorbed surfactants are expected to rise to the surface of the liquid where they form a froth layer that can be removed.
6.1 Sampling

Table 21 contains an accounting of sample storage times.

Table 21: Date on which sample analyses were conducted during the treatment study.

<table>
<thead>
<tr>
<th>February 26</th>
<th>February 27</th>
<th>February 28</th>
<th>March 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration and SPE</td>
<td>Microtox™</td>
<td>Whole Sample MBAS</td>
<td>Fractionated MBAS</td>
</tr>
<tr>
<td>1,2,3,4,5,6,7</td>
<td>1,2,3</td>
<td>1,2,3,4,5,6,7</td>
<td>1 (n=1&amp;2)</td>
</tr>
<tr>
<td>Fractionated MBAS</td>
<td>Fractionated MBAS</td>
<td>Fractionated MBAS</td>
<td></td>
</tr>
<tr>
<td>1,2,3</td>
<td>1 (n=3) 2,3</td>
<td>4,5,6,7</td>
<td></td>
</tr>
</tbody>
</table>

1 = raw filtered effluent, 2 = air flotation effluent, 3 = ozonation effluent, 4 = UBC Pilot Plant waste activated sludge, 5 = biological effluent, 6 = partitioning to abiotic bio-solids effluent, 7 = alum coagulation/flocculation with gravity settling effluent. n = number of replicates.

A) Lions Gate WWTP

Primary effluent was obtained from Lions Gate WWTP on the morning of February 26th, 2004 for use in the treatability study. 8 L of primary effluent was collected from the Lions Gate WWTP (see Section 3.1.1). The primary effluent was stored in 2-4 L brown glass bottles at 4 °C until needed.

B) UBC Pilot Plant

A waste activated sludge sample was collected from the UBC Pilot Plant on the morning of February 26th, 2004. This sample was used in the bio-treatment test (see Sections 3.2.1.A and B). 1.5 L of waste activated sludge was collected from the UBC Pilot Plant, and was stored in a 2 L glass bottle at 4 °C until needed.

6.2 Removal of MBAS

The MBAS removal efficiency was determined by comparing the MBAS concentrations in the treated effluent to that present in the primary effluent before treatment. For bio-treatment where the primary effluent was diluted with waste activated sludge, the starting MBAS concentrations were calculated using a mass balance approach (see Section 3.2.7). The results from the treatability study are presented in Table 22. Tables E.1 through to E.8 (Appendix E)
include the raw MBAS data for the different treatments considered. Each treatment was conducted once, but MBAS measurements were done in triplicate.

Table 22: Treatability study: MBAS removal efficiencies.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Partitioning to solids</th>
<th>Biodegradation</th>
<th>Alum</th>
<th>Ozone</th>
<th>Air Flotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole filtered sample</td>
<td>64 ± 97</td>
<td>96 ± 83</td>
<td>46 ± 32</td>
<td>95 ± 39</td>
<td>77 ± 36</td>
</tr>
<tr>
<td>65%</td>
<td>56 ± 42</td>
<td>85 ± 49</td>
<td>63 ± 38</td>
<td>94 ± 29</td>
<td>66 ± 25</td>
</tr>
<tr>
<td>75%</td>
<td>100 ± 95</td>
<td>98 ± 95</td>
<td>49 ± 43</td>
<td>97 ± 48</td>
<td>77 ± 44</td>
</tr>
<tr>
<td>90%</td>
<td>67 ± 100</td>
<td>95 ± 100</td>
<td>0 ± 73</td>
<td>82 ± 78</td>
<td>74 ± 77</td>
</tr>
</tbody>
</table>

± Corresponds to 95% confidence interval

I Bio-Treatment

A) Biological Degradation

Biological treatment resulted in the highest overall MBAS removal efficiency with approximately 96% of the MBAS being removed during treatment. The suspended solids concentration was 1850 mg/L. The MBAS removal efficiency is slightly higher than removal efficiencies reported in the literature in which LAS removals of 80-85% are reported (Fauser et al., 2003; Kolbener et al., 1995).

Of the 96% of the MBAS removed, a large fraction was likely removed simply by the MBAS molecules partitioning to bio-solids. No attempt was made to quantify the relative contribution of both biological degradation and partitioning to bio-solids to the overall removal of MBAS.

B) Partitioning to Abiotic Bio-Solids

Overall, a 64% reduction in the MBAS concentration could be achieved through partitioning to abiotic bio-solids alone.
Bio-treatment was performed as a control to verify that the high MBAS removal efficiencies reported in the literature can be achieved (Fauser et al., 2003; Kolbener et al., 1995). However, bio-treatment methods are not a viable interim solution at the Lions Gate WWTP, as bio-treatment methods do not fulfill the objective of being an interim treatment method.

