QUANTITATIVE ANALYSIS OF EARTHY AND MUSTY ODORS IN DRINKING
WATER SOURCES IMPACTED BY WASTEWATER AND ALGAL DERIVED
CONTAMINANTS

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QUANTITATIVE ANALYSIS OF EARTHY AND MUSTY ODORS IN DRINKING
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ABSTRACT

The aim of this study was to optimize a method to quantify compounds responsible for taste and odor problems (i.e., geosmin and 2-methylisoborneol) at very low concentrations that are in finished drinking water in the presence of wastewater and algal derived contaminants/interferents. A polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was used for headspace - solid phase microextraction (HS-SPME) to improve the extraction efficiency of the taste and odor compounds from model and raw source water samples. Gas Chromatography coupled with Mass Spectrometry (GC/MS) in full scan mode was used to analyze the compounds adsorbed onto the fiber.

The following parameters were optimized in order to enhance extraction efficiency: extraction temperature, extraction time, desorption time, sonication temperature, sonication time and GC/MS configuration and temperature program. After optimization, the method provided a linear response from 1 to 300 ng/L for 2-MIB, geosmin and camphor. The Method Detect Limits (MDLs) for this method were 0.81 ng/L, 0.42 ng/L and 0.84 ng/L for 2-MIB, geosmin and camphor respectively, and the limit of quantification (LOQ) for 2-MIB, geosmin and camphor were 7.43 ng/L, 6.93 ng/L and 6.93 ng/L respectively. After HS-SPME optimization, raw source water samples were successfully analyzed for taste and odor compounds with interfering analytes with similar mass charged fragments and elution times. This method proves that low detection limits as well as exact molecular confirmation for taste and odor compounds can be achieved in GC/MS full scan mode.

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CHAPTER I

INTRODUCTION

1.1 Motivation of the Proposed Research

Eutrophication or algal blooms are one of the most significant causes of source water deterioration around the world and in the agricultural Midwest of the United States (Bell and Codd, 1996). Cyanobacteria or blue-green algal blooms are one of the most harmful consequences of eutrophication due to excessive nutrient loading in drinking water sources (Paerl and Pinckney, 1996). Cyanobacteria not only produce toxic metabolites but also taste and odor (T&O) compounds, which are generally not removed during conventional drinking water treatment (Bruchet et al., 2004).

2-Methylisoborneol (2-MIB) and geosmin are produced by certain species of actinomyces and cyanobacteria and are typically responsible for earthy-musty taste in finished drinking water. The odor threshold of these two compounds can be detected by humans at 4 ng/L for geosmin and 9 ng/L for 2-MIB (Mallevalle et al., 1987). Therefore, a sensitive, rapid and simple method to determine and quantify 2-MIB and geosmin at low concentration level is required.

Current methods, for detection and quantification of these compounds at low concentration levels, such as liquid–liquid extraction (LLE) (Rashash et al., 1996),

solid-phase extraction (SPE), or closed loop stripping analysis (Hwang et al., 1984) require large sample volumes, multiple sample prepare procedures or relatively complex equipment. These methods can be time consuming, resulting in low sample throughput, and may require expensive high-resolution mass spectrometers. Solid phase micro extraction (SPME) methods, a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Arthur et al., 1992; Magdic and Pawliszyn, 1996), has greatly minimized the sample preparation and increased the recovery of volatile organic compounds (VOCs) from aqueous samples.

In 2000, Watson and coworkers successfully applied and developed an SPME method for T&O compounds geosmin and 2-MIB (Watson et al., 2000; Watson et al., 2003). The concentrations of geosmin and 2-MIB were measured over the range of 1 to 1000 ng/L. Watson's work showed the absorption in sample headspace and desorption in the GC inlet were the main factors influencing extraction efficiency and recovery of geosmin and 2-MIB. Fiber type, extraction time, extraction temperature, ionic strength, desorption temperature and time are the parameters needed to be optimized in order to improve the extraction efficiency of T&O compounds. In the recent research, Ligor and Buszewski (2006) applied headspace(HS)-SPME using DVB/PDMS fiber on 25 mL samples, 40 min 40°C extraction conditions, 1 min at 220°C desorption condition to quantify geosmin, 2-MIB, and 2,4,6-trichloroanisole (TCA), which resulted in the ability to quantify the three compounds from 20-168 ng/L. However, most methods use selected ion monitoring (SIM) to increase method sensitivity to achieve low ng/L concentrations. Compared to full scan mode, SIM mode does not allow for exact molecular confirmation due to the fact that SIM is only scanning of one mass fragment at

a time. Therefore, other algal or wastewater derived contaminants could interfere with T&O analysis.

1.2 Scope of Work

A headspace SPME (HS-SPME) method was optimized to determine the concentrations of taste and odor compounds in the presence of wastewater and algal derived interferents in full scan mass detection mode at ng/L concentrations.