II Alum Coagulation/Flocculation with Gravity Settling

Overall, alum coagulation/flocculation followed by gravity settling resulted in poor MBAS removals. The overall MBAS removal in the whole filtered sample was 46%. This relatively low removal efficiency is consistent with those reported in a previous study (see Section 2.8). Of particular interest is the complete absence of any MBAS removal from the 90% elution fraction. Therefore, the presence of high molecular weight anionic surfactant molecules, following treatment with alum coagulation/flocculation with gravity settling, may contribute to the residual toxicity contained in the treated effluent measured in a previous study using primary effluent from the Lions Gate WWTP (CH2MHILL, 2002). Although this previous study confirmed that alum coagulation/flocculation with gravity settling can remove some of the MBAS from a primary effluent, results obtained in the present study suggest that alum coagulation/flocculation followed by gravity settling is not capable of removing the more toxic, high molecular weight fractions of the MBAS.

III Ozonation

Ozone effectively removed MBAS from the primary effluent with removal efficiencies comparable to those that could be achieved with biological treatment. However, the MBAS contained in the 90% reconstituted SPE extract was not as effectively removed in comparison to that which could be removed using biological treatment.

IV Air Flotation

Air flotation was effective at removing MBAS from the Lions Gate WWTP primary effluent. Approximately 66 to 77% of the MBAS present in the different elution fractions was
removed via air flotation. MBAS removals in the 75% and 90% elution fractions were higher than observed for the 65% elution fractions. This is likely due to the greater affinity of the more hydrophobic MBAS, contained in the 75% and 90% elution fractions, for the surface of rising air bubbles. The froth formed by the rising air bubbles and the associated partitioned MBAS was removed from the surface of the liquid. The volume of froth removed from the air flotation system during treatment was equivalent to approximately 16% of the total volume treated. This waste stream, containing the recovered anionic surfactants, will require further treatment. Biological treatment of the pre-concentrated waste stream may be a viable treatment method, since the volume of wastewater requiring treatment is significantly reduced following air flotation. However, further studies, beyond the scope of the present study, are needed.

6.3 Removal of Toxicity

The Microtox™ system was used to measure the toxicity contained in samples of the primary effluent from the Lions Gate WWTP before and after treatment. Gradient methanol elution using 65% and 75% methanol in water preceded elution using 90% methanol in water. However, the toxicities were only determined in the whole filtered sample and in the 90% elution fraction. The 90% elution fraction was the only fraction tested because previous results indicated that the 90% elution fraction was more toxic than the other elution fractions (see Section 5.2.3).

Toxicities were measured following treatment by ozonation and air flotation. Toxicities were not determined following bio-treatment and alum coagulation/flocculation with gravity settling. As part of these treatments, foreign materials (i.e., alum, bio-solids or sodium azide) were added to the primary effluent. As a result, it was not possible to precisely determine the impact of treatment on the original primary effluent matrix.

The results of the treatability study are presented in Table 23. The raw data, used to calculate the toxicity values, is presented in Figures E.1 through to E.6 (Appendix E). A non-toxic response is one in which greater than 100% sample by volume would be required to cause a 20% reduction in bioluminescence.
Table 23: Treatability study: toxicity before and after treatment.

<table>
<thead>
<tr>
<th>Sample</th>
<th>15 min IC50 (% V/V)</th>
<th>Percent Decrease in Toxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Effluent</td>
<td>Treated Ozone</td>
</tr>
<tr>
<td>Whole filtered sample</td>
<td>17 ± 5</td>
<td>12 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td>68* ± 35</td>
<td>Non-Toxic</td>
</tr>
</tbody>
</table>

± corresponds to 95% confidence intervals
* value obtained by extrapolation
( ) denotes an increase in toxicity

6.3.1 Ozonation

Ozone treatment rendered the 90% elution fraction non-toxic (i.e., greater than 100% sample by volume would be required to cause a 20% reduction in bioluminescence). However, the toxicity of the whole filtered sample increased slightly, although not significantly, based on a 95% confidence interval. It is possible that by-products of the oxidation process were formed and were responsible for the increase in toxicity. These results are consistent with those found by Monarco, et al., (2000) in which the toxicity of a secondary effluent increased following ozonation (Monarco, et al., 2000). However, further investigation in this area was beyond the scope of the present study.

6.3.2 Air Flotation

Air flotation significantly reduced the toxicity of the treated whole filtered sample by 45 ± 24%, based on a 95% confidence interval. Following treatment, the 90% reconstituted SPE extract was rendered non-toxic (i.e., greater than 100% sample by volume would be required to cause a 20% reduction in bioluminescence). The more hydrophobic anionic surfactant molecules, contained in the 90% reconstituted SPE extract, may be preferentially removed from solution by partitioning to the rising air bubbles forming a froth that is removed from the surface. It should be noted that the removed froth product likely contains a high concentration of anionic surfactants. Therefore, this waste stream will likely require further treatment. Biological treatment of the pre-concentrated waste stream may be a viable treatment method, as the volume
of wastewater requiring treatment is significantly reduced following air flotation and since biological treatment resulted in the highest MBAS removals as found in the present study.
Chapter 7

Conclusions

A series of analytical methods were developed to quantify the concentration of anionic surfactants in the primary effluent, to fractionate the anionic surfactants on the basis of molecular weight, and to measure the toxicity of the whole and discrete molecular weight fractions. A modified methylene blue active substances (MBAS) test was used to quantify the concentration of anionic surfactants contained in the primary effluent of the Lions Gate WWTP. Solid phase extraction columns and gradient methanol elution were used to fractionate the anionic surfactants on the basis of hydrophobicity and, therefore, molecular weight. Following sample preparation procedures, the modified MBAS method produced results with good precision and with high recoveries. The toxicity of the whole and discrete molecular weight fractions was determined by measuring the bioluminescence of *Vibrio fischeri* using the Microtox™ toxicity test system. The response of the *Vibrio fischeri*, used in the Microtox™ system, to anionic surfactants was consistent with the responses of three marine aquatic organisms to anionic surfactants (EVS, 2003).

The primary effluent from the Lions Gate WWTP was characterized in terms of the concentration of anionic surfactants and the anionic surfactant induced toxicity contained in whole samples and in discrete molecular weight fractions. The concentration of MBAS, contained in the primary effluent of the Lions Gate WWTP, was found to increase throughout the day. As the concentration of MBAS increased, so too did the toxicity. The concentration of MBAS contained in the three reconstituted SPE elution fractions was greatest in the 75% fraction, followed by the 65% fraction and lowest in the 90% fraction. Despite having the lowest amount of MBAS, the toxicity of the material contained in the 90% reconstituted SPE elution fraction was the highest. In addition, the toxicological impact, defined as the percent of MBAS in excess of the IC$_{20}$, was greatest for the 90% elution fraction.

A treatability study was conducted to investigate the removal of MBAS and the associated toxicity from the Lions Gate WWTP primary effluent. The treatability study was
designed to screen the treatment methods, and it was a minor aspect in the overall work completed as part of the present study. Four treatment methods were considered: bio-treatment, alum coagulation/flocculation with gravity settling, ozonation, and air flotation. Biological treatment resulted in the highest MBAS removals followed by ozonation, air flotation and finally alum coagulation/flocculation with gravity settling. Air flotation effectively decreased the toxicity of the whole filtered sample by 45%, and the toxicity of the most toxic components of the MBAS (i.e., the 90% elution fraction) by 100%. The recovered froth product, requiring further treatment, had a significantly reduced volume.
Chapter 8

Recommendations

When *Vibrio fischeri* are exposed to fractionated anionic surfactants (on the basis of hydrophobicity and therefore molecular weights), the toxicity trends determined using the Microtox™ test system were similar to those found in a previous study in which three marine aquatic organisms were used (EVS, 2003). The Microtox™ system is fast and relatively simple to use. For these reasons, the continued use of the Microtox™ system is recommended.

Full treatment using activated sludge is not a viable interim treatment method at Lions Gate WWTP. Air flotation, however, appears to be a useful means of pre-concentrating the toxicant and reducing anionic surfactant induced toxicity from a primary effluent. Further studies investigating the efficiency of toxicant removal, using air flotation and subsequent treatment of the recovered foam, are warranted.
List of References


List of References


EVS. 2003. Toxicity Tests Using Marine Species and Identification of Causes of Toxicity in Effluent from the Lions Gate Wastewater Treatment Plant. Prepared for the Greater Vancouver Regional District (GVRD), Burnaby, BC by EVS Environment Consultants, North Vancouver, BC.


List of References


Environmental Health Criteria 169 Linear Alkylbenzene Sulfonates and Related Compounds. *International Programme On Chemical Safety INCHEM.*


APPENDIX A: Analytical Method Development of Microtox™

A) Standard Zinc Toxicant

Figure A.1: Standard Zinc Toxicant
IC₅₀ - 15 Minute Acute Toxicity (n=3)
50.8 mg/L ZnSO₄

B) Methanol Toxicity to Microtox™

Figure A.2: Methanol Toxicity to Microtox™ (791 mg/L Methanol)
Figure A.3: Methanol Toxicity to Microtox™ (1582 mg/L Methanol)

\[ y = 0.5973x - 1.7193 \]
\[ r^2 = 0.909 \]

Figure A.4: Methanol Toxicity to Microtox™ (3955 mg/L Methanol)

\[ y = 1.6486x - 3.2777 \]
\[ r^2 = 0.9889 \]
C) Impact of Laboratory water on the Bioluminescence of *Vibrio fischeri*

Table A.1: Effect of Laboratory Water Source on Bioluminescence - Results

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<tr>
<th>Sample</th>
<th>Concentration (%)</th>
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<tbody>
<tr>
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<td>5.625</td>
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<tr>
<td>Reduction in Bioluminescence (%)</td>
<td></td>
</tr>
<tr>
<td>Milli-Q Trial 1</td>
<td>11.2</td>
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<tr>
<td>Milli-Q Trial 2</td>
<td>0.8</td>
</tr>
<tr>
<td>Distilled De-ionized water Trial 1</td>
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<td>Distilled De-ionized water Trial 2</td>
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<td>Distilled De-ionized water Trial 3</td>
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APPENDIX B: SPE Bioluminescence Correction Factors

Table B.1: Solid Phase Extraction Blank Correction Factors - Trials 1, 2 and 3

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<th>Reduction in Bioluminescence (%)</th>
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<td>1.09</td>
</tr>
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# APPENDIX C: Lions Gate Characterization Study