4-bromofluorobenzene (BFB), was used as the internal standard to verify the method optimization and increase method reliability. Extraction parameters optimized included extraction temperature, extraction time, and GC inlet desorption time. Sonication, a procedure used to prepare raw samples to release cell bound taste and odor compounds, was evaluated as a function of time (10, 20 and 30 min) and temperature (4 °C and 25 °C) in a temperature controlled water bath sonicator. Two different GC/MS configurations and oven profiles were used to separate overlapping signal peaks. The optimized method yielded linear responses and low detection limits for 2-MIB, geosmin, camphor and decanal in full scan MS detection mode. After HS-SPME method optimization, raw water samples were collected and examined for the presence of taste and odor compounds from two different source waters in Northeast Ohio impacted by both wastewater and algal blooms.

CHAPTER II

LITERATURE REVIEW

The occurrence of objectionable tastes and odors in drinking water is a common and widespread problem in the agricultural Midwest of the United States. The most troublesome odors are usually those described as muddy or earthy-musty. The two organic compounds most frequently associated with earthy-musty odor in drinking water are geosmin and 2-methylisoborneol (2-MIB) (Suffet et al., 1995; Wood et al., 2001), which are known metabolites produced by cyanobacteria. Odor thresholds for geosmin and 2-MIB are as low as few ng/L (Pirbazari et al., 1993; Lloyd et al., 1998). For the isolation and determination of these compounds, only sensitive and selective methods such as the solid phase microextraction (SPME) coupled with gas chromatography/mass spectrometry (GC/MS) are capable of extracting and analyzing compounds in that concentration range (Lloyd et al., 1998; Watson et al., 2000).

This literature review has been presented in four sections. The first section will discuss eutrophication, cyanobacteria taxonomy and algal bloom dynamics. The second section will briefly describe toxins produced by different strains of cyanobacteria. The third section will focus on several odor compounds produced by cyanobacteria and other odor compounds in source and finished drinking water. The last section will introduce

extraction and analytical methods that have been used to detect taste and odor compounds, specifically 2-MIB and geosmin.

2.1 Eutrophication and algal blooms

2.1.1. Introduction of Eutrophication

Since the industrial revolution, anthropogenic activities have caused profound alterations in the structure and function of the environment. Anthropogenic activities have been shown to have strong impacts upon the global biogeochemical cycles of carbon (C), nitrogen (N) and phosphorus (P). Eutrophication is caused by excessive concentrations of growth rate-limiting chemical nutrients, typically compounds containing nitrogen or phosphorus in an ecosystem (Paerl, 1997, 1999; Paerl et al., 2011). It may occur in lakes, rivers, ponds and coastal waters. When an ecosystem experiences an increase in nutrients, primary producers reap the benefits of available rate-limiting nutrients. In freshwater aquatic ecosystems, species such as algae and cyanobacteria experience an exponential population increase. Eutrophication or algae blooms are one of the most significant causes of source water quality deterioration in North America (Bell and Codd, 1996). Reservoirs and lakes in agriculturally land dominated watersheds are particularly vulnerable to eutrophication.

The major sources of nutrients to watersheds are separated by point sources and nonpoint sources. Precipitation, dissolution of natural minerals from soil, geologic formations, and agriculture runoff are defined as nonpoint sources (Richards, 1985; Strickland et al., 2010; Han et al., 2012). Waste water treatment plant effluents and industrial waste discharge are point sources. In coastal and lake areas, eutrophication is generally associated with nutrient runoff from intensive agriculture land use and waste

water treatment plant effluents (Han et al., 2012). Based on Ohio EPA Phosphorus Task Force Report (2010), nonpoint sources are responsible for about the two thirds of the total phosphorus load in comparison to about 20.7% for point sources. After 1980 with many of the waste water treatment plants in Ohio meeting the 1 mg/L total phosphorus limit, agricultural runoff became the major contributor of phosphorus into Lake Erie. Although, during 1975 to 1995, the amount of dissolved reactive phosphorus (DRP) decrease nearly 85% from its historic level of 0.3 mg/L-P. In 2009 the DRP concentrations in Lake Erie has been measured at its historically high levels due to agricultural runoff (Strickland et al., 2010; Han et al., 2012).

2.1.2. Cyanobacteria Bloom Development and Effect on Ecosystems

Cyanobacteria (blue-green algae) as one of the earth's oldest oxygen producing organisms are remains within fossil dating back approximately 3.5 billion years (Schopf, 2000). Cyanobacterial proliferation during the Precambrian period is largely responsible for the modern day oxygen enriched atmosphere and subsequent evolution of higher order plant and animal life (Schopf, 2000). This long evolutionary history has served cyanobacteria well, for it has enabled them to develop diverse and highly effective ecophysiological adaptations and strategies for ensuring survival and dominance in aquatic environments undergoing natural and human-induced environmental change (Huisman et al., 2005; Paerl and Fulton, 2006). Today, they enjoy a remarkably broad geographic distribution, ranging from polar to tropical regions in northern and southern hemispheres, where they are capable of dominating planktonic and benthic primary production in diverse behaviors.

One of the most detrimental consequences of eutrophication is the development of cyanobacterial or blue-green algal blooms. As a “microalgal” group, the cyanobacteria exhibit highly efficient nutrient (i.e., N, P, Fe and trace metal) uptake and storage capabilities, and they are the only oxygenic phototrophs capable of utilizing atmospheric nitrogen (N_2) to support growth via nitrogen fixation (Gallon, 1992). Furthermore, many planktonic genera are capable of rapid vertical migration by altering their buoyancy, allowing them to exploit deeper, nutrient-rich waters while also taking advantage of radiant-rich conditions near the surface (Reynolds et al., 2006). Lastly, some genera have formed symbioses (as endosymbionts) with diatoms, sponges, corals, lichens, ferns, and mutualistic associations with a variety of other organisms, which provide protection and enhance nutrient cycling and availability in nutrient-deplete waters (Paerl and Pinckney, 1996; Rai et al., 2002).

But over the past several centuries, human nutrient enrichment (particularly nitrogen and phosphorus) associated with urban, agricultural and industrial development, has promoted accelerated rates of primary production, or eutrophication. Eutrophication favors periodic proliferation and dominance of harmful blooms of cyanobacteria in both planktonic (Steinberg and Hartmann, 1988; Paerl and Fulton, 2006) and benthic (Izaguirre et al., 2007; Paerl and Piehler, 2008) environments. In freshwater ecosystems, P availability has traditionally been viewed as a key factor limiting cyanobacteria proliferation, and excess P (relative to N) loading has been identified as favoring cyanobacteria bloom development. The emphasis on P control is based on the N_2 fixing capabilities of some cyanobacteria genera, which help satisfy cellular N requirements under P-limited conditions (Paerl and Fulton, 2006). At the ecosystem

level, only a fraction, usually far less than 50%, of primary and secondary production demands are met by N₂ fixation, even when P supplies are sufficient (Howarth et al., 1988; Lewis and Wurtsbaugh, 2008). Nutrient loading dynamics have changed substantially over the past several decades. While P reductions have been actively pursued, human population growth in watersheds has been paralleled by increased N loading, often at higher rates than P (Paerl and Fulton, 2006; Paerl and Piehler, 2008). Excessive N loads are now as large of a concern as P loads as stimulants of freshwater, estuarine and marine eutrophication of harmful algal (including cyanobacterial) blooms (Paerl and Huisman, 2009).

Cyanobacterial blooms, a common feature of eutrophication, occur worldwide and have been well documented in the past years. Mass development of cyanobacteria increases turbidity and, hence, restricts light penetration in affected ecosystems. This, in turn, suppresses the establishment and growth of aquatic macrophytes and benthic microalgae and, thereby, negatively affecting the underwater habitat for benthic flora and fauna (Scheffer, 2005; Jeppesen et al., 2007). Cyanobacteria also cause night time oxygen depletion through respiration and bacterial decomposition of dense blooms, which can result in fish kills and loss of benthic infauna and flora (Paerl et al., 2004; Paerl and Fulton, 2006).

2.2 Cyanobacteria Toxins

Hence, one of the main reason cyanobacterial blooms become a serious problem is due to the ability of cyanobacteria to produce toxic secondary metabolites, which create major concerns for freshwater ecosystems and drinking water sources, irrigation, marine fishing and recreational purposes (Codd, 1995; Codd, 2000; Osborne et al., 2001;

Haider et al., 2003). Those toxic compounds can cause deleterious effects on aquatic organisms, wild life, domestic animals and humans upon drinking or contacting water containing these compounds. For instance, Microcystins (MCs) are one of the most commonly occurring cyanotoxins generated by cyanobacteria, usually by *Microcystis* sp., in freshwaters globally (Graham et al., 2004; Via-Ordorika et al., 2004). As a family of cyclic peptide of hepatotoxins, MCs have differential functions as inhibitors of several serine/threonine protein phosphatases including PP1 and PP2A, and can lead to the disruption of normal cell metabolism and functions (Yoshizawa et al., 1990). Exposure to MCs can lead to liver failure in wild animals, livestock, and aquatic life. as well as human illnesses and mortality (Azevedo et al., 2002).

Cyanotoxins are very diverse in their chemical structure and toxicity (Kaebernick and Neilan, 2001). There are three main groups of cyanobacterial toxins that have been classified based on their mechanism of toxicity: 1). dermatotoxins (lipopolysaccharides, lyngbyatoxin-a, and aplysiatoxins), 2). neurotoxins (anatoxin-a, homoanatoxin-a, anatoxin-a(s), and saxitoxins), and 3). hepatotoxins (microcystins, nodularin, and cylindrospermopsin). The table below classifies the toxic compounds generated from different cyanobacteri.

Table 2.1 Toxin and taste and odor producing cyanobacteria.