## A) MBAS - December 16,\textsuperscript{th} 2003

### Tables C.1: MBAS in Lions Gate effluent December 16,\textsuperscript{th} 2003

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<th>Absorbance</th>
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<td>1.34</td>
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<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
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<td>4.13</td>
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<table>
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<th>Sample</th>
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<th>MBAS Concentration (mg/L)</th>
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**APPENDIX C: Lions Gate Characterization Study**

B) MBAS - February 3,\textsuperscript{rd} 2004

Tables C.2: MBAS in Lions Gate effluent February 3,\textsuperscript{rd} 2004

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C) MBAS - February 26th, 2004

Tables C.3: MBAS in Lions Gate effluent February 26th, 2004

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<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
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<td>1.25</td>
<td>0.056</td>
<td>1.86</td>
</tr>
<tr>
<td>0.5</td>
<td>March 1st</td>
<td>0.03</td>
<td>75-2</td>
<td>1.25</td>
<td>0.072</td>
<td>2.39</td>
</tr>
<tr>
<td>1</td>
<td>March 1st</td>
<td>0.047</td>
<td>90-2</td>
<td>0.50</td>
<td>0.023</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>March 1st</td>
<td>0.104</td>
<td>65-3</td>
<td>1.25</td>
<td>0.048</td>
<td>1.59</td>
</tr>
<tr>
<td>4</td>
<td>March 1st</td>
<td>0.186</td>
<td>75-3</td>
<td>1.25</td>
<td>0.062</td>
<td>2.06</td>
</tr>
<tr>
<td>90-3</td>
<td>March 1st</td>
<td>0.036</td>
<td></td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
</tbody>
</table>
D) Methanol - December 16,\textsuperscript{th} 2003

Figure C.1 and Tables C.4: Methanol concentrations December 16,\textsuperscript{th} 2003

![Methanol Standard Curve](image_url)

---

### Reconstituted SPE Extracts
9:30 AM, Dec. 16, 2003

<table>
<thead>
<tr>
<th>Sample</th>
<th>methanol conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-1</td>
<td>226.32*</td>
</tr>
<tr>
<td>75-1</td>
<td>19.66</td>
</tr>
<tr>
<td>90-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-2</td>
<td>5.56</td>
</tr>
<tr>
<td>90-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-3</td>
<td>11.43</td>
</tr>
<tr>
<td>90-3</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*concentration obtained by extrapolation
N.D. Non-detect

### Reconstituted SPE Extracts
6:30 PM, Dec. 16, 2003

<table>
<thead>
<tr>
<th>Sample</th>
<th>methanol conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-1</td>
<td>154.81*</td>
</tr>
<tr>
<td>75-1</td>
<td>22.18</td>
</tr>
<tr>
<td>90-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-3</td>
<td>32.92</td>
</tr>
<tr>
<td>75-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-3</td>
<td>477.85*</td>
</tr>
</tbody>
</table>

*concentration obtained by extrapolation
N.D. Non-detect

### Reconstituted SPE Extracts
11:30 PM, Dec. 16, 2003

<table>
<thead>
<tr>
<th>Sample</th>
<th>methanol conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-1</td>
<td>25.43</td>
</tr>
<tr>
<td>75-1</td>
<td>79.21</td>
</tr>
<tr>
<td>90-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-3</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. Non-detect
E) Methanol - February 3,rd 2004

Figure C.2 and Tables C.5: Methanol concentrations February 3,rd 2004

![Methanol Standard Curve]

\[ y = 2.4255x \]
\[ R^2 = 0.9915 \]

<table>
<thead>
<tr>
<th>Sample</th>
<th>methanol conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-1</td>
<td>9.80</td>
</tr>
<tr>
<td>75-1</td>
<td>12.70</td>
</tr>
<tr>
<td>90-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-3</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. Non-detect

<table>
<thead>
<tr>
<th>Sample</th>
<th>methanol conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-1</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>90-2</td>
<td>N.D.</td>
</tr>
<tr>
<td>65-3</td>
<td>N.D.</td>
</tr>
<tr>
<td>75-3</td>
<td>14.30</td>
</tr>
<tr>
<td>90-3</td>
<td>54.30</td>
</tr>
</tbody>
</table>

N.D. Non-detect
F) Toxicity - December 16, 2003

Figure C.3: 9:30 AM, 65% elution fraction, Dec. 16, 2003.

Figure C.4: 9:30 AM, 75% elution fraction, Dec. 16, 2003.
APPENDIX C: Lions Gate Characterization Study

Figure C.5: 9:30 AM, 90% elution fraction, Dec. 16, 2003.

Figure C.6: 9:30 AM, whole filtered sample, Dec. 16, 2003.

Figure C.7: 6:30 PM, 65% elution fraction, Dec 16, 2003.
Figure C.8: 6:30 PM, 75% elution fraction, Dec. 16, 2003.
Figure C.9: 6:30 PM, 90% elution fraction, Dec 16, 2003.