Cyanobacterial Genera	Dermatotoxins			Hepatotoxins			Neurotoxins					T&O	
	LYN	APL	LPS	CYL	MC	NOD	ANA	BMAA	NEO	SAX	GEO	MIB	
Colonial/Filamentous			Y	Y	Y		Y	Y	Y	Y	Y		
Anabaena			Y	Y	Y								
Anabaenopsis			Y										
Aphanizomenon			Y	Y			Y	Y	Y	Y	Y		
Aphanocapsa			Y		Y								
Cylindrospermopsis			Y	Y				Y		Y			
Fischerella			Y					Y			Y		
Haploisiphon			Y		Y								
Hyella			Y								Y	Y	
Lyngbya (Plectonema)	Y	Y	Y	Y				Y		Y	Y	Y	
Microcystis			Y		Y			Y					
Nodularia			Y			Y		Y					
Nostoc			Y		Y			Y			Y	Y	
Oscillatoria (Planktothrix)	Y	Y	Y		Y		Y	Y		Y	Y	Y	
Phormidium			Y				Y	Y			Y	Y	
Pseudanabaena			Y		Y							Y	
Raphidiopsis			Y	Y			Y						
Schizothrix	Y	Y	Y										
Synechococcus			Y		Y			Y			Y	Y	
Synechocystis			Y	Y	Y			Y					

(LYN, lyngbyatoxin-a; APL, aplysiatoxins; LPS, lipopolysaccharides; CYL, cylindrospermopsins; MC, nanatoxins; BMAA, β -N-methylamino-L-alanine; NEO, neosaxitoxins; SAX, saxitoxins; GEO, geosmin)

Many cyanotoxins have multiple variants with a range of toxicities. For example, there are over 80 known microcystin variants and toxicity differences among them, which can vary by an order of magnitude. Such diversity complicates risk assessment and development of regulations for human-health protection. Regardless, several countries have set national standards or guidelines for cyanotoxins in drinking and recreational waters. Currently, cyanotoxins are on the U.S. Environmental Protection Agency's drinking water contaminant candidate list (CCL) and several states include cyanotoxins, typically microcystins measured by enzymelinked immunosorbent assay (ELISA), in their freshwater beach monitoring programs.

2.3 Taste and Odor Compounds Source and Finished Drinking Water

Cyanobacteria cause many drinking water quality concerns due to the formation of taste and odor (T&O) compounds, which are not toxic but lead to a musty nasty taste to finished drinking water. These T&O compounds are generally not removed during conventional drinking water treatment (i.e., coagulation, flocculation, sedimentation, and filtration). Although the cyanobacterial cells can be removed during coagulation, residual cells can release T&O compounds during drinking water disinfection.

Three main origins of T&O problems have been identified. Firstly, a large fraction of the T&O compounds are formed in surface waters. These compounds are produced by cyanobacteria and often show a strong spatial and seasonal pattern according to the growth (Paerl and Ustach, 1982). While it is probable that some T&O compounds are biologically active, others are formed as metabolic by-products and only released to the water when the cell membrane is damaged. This cell-bound fraction can be a substantial source of T&O problems when they are released from damaged cells

during drinking water treatment or due to algal bloom die-off (Hoeger et al., 2005; Jüttner and Watson, 2007). Table 2.2 gives an overview of the most important T&O compounds related to production in surface waters.

Table 2.2 Nature relevant T&O compounds in surface drinking water.

Compound	Odor	Odor threshold (ng/L)	Source
β -cyclocitral	fruity	19000	cyanobacteria
2-trans, 4-cis, 7-cis-decatrienal	fishy	20000	green algae
dimethyl trisulfide	decaying vegetable	10	bacterial decomposition of algal blooms and grass
geosmin	earthy	4	cyanobacteria and actinomycetes
trans, trans-2,4-heptadienal	fishy	5000	green algae
cis-3-hexen-1-ol	grassy	70000	green algae
β -ionone	violets	7	green algae
2-isopropyl-3-methoxypyrazine	decaying vegetation	0.2	Actinomycetes; biochemical decay of grass
3-methyl-1-butanol	fusel oil	150	cyanobacteria
2-methylisoborneol	musty	9	cyanobacteria and actinomycetes
trans, cis-2,6-nonadienal	cucumber	20	green algae
1-penten-3-one	fishy-rancid	1250	green algae, cyanobacteria

Secondly, some potent T&O compounds can be formed as by-products during oxidation and disinfection of drinking water (Table 2.2). The extent of the formation of these by-products depends strongly on the composition of the water matrix and can be minimized by adequate treatment trains (Bruchet et al., 2004). The use of chlorine, for instance, can lead to the formation of bromophenols, such as 2,6-dibromophenol (Whitfield et al., 1988). Also, the use of ozone results in the formation of short-chain aldehydes, intermediate reaction products, that could impart odors in drinking water. However, by-products of ozonation have much higher T&O thresholds than by-products of chlorination and are usually easily removed by the subsequent biologically active activated carbon filtration (Whitfield et al., 1988; Bruchet et al., 2004).

Finally, some T&O compounds can be generated downstream of water treatment or in the drinking water distribution system (Khiari et al., 2002; Jüttner and Watson, 2007). Distribution generated T&O problems are a major concern to water suppliers because there is no barrier available to remove these compounds before they reach the tap. Therefore, special attention has to be paid on the choice of appropriate components and the maintenance of distribution systems. For instance, trichloroanisole, which is a potent musty smelling metabolite, generated by fungi during activated carbon filtration process and in distribution pipes (Khiari et al., 2002). But some of distribution system related T&O problems can be avoided or masked by the use of a residual disinfectant (i.e., chlorination), or by 2,6-di-tert-butyl-4-methylphenol (BHT), which can leach from polyethylene pipes. However, chlorination will produce disinfection byproducts and the chlorine odor in the tap water, which is likely to cause consumer complaints.