Figure C.10: 6:30 PM, whole filtered sample, Dec 16, 2003.
Figure C.11: 11:30 PM, 65% elution fraction, Dec. 16, 2003.

\begin{align*}
\text{Trial 1, } y &= 1.3302x - 2.7873, \quad R^2 = 0.9944 \\
\text{Trial 2, } y &= 1.5186x - 3.0932, \quad R^2 = 0.9567 \\
\text{Trial 3, } y &= 1.2977x - 2.9925, \quad R^2 = 0.9281
\end{align*}

Figure C.12: 11:30 PM, 75% elution fraction, Dec. 16, 2003.

\begin{align*}
\text{Trial 1, } y &= 1.0927x - 1.6941, \quad R^2 = 0.9974 \\
\text{Trial 2, } y &= 0.9224x - 1.6843, \quad R^2 = 0.9967 \\
\text{Trial 3, } y &= 1.6277x - 2.9587, \quad R^2 = 0.9961
\end{align*}
Figure C.13: 11:30 PM, 90% elution fraction, Dec. 16, 2003.

Figure C.14: 11:30 PM, whole filtered sample, Dec. 16, 2003.
G) Toxicity - February 3, 2004

Figure C.15: 9:45 AM, 65% elution fraction, Feb 3, 2004.

\[ y = 0.4332x - 1.6366, r^2 = 0.6091 \]
\[ y = 0.8607x - 2.1955, r^2 = 0.8893 \]
\[ y = 0.3578x - 1.4814, r^2 = 0.4848 \]

Figure C.16: 9:45 AM, 75% elution fraction, Feb 3, 2004.

\[ y = 0.9247x - 1.9170, r^2 = 0.9247 \]
\[ y = 0.9794x - 1.9329, r^2 = 0.9989 \]
\[ y = 1.1832x - 2.3480, r^2 = 0.9939 \]
Figure C.17: 9:45 AM, 90% elution fraction, Feb 3, 2004.

Log Cone.

- Trial 1, \( y = 0.9581x - 1.9795, R^2 = 0.9167 \)
- Trial 2, \( y = 0.7734x - 1.5826, R^2 = 0.9496 \)
- Trial 3, \( y = 0.7039x - 1.5723, R^2 = 0.9705 \)

Figure C.18: 9:45 AM, whole filtered sample, Feb 3, 2004.

- Trial 1, \( y = 0.6662x - 1.5236, R^2 = 0.8626 \)
- Trial 2, \( y = 0.7279x - 1.6291, R^2 = 0.9554 \)
- Trial 3, \( y = 0.5484x - 1.4114, R^2 = 0.9414 \)
Figure C.19: 6:45 PM, 65% elution fraction, Feb 3, 2004.

- Trial 1, $y = 1.7280x - 3.4942$, $R^2 = 0.9634$
- Trial 2, $y = 1.0759x - 2.2718$, $R^2 = 0.9336$
- Trial 3, $y = 1.1044x - 2.4429$, $R^2 = 0.5460$

Figure C.20: 6:45 PM, 75% elution fraction, Feb 3, 2004.

- Trial 1, $y = 1.3291x - 2.1246$, $R^2 = 0.9862$
- Trial 2, $y = 1.4769x - 2.7356$, $R^2 = 0.9677$
- Trial 3, $y = 1.4253x - 2.5731$, $R^2 = 0.9963$
APPENDIX C: Lions Gate Characterization Study

Figure C.21: 6:45 PM, 90% elution fraction, Feb 3, 2004.

\[ y = 0.7615x - 1.2635, \quad R^2 = 0.9969 \]

Trial 1, \( y = 0.7615x - 1.2635, \quad R^2 = 0.9969 \)

Figure C.22: 6:45 PM, whole filtered sample, Feb 3, 2004.

\[ y = 0.9488x - 1.6300, \quad R^2 = 0.9826 \]

Trial 1, \( y = 0.9488x - 1.6300, \quad R^2 = 0.9826 \)
Figure C.23: 11:30 PM, 65% elution fraction, Feb 3, 2004.

Figure C.24: 11:30 PM, 75% elution fraction, Feb 3, 2004.
Figure C.25: 11:30 PM, 90% elution fraction, Feb 3, 2004.

![Graph showing log gamma vs log conc. for three trials with regression lines and R^2 values.](image1)

- Trial 1, $y = 0.8501x - 1.3732$, $R^2 = 0.9976$
- Trial 2, $y = 0.7232x - 1.3709$, $R^2 = 0.9958$
- Trial 3, $y = 0.7499x - 1.1645$, $R^2 = 0.9994$

Figure C.26: 11:30 PM, whole filtered sample, Feb 3, 2004.

![Graph showing log gamma vs log conc. for three trials with regression lines and R^2 values.](image2)

- Trial 1, $y = 0.9815x - 1.6321$, $R^2 = 0.9866$
- Trial 2, $y = 1.0904x - 1.7680$, $R^2 = 0.9932$
- Trial 3, $y = 1.1058x - 1.8146$, $R^2 = 0.9946$
H) Toxicity - February 26th, 2004

Figure C.27: Morning, 90% elution fraction, Feb 26, 2004.