However, most taste and odor events associated with source waters are produced by cyanobacteria (i.e., geosmin or 2-MIB) (Watson, 2003). Currently, outbreaks of geosmin and 2-MIB are a major problem in many watersheds of the United States, since the ancient seabed saline and dynamic of surface waters (Kenefick et al., 1992). Unlike toxins, geosmin and 2-MIB have no known human health effects and there are no regulations for these compounds. Aesthetic issues associated with geosmin and 2-MIB occurs at low concentrations (i.e., 10 ng/L) and remedial actions often are taken as soon as taste or odor is detected in finished drinking water. Geosmin and 2-MIB are known to be produced by at least 40 cyanobacteria and noncyanobacterial, including *Aphanizomenon*, *Geitlerinema*, *Symploca*, *Oscillatoria*, *Phormidium*, *Nostoc*, *Pseudanabaena* and *Lyngbya* (Medsker et al., 1968; Izaguirre and Taylor, 1995; Rashash et al., 1996; Jüttner and Watson, 2007). Actually, both of compounds were firstly identified by isolation *Streptomyces* (Medsker et al., 1968; Medsker et al., 1969). Recently, studies have focused on several environmental characteristics and their effect on the production of these two off-flavor compounds. Schrader and Blevins (2001), proved that Zn, Fe, and Cu had major effect on the biomass and in the production of geosmin. Study recently showed the importance of microbiological, mineral, organic and tributary basin aspects in the origins and causes of geosmin and 2-MIB in water used for drinking source (Jüttner and Watson, 2007; Srinivasan and Sorial, 2011).

Table 2.3 T&O compounds generated in drinking water treatment plant and distribution system.

Compounds	Odor	Odor threshold (ng/L)	Source
low molecular weight aldehydes (>heptanal)	Fruity	>30(undecanal)	ozonation
low molecular weight aldehydes (< heptanal)	very individual (swampy-swimming pool)	200 (heptanal)-12000 (propanal)	ozonation
2-chlorophenol	Medicinal	360	chlorination of phenols
2,6-dibromophenol	Medicinal	0.5	chlorination of phenols in the presence of bromide
free chlorine	Chlorinous	25000	disinfection with chlorine
Iodoform	Medicinal	30	chlorination in the presence of iodide
monochloramin	Chlorinous	280000	disinfection with chloramine
2,6-di-tert-butyl-4-methylpneonol (BHT)	Plastic	not available	leaching from polyethylene pipes
2,4,6-trichloroanisole	Musty	0.03	methylation of 2,4,6-trichlorophenols by biofilms

Biodegradation of 2-MIB and geosmin is attributed to their structures, alicyclic alcohols and ketones (Trudgill, 1984) (Figure 2.1). To date, there have been no definitive pathways for the biodegradation of 2-MIB and geosmin. Trudgill (1984) made an assumption that the biodegradation pathway of 2-MIB may be through the Baeyer-Villiger biological reaction responsible for camphor degradation, a bicyclic ketone documented to be biodegraded. In this process, the ring structures of camphor are cleaved by mono-oxygenase enzymes (Trudgill, 1984). Eaton and Sandusky (2009) demonstrated this assumption, three known camphor-degrading microbes were isolated after the addition of camphor, including *Pseudomonas putida* G1, which resulted in the production of metabolites resulting from hydroxylation at all of the three available secondary carbons on the six-member ring of 2-MIB. Eaton and Sandusky (2010) demonstrated geosmin can be biodegraded by two terpene-degrading bacteria, *Rhodococcus wratislaviensis* DLC-cam and *Pseudomonas* sp. SBR3-tpnb when addition of camphor or terinene.

2-Methylisoborneol(2-MIB)

Geosmin

Camphor

Figure 2.1 Molecular structures of 2-MIB, geosmin and camphor.

2.4 Cyanobacterial Derived T&O Compounds Extraction Methods

2.4.1. Current methods for detect T&O compounds

Current methods for quantification or detection T&O compounds at OTCs levels

requires large sample volumes and intensive sample pre-concentration procedures such as liquid–liquid extraction (LLE) (Rashash et al., 1996) or relatively complex equipment, e.g., closed loop stripping analysis (Hwang et al., 1984), simultaneous distillation extraction (Mallevalle et al., 1987) or purge and trap (Lloyd et al., 1998). In the 1980s, solid-phase extraction (SPE) was developed, and SPE cartridges and membranes have been employed to extract organic toxicants or drugs from complex aqueous matrixes. However, in both LLE and SPE, organic solvents are used and multiple operational steps are needed. These procedures can be time consuming, resulting in low sample throughput, and may require expensive high-resolution mass spectrometers to provide detection at ng/L concentrations (Palmentier et al., 1998). Solid-phase microextraction (SPME), a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Arthur et al., 1992), has eliminated most of the drawbacks in the preparation of an aqueous samples for geosmin and 2-MIB analysis. Arthur and Pawliszyn (1990) coupled headspace solid-phase microextraction (HS-SPME) with high-performance gas chromatography (GC), which allowed for quantification of a large number of molecules with low detection limits and reproducibility over a considerable dynamic concentration range. Mass spectrometry used in specific fragmentometry after ionization by constant-energy electron impact in the selected ion monitoring mode (EI-MS-SIM) allows detection that is simultaneously versatile, sensitive and very specific to the analyte.

2.4.2. Solid-phase microextraction (SPME) method development

There are currently three SPME modes that require either fused-silica fibers or GC capillary columns. Headspace (HS) and direct insertion (DI) SPME are the two

fiber extraction modes, while the GC capillary column mode is referred to as in-tube SPME. The HS-SPME mode has been adapted for the analysis of volatile analytes. The primary advantage of HS-SPME is prevention of direct fiber contact with the aqueous sample thus lowering background noise.

HS-SPME is based on partitioning of the analyte between an aqueous sample and adsorption to a polymeric stationary phase in the headspace of the extraction vial. The quantitative basis for SPME is the linear relationship between the aqueous phase analyte concentration and the amount of the analyte absorbed by the fiber. HS-SPME is a two-step process that simultaneously extracts and pre-concentrates analytes from sample matrices. In the first step, a fused-silica fiber with a syringe is coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) for adsorption of volatile/polar analytes. The fiber is exposed to the headspace above the sample, allowing organic compounds to adsorb onto and partition into the coating phase. In the second step, the fiber with the adsorbed analyte is transferred to the GC inlet for thermal desorption, GC capillary column for separation, and quantification by GC-MS.

There are two basic factors that will affect the extraction efficiency of SPME, absorption in headspace and desorption in GC inlet (Watson et al., 2000; Nakamura and Daishima, 2005). The effect of ionic strength is considered to be the one of the most important absorption parameter. The suitability of the headspace SPME technique for the extraction of compounds in water depends on the transfer of compounds from the aqueous phase to the gaseous phase. Salt addition could significantly decrease their solubility in water, resulting in an equilibrium shift of these compounds from the solution toward the headspace. Guan (1998) proved the Na_2SO_4 shows higher responses

compared to add NaCl, NaH₂PO₄, NH₄Cl, KCl, NaHCO₃, (NH₄)₂SO₄, Na₂SO₄, CaCl₂, K₂B₄O₇, Na₂CO₃ and NaOH in herbicides samples. Watson et al.(2000) indicated that HS-SPME with the addition of NaCl would result in 3 and 4 times improved recoveries compare to unsalted geosmin and 2-MIB samples. The responses of most VOCs with addition of 30% NaCl were 2-3 times higher than those without salt addition NaCl (Nakamura and Daishima, 2005). Sung et al. (2005) also investigated NaCl concentrations ranging from 0 to 30% for geosmin, 2-MIB 2,4,6-TCA samples and found that a 30% NaCl addition improved extraction efficiency approximately 2.6–3.2 times. On the other hand, fiber type can be just as critical desorption parameter. Möder et al. (1999) suggests that the extraction efficiency for chloroacetamides, amides, thiocarbamates, triazines, uracils, and triazine metabolites is optimized using CW(Carbowax)/DVB(Divinylbenzene). Fiber with DVB polymer coat increases the available surface area and thus improves the extraction of small polar molecules. Watson et al.(2000) and Ligor and Buszewski (2006) proved that DVB/ PDMA is considered to be the best overall recovery for 2-MIB and geosmin. Nakamura and Daishima (2005) further confirmed this result and found CAR/PDMS is suitable for 22 VOCs and MTBE.

SPME has been widely used to detect taste and odor compounds after Watson and coworkers first successfully developed the method for qualitative and quantitative analysis T&O compounds (geosmin and 2-MIB) in source water and drinking water samples (Watson et al., 2000; Watson et al., 2003). Concentrations of geosmin and 2-MIB can be measured over the concentration ranged of 1 to 1000 ng/L. The HS-SPME method achieved typical precision of 5-12% in a wide variety of sample

matrices (Watson et al., 2000; Watson et al., 2003). Lin et al. (2003) proved residual chlorine negatively effects extraction on geosmin, 2-MIB, and MTBE in chlorinated drinking water sample and found dechlorinating samples with sodium thiosulfate improved extraction and analysis of these volatile compounds. Sung et al. (2005) optimized the HS-SPME method by varying desorption time, ionic strength of the aqueous sample, and pH of samples, which show good precision (5.9-9.8%) by using 2-isobutyl-3-methoxy pyrazine (IBMP) as internal standard. Nakamura and Daishima (2005) further evaluated three types of SPME fiber capable of analyzing for 22 volatile organic compounds, and found DVB/PDMS is suitable for 1,4-dioxane, 2-MIB and geosmin. Ligor and Buszewski (2006) further optimized the sorption and desorption conditions, and proved 20 min, 40 C° and 25% NaCl addition as the optimal sorption condition to extracted 2-MIB, 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine. desorption temperature for taste and odor compounds.