Figure C.28: Morning, whole filtered sample, Feb 26, 2004.
APPENDIX D: UBC Pilot Plant

The UBC Environmental Engineering Pilot Plant is located on the south campus of UBC where it draws wastewater from the south sewer trunk. The south sewer collects domestic wastewater mainly from Acadia Park family housing and Hampton Place developments.

A) MBAS

The UBC Environmental Engineering Pilot Plant offered a convenient location from which primary effluent and waste activated sludge could be obtained. The primary effluent was used in method development and quality control investigations (Sections 4.1.1, 4.1.5.3.A, 4.1.5.4). The waste activated sludge was used in treatment studies that are discussed in Chapter Six. MBAS concentrations contained in the UBC Pilot Plant primary effluent are presented in Table D.1. Tables D.3, D.4, and D.5 contain the raw data for the MBAS measured on November 21, 26th and December 11, 2003 respectively. Table D.6 contains the raw data for the MBAS concentration of the waste activated sludge.

Table D.1: UBC Pilot Plant primary effluent: MBAS concentration.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>60%</th>
<th>65%</th>
<th>70%</th>
<th>75%</th>
<th>90%</th>
<th>Whole Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov 21, 03</td>
<td>Primary Effluent</td>
<td>0.8</td>
<td>1.0</td>
<td>2.2</td>
<td>6.1 ±2.5</td>
<td>n=2</td>
<td></td>
</tr>
<tr>
<td>Nov 26, 03</td>
<td>Primary Effluent</td>
<td>2.6 ±0.6</td>
<td>3.1 ±0.6</td>
<td>0.7 ± 3.2</td>
<td>7.2 ± 0</td>
<td>n=2</td>
<td></td>
</tr>
<tr>
<td>Dec 11, 03</td>
<td>Primary Effluent</td>
<td>1.4</td>
<td>3.4</td>
<td>0.64</td>
<td>6.4</td>
<td>n=2</td>
<td></td>
</tr>
<tr>
<td>Feb 26, 04</td>
<td>Waste Activated Sludge</td>
<td>0.17 ±0.04</td>
<td>0.02 ±0.07</td>
<td>0.04 ±0.05</td>
<td>0.5 ± 0.06</td>
<td>n=3</td>
<td></td>
</tr>
</tbody>
</table>

± corresponds to the 95% confidence interval
The distribution of MBAS in each elution fraction is presented in Figure D.1.

Figure D.1: MBAS distribution in elution fractions - UBC Pilot Plant

![Figure D.1: MBAS distribution in elution fractions - UBC Pilot Plant](image)

The distribution of MBAS between the three elution fractions (65%, 75%, and 90%) is similar to the distributions observed during the morning, evening and night sampling events that took place at the Lions Gate WWTP (see Section 5.2.1).

B) Toxicity

The toxicity contained in three elution fractions using the UBC pilot plant effluent was measured using the Microtox™ system and results are presented in Table D.2. The raw data is presented in Figure D.2.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>IC&lt;sub&gt;20&lt;/sub&gt; (V/V)</th>
<th>IC&lt;sub&gt;20&lt;/sub&gt; (mg/L MBAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov 26, 03</td>
<td>65%</td>
<td>11</td>
<td>0.3</td>
</tr>
<tr>
<td>Nov 26, 03</td>
<td>75%</td>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>Nov 26, 03</td>
<td>90%</td>
<td>8</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The UBC Pilot Plant reconstituted SPE extracts appear to contain greater toxicity than the toxicity contained in the Lions Gate WWTP (see Section 5.2.2).
C) Raw data

Table D.3: UBC Pilot Plant - Nov 21, 2003
Primary Effluent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/L std.</td>
<td></td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>1.0 mg/L std.</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>2.0 mg/L std.</td>
<td></td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>60-1</td>
<td>0.5</td>
<td>0.063</td>
<td>0.8</td>
</tr>
<tr>
<td>70-1</td>
<td>1.25</td>
<td>0.037</td>
<td>1</td>
</tr>
<tr>
<td>90-1</td>
<td>1.25</td>
<td>0.07</td>
<td>2.2</td>
</tr>
<tr>
<td>65-1</td>
<td>1.25</td>
<td>0.07</td>
<td>2.2</td>
</tr>
<tr>
<td>75-1</td>
<td>1.25</td>
<td>0.111</td>
<td>3.6</td>
</tr>
<tr>
<td>90-1</td>
<td>1.25</td>
<td>0.028</td>
<td>0.7</td>
</tr>
<tr>
<td>Whole Sample</td>
<td>1</td>
<td>0.112</td>
<td>6.3</td>
</tr>
<tr>
<td>Whole Sample + formaldehyde</td>
<td>1</td>
<td>0.106</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Table D.4: UBC Pilot Plant - Nov 26, 2003
Primary Effluent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mg/L std.</td>
<td></td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td>4 mg/L std.</td>
<td></td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td>75-1</td>
<td>5</td>
<td>0.021</td>
<td>3</td>
</tr>
<tr>
<td>90-1</td>
<td>2.5</td>
<td>0.013</td>
<td>1</td>
</tr>
<tr>
<td>65-2</td>
<td>2.5</td>
<td>0.039</td>
<td>2.6</td>
</tr>
<tr>
<td>75-2</td>
<td>2.5</td>
<td>0.052</td>
<td>3.4</td>
</tr>
<tr>
<td>65-3</td>
<td>2.5</td>
<td>0.038</td>
<td>2.5</td>
</tr>
<tr>
<td>75-3</td>
<td>2.5</td>
<td>0.046</td>
<td>3</td>
</tr>
<tr>
<td>90-3</td>
<td>2.5</td>
<td>0.005</td>
<td>0.5</td>
</tr>
<tr>
<td>Whole Sample</td>
<td>5</td>
<td>0.056</td>
<td>7.2</td>
</tr>
<tr>
<td>Whole Sample</td>
<td>5</td>
<td>0.056</td>
<td>7.2</td>
</tr>
<tr>
<td>SPE underflow</td>
<td>1</td>
<td>0.009</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table D.5: UBC Pilot Plant - Dec 11, 2003
Primary Effluent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/L std.</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>65%</td>
<td>25/14</td>
<td>0.031</td>
<td>1.43</td>
</tr>
<tr>
<td>75%</td>
<td>1.25</td>
<td>0.11</td>
<td>3.45</td>
</tr>
<tr>
<td>90%</td>
<td>0.5</td>
<td>0.049</td>
<td>0.64</td>
</tr>
<tr>
<td>Whole Sample</td>
<td>5/3</td>
<td>0.155</td>
<td>6.43</td>
</tr>
<tr>
<td>SPE Underflow</td>
<td>1</td>
<td>0.006</td>
<td>0.22</td>
</tr>
<tr>
<td>40% Wash Underflow</td>
<td>0.5</td>
<td>0.017</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table D.6: UBC Pilot Plant - Feb 26, 2004
filtered waste activated sludge