CHAPTER III

EXPERIMENTAL

3.1 Materials and Reagents

Standard solutions of 100 g/ml of geosmin (purity 99.5%), 2-methylisoborneol (2-MIB, purity 99.9%), decyl aldehyde (99.2% purity), and camphor (99.3% purity) were obtained from Chemservice (West Chester, PA, USA). The internal standard, 4-bromofluorobenzene (99.0% purity), was obtained from Fisher Scientific (New Jersey, USA). ACS reagent grade Methanol (99.8% purity) was obtained from Sigma–Aldrich (St. Louis, MO, USA), and sodium chloride (purity $\geq$ 99%) was purchased from EMD Chemical INC (Gibbstown, NJ, USA). Working solutions of geosmin and 2-MIB (1 mL each) were diluted to create 200ug/L, 40ug/L, 4ug/L in methanol. The internal standard, 4-bromofluorobenzene, working solution was 2mg/L in methanol. All the standards and working solutions were stored 4°C .

The 65 μ m PDMS/DVB fibers (Supelco, Bellefonte, PA, USA) were used to perform solid phase micro-extraction (SPME). PDMS/DVB fiber has been considered to the best overall recovery effective for 2-MIB and geosmin (Watson et al., 2000; Ligor and Buszewski, 2006). The fibers were thermally conditioned in the GC injector port at 220°C for 15min. Aqueous stock solutions and experiments used laboratory prepared deionized (DI) water (18.2 M Ω /cm) from a Barnstead NANO pure system (Barnstead-Thermolyne Corporation, Dubuque, IA). All pH measurements were taken with an Orion 5 Star pH meter and ROSS ultra combination pH probe with temperature

compensation (ThermoFisher Scientific, Pittsburgh, PA). All other organic and inorganic chemicals were certified ACS reagent grade and used without further purification. Digital hot plates and table top ultra-sonicator were purchased from Fisher Scientific (Pittsburg, PA, USA). The Omega balance was obtained from Mettler Toledo (Blairsville, PA, USA). The raw water samples were filtered through a 25mm 0.45uM PTFE membrane syringe filter (VWR international, USA).

The 40mL screw-capped amber vials were soaked in a 0.5 M NaOH bath overnight. Then sample bottles were rinsed with DI water and baked at 400°C in an oven for at least 4 hours. Prior to use, the vials were cooled to room temperature. The sample caps were soaked overnight in DI water and then rinsed 3 times. These cleaning procedures ensured minimum cross contamination.

3.1 Experimental Methods

3.2.1 Extraction and desorption procedure

SPME extractions were performed on 20 mL of aqueous samples unless otherwise noted. Analysis and quantification of geosmin, 2-MIB, decanal, or camphor was performed by the following procedure. First, seven grams of NaCl was added to the 20 mL aqueous sample. Recent research has shown the addition of NaCl creating a 35% salt solution provides the best recovery for 2-MIB (Ligor and Buszewski, 2006); therefore, we also used a 35% NaCl solution. Then the internal standard (4-bromofluorobenzene, 0.5µL, 1mg/L), and a small PTFE-coated magnetic stirring bar (2mm*7mm) was added was added to the sample. The vial was then capped with a PTFE-faced silicone rubber septum that was pre-pierced in order not to damage the SPME needle. Each sample was then stirred at 500rpm and heated to equilibrate the

sample to the extraction temperature for 20min in the water bath. The SPME fiber was then pierced through the septum and exposed to the headspace of sample under specified extraction conditions. Extraction times (30-70 minutes) and extraction temperatures (30-80 °C) were varied to optimize analyte adsorption to the SPME fiber.

Figure 3.1 The extraction procedure and digital hot plates water bath.

After the fiber was exposed to the headspace for a specified amount of time, it was removed from the vial and immediately inserted into a GC injection port for thermal desorption (Figure 3.2) at 220°C . According to previous research (Mattheis and Roberts, 1992; Izaguirre and Taylor, 1995; Ligor and Buszewski, 2006), this is the optimal temperature for total desorption of taste and odor compounds without destroying the fiber coating. Thermal desorption times for 2-MIB and geosmin were varied from 0-2 minutes to optimize analyte desorption from the SPME fiber. After 20 min, the fiber was re-inserted in the GC injection port for 15 minutes for fiber reconditioning.

Figure 3.2 The desorption procedure and GC/MS injection port.

3.2.2 Experimental Apparatus: Gas Chromatograph/Mass Spectrometer (GC/MS)

Gas Chromatography/Mass Spectrometry was used to analyze the analytes of interest. The gas chromatograph/mass spectrometer (GC/MS) was an Agilent 7890A GC with a 5975C mass selective detector obtained from Agilent Technologies (Wilmington, DE, USA). Chromatic data was analyzed with MSD Chemstation. The GC column was an Rtx-5MS (30 m, 0.25 mm ID, 0.5um df, Agilent Technologies. The guard column was Fused silica intermediate polarity 25760-U (15m, 0.25 mm ID, Supelo analytical). Helium was used as carrier gas at a flow rate of 1.12 ml/min under constant flow and splitless mode. Two GC temperature programs were developed to compare to ensure minimal co-elution of analytes. The first GC program was as follows: isothermal period at 65 °C for 1 min, linear increase by 5 °C/min to 95 °C, isothermal period at 125 °C for 0 min, linear increase by 10 °C/min to 280 °C, isothermal period at 280 °C for 0 min and cooling down to 220 °C in 1 min. The total GC runtime was 25.5 min. The