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>0.022</td>
<td>0.58</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.00</td>
<td>0.021</td>
<td>0.56</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.017</td>
<td>0.18</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.006</td>
<td>0.06</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.014</td>
<td>0.15</td>
</tr>
<tr>
<td>75-2</td>
<td>0.50</td>
<td>N.D.</td>
<td>0.00</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>65-3</td>
<td>0.50</td>
<td>0.016</td>
<td>0.17</td>
</tr>
<tr>
<td>75-3</td>
<td>0.50</td>
<td>0.005</td>
<td>0.05</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.005</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Non-detect
Figure D.2: UBC Pilot Plant primary effluent toxicity - Nov. 26, 2003

- 65% Elution Fraction, \( y = 1.5059x - 2.0343, I^2 = 0.7301 \)
- 75% Elution Fraction, \( y = 0.7954x - 0.9629, I^2 = 0.8896 \)
- 90% Elution Fraction, \( y = 0.7136x - 0.7679, I^2 = 0.7657 \)
APPENDIX E: Treatability Study

A) MBAS

Table E.1: MBAS standards.

<table>
<thead>
<tr>
<th>MBAS Conc. (mg/L)</th>
<th>Date</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Feb 27th</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>Feb 27th</td>
<td>0.017</td>
</tr>
<tr>
<td>1</td>
<td>Feb 27th</td>
<td>0.036</td>
</tr>
<tr>
<td>2</td>
<td>Feb 27th</td>
<td>0.068</td>
</tr>
<tr>
<td>4</td>
<td>Feb 27th</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Table E.2: Lions Gate primary effluent (untreated)
8:00 AM Feb 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.67</td>
<td>0.085</td>
<td>3.76</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.67</td>
<td>0.074</td>
<td>3.27</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.67</td>
<td>0.093</td>
<td>4.11</td>
</tr>
<tr>
<td>65-1</td>
<td>1.25</td>
<td>0.05</td>
<td>1.66</td>
</tr>
<tr>
<td>75-1</td>
<td>1.25</td>
<td>0.082</td>
<td>2.72</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.029</td>
<td>0.38</td>
</tr>
<tr>
<td>65-2</td>
<td>1.25</td>
<td>0.056</td>
<td>1.86</td>
</tr>
<tr>
<td>75-2</td>
<td>1.25</td>
<td>0.072</td>
<td>2.39</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.023</td>
<td>0.31</td>
</tr>
<tr>
<td>65-3</td>
<td>1.25</td>
<td>0.048</td>
<td>1.59</td>
</tr>
<tr>
<td>75-3</td>
<td>1.25</td>
<td>0.062</td>
<td>2.06</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.036</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table E.3: UBC pilot plant waste activated sludge (untreated)  
Feb 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>0.022</td>
<td>0.58</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.00</td>
<td>0.021</td>
<td>0.56</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.017</td>
<td>0.18</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.006</td>
<td>0.06</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.014</td>
<td>0.15</td>
</tr>
<tr>
<td>75-2</td>
<td>0.50</td>
<td>N.D.</td>
<td>0.00</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>65-3</td>
<td>0.50</td>
<td>0.016</td>
<td>0.17</td>
</tr>
<tr>
<td>75-3</td>
<td>0.50</td>
<td>0.005</td>
<td>0.05</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.005</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Non-detect

Table E.4: Partitioning to abiotic bio-solids (treated)  
February 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.67</td>
<td>0.003</td>
<td>0.13</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.67</td>
<td>0.033</td>
<td>1.46</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.67</td>
<td>0.016</td>
<td>0.71</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.039</td>
<td>0.41</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.038</td>
<td>0.40</td>
</tr>
<tr>
<td>75-2</td>
<td>0.50</td>
<td>N.D.</td>
<td>0.00</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.012</td>
<td>0.13</td>
</tr>
<tr>
<td>65-3</td>
<td>0.50</td>
<td>0.039</td>
<td>0.41</td>
</tr>
<tr>
<td>75-3</td>
<td>0.50</td>
<td>N.D.</td>
<td>0.00</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.007</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Non-detect
### Table E.5: Biological treatment (treated with activated sludge)

February 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.006</td>
<td>0.16</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>N.D.</td>
<td>0.00</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.00</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.011</td>
<td>0.12</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.012</td>
<td>0.13</td>
</tr>
<tr>
<td>75-2</td>
<td>0.50</td>
<td>0.004</td>
<td>0.04</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>65-3</td>
<td>0.50</td>
<td>0.016</td>
<td>0.17</td>
</tr>
<tr>
<td>75-3</td>
<td>0.50</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Non-Detect
N.A. Not Available

### Table E.6: Alum coagulation/flocculation with gravity settling (treated)

February 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.075</td>
<td>1.99</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>0.072</td>
<td>1.91</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.00</td>
<td>0.081</td>
<td>2.15</td>
</tr>
<tr>
<td>65-1</td>
<td>0.83</td>
<td>0.035</td>
<td>0.61</td>
</tr>
<tr>
<td>75-1</td>
<td>0.83</td>
<td>0.065</td>
<td>1.14</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.05</td>
<td>0.52</td>
</tr>
<tr>
<td>65-2</td>
<td>0.83</td>
<td>0.033</td>
<td>0.58</td>
</tr>
<tr>
<td>75-2</td>
<td>0.83</td>
<td>0.083</td>
<td>1.45</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.037</td>
<td>0.39</td>
</tr>
<tr>
<td>65-3</td>
<td>0.83</td>
<td>0.04</td>
<td>0.70</td>
</tr>
<tr>
<td>75-3</td>
<td>0.83</td>
<td>0.062</td>
<td>1.08</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.036</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Table E.7: Ozonation (treated)  
February 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.008</td>
<td>0.21</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>0.007</td>
<td>0.19</td>
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<tr>
<td>whole-3</td>
<td>1.00</td>
<td>0.005</td>
<td>0.13</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.006</td>
<td>0.08</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0.007</td>
<td>0.09</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.003</td>
<td>0.04</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.005</td>
<td>0.07</td>
</tr>
<tr>
<td>75-2</td>
<td>0.50</td>
<td>0.007</td>
<td>0.09</td>
</tr>
<tr>
<td>90-2</td>
<td>0.50</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>65-3</td>
<td>0.50</td>
<td>0.012</td>
<td>0.16</td>
</tr>
<tr>
<td>75-3</td>
<td>0.50</td>
<td>0.002</td>
<td>0.03</td>
</tr>
<tr>
<td>90-3</td>
<td>0.50</td>
<td>0.002</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table E.8: Air flotation (treated)  
February 26, 2004

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multiplication Factor</th>
<th>Absorbance</th>
<th>MBAS Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-1</td>
<td>1.00</td>
<td>0.031</td>
<td>0.82</td>
</tr>
<tr>
<td>whole-2</td>
<td>1.00</td>
<td>0.036</td>
<td>0.95</td>
</tr>
<tr>
<td>whole-3</td>
<td>1.00</td>
<td>0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>65-1</td>
<td>0.50</td>
<td>0.042</td>
<td>0.56</td>
</tr>
<tr>
<td>75-1</td>
<td>0.50</td>
<td>0.037</td>
<td>0.49</td>
</tr>
<tr>
<td>90-1</td>
<td>0.50</td>
<td>0.012</td>
<td>0.16</td>
</tr>
<tr>
<td>65-2</td>
<td>0.50</td>
<td>0.04</td>
<td>0.53</td>
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<tr>
<td>75-2</td>
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<td>0.043</td>
<td>0.57</td>
</tr>
<tr>
<td>90-2</td>
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<td>0.003</td>
<td>0.04</td>
</tr>
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<td>0.046</td>
<td>0.61</td>
</tr>
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<td>75-3</td>
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<td>0.045</td>
<td>0.60</td>
</tr>
<tr>
<td>90-3</td>
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<td>0.008</td>
<td>0.11</td>
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</tbody>
</table>
B) Toxicity

Figure E.1: 90% reconstituted SPE extract
Lions Gate primary effluent (untreated)
February 26, 2004

Figure E.2: Whole filtered sample
Lions Gate primary effluent (untreated)
February 26, 2004
Figure E.3: 90% reconstituted SPE extract ozonation (treated) February 26, 2004

Figure E.4: Whole filtered sample ozonation (treated) February 26, 2004
Figure E.5: 90% reconstituted SPE extract
air flotation (treated)
February 26, 2004

Figure E.6: Whole filtered sample
air flotation (treated)
February 26, 2004