following MSD temperature parameters were: transfer line 250 °C, MS-source 230 °C, and quadrupole 150 °C. Scan parameters were: low mass: 40, high mass: 550, and threshold: 150. The second program was as follows: isothermal period at 65 °C for 2 min, linear increase by 5 °C/min to 125 °C, isothermal period at 125 °C for 0 min, linear increase by 10 °C/min to 280 °C, isothermal period at 280 °C for 5 min and cooling down to 220 °C in 1 min. The total GC runtime was 34.5 min. The following MSD temperature parameters were: transfer line 280 °C, MS-source 230 °C, and quadrupole 150 °C. For the full scan mode, scan parameters were: low mass: 40, high mass: 550, and threshold: 150. The following quantification m/z ratios were monitored: geosmin: m/z 112 (target ion)/111/125/126, 2-MIB: m/z 95 (target ion)/95, Decanal: m/z 43 (target ion)/41/43/57, 4-bromofluorobenzene (internal standard): m/z 175 (target ion)/95/174/175.

3.3 Preparation of raw drinking water samples for analysis

3.3.1 Akron Source Water and Distribution Samples

The Cuyahoga watershed begins in Geauga County with the West Branch and the East Branch tributaries. It takes its 84.9 mile journey flowing through six counties and emptying into Lake Erie just west of downtown Cleveland, OH. During this study we focus on the upper Cuyahoga River watershed. The upper Cuyahoga River Watershed is located in northeast Ohio, flowing through Geauga and Portage counties. There are three large reservoirs located in this segment of the watershed, East Branch, LaDue, and Rockwell that serve as drinking water source for the city of Akron (Figure 3.3). There are 7 municipal and 2 industrial wastewater plants that discharge their effluents into the this agriculturally dominate watershed, as well as multiple failing septic systems near the East Branch Reservoir.

Upper Cuyahoga River Watershed

Figure 3.3 Upper Cuyahoga River watershed and the location of Akron water plant reservoirs, East Branch, LaDue Reservoirs, and Rockwell

The water samples were collected directly from Rockwell, Ladue, East Branch once a week. The samples were stored in 40 mL amber vial headspace-free and stored at 4°C before analysis. The samples were filtered and then transferred into two 20 ml

40ml amber bottles and internal standard, salt, and the PTFE stir bar were added prior to be capped with PTFE septa.

3.3.2 Alliance water sample

Source water for the City of Alliance is comprised of two contiguous reservoirs, Walborn and Deer Creek. The watershed is mostly dominated with agriculture, especially dairy farms that leach untreated manure into Walborn Reservoir. Also, there are multiple septic systems that have failed as well as two cities that directly discharge their sewage untreated into Walborn (Marlboro, OH) and Deer Creek (Limaville, OH) Reservoirs. The 40 mL samples were separated into two 20 mL samples. One sample was sonicated at two different temperatures (4 °C and room temperature) to optimize analyte extraction. Another 20 mL sample was filtered to compare total 2-MIB and geosmin concentrations (i.e., cell bound and aqueous) to just aqueous phase concentrations. After either filtration or sonication, internal standard, salt, and a PTFE stir bar were added and the sample was placed in the water bath for extraction.

Figure 3.4 The location of Alliance water plant reservoirs.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter has been divided into three sections to discuss the development of a Solid Phase Micro Extraction (SPME) method coupled with GC/MS detection for 2-MIB, geosmin, camphor and decanal. In the first section, four parameters that influence SPME extraction efficiency were optimized during method development such as extraction temperature (30, 40, 50, 60, 70, and 80°C), extraction time (30, 40, and 50 min), GC inlet desorption time (0.5, 1.0, 1.5, and 2.0 min), and sonication time (10, 20, and 30 min) at two temperatures (4°C and 25°C). The second section developed calibration curves of 2-MIB, geosmin, camphor and decanal, and calculated their method detection limit (MDL) and limit of quantification (LOQ). The last section was to apply the optimized method to raw water samples from two drinking water sources in the Northeast Ohio, Akron and Alliance.

4.1 Headspace SPME-GC/MS method development and optimization

Previous work has shown there are two basic factors that effect analyte extraction efficiency when performing headspace SPME: absorption in headspace and desorption in GC inlet (Watson et al., 2000; Nakamura and Daishima, 2005). The primary parameters influencing analyte adsorption onto the stationary phase are fiber type, extraction time, ionic strength, temperature, sample volume, agitation and occasionally pH. In the GC/MS inlet, analyte desorption is a function of time and temperature in the GC inlet.

4.1.1 Internal standard 4-bromofluorobenzene (BFB) area as a function of extraction condition.

To ensure exact quantification 2-MIB and geosmin, an internal standard, 4-bromofluorobenzene (BFB), was added to samples and standards prior to extraction to account for sample matrix effects. BFB is a volatile organic compound (VOC) that can be used as an internal standard for surface water and drinking water samplings using GC-MS detection, and it has been found to be a suitable internal standard for the determination of volatile organic compounds (VOCs) in water as well as fish, sediment, soil, analysis of hazardous air pollutants and air quality in car parking lots (Mehran et al., 1996; Schumacher and Ward, 1997). BFB was chosen as internal standard since it elutes quickly and does not interfere with target analytes. Comparing the raw peak area of BFB to a target compound's raw peak area will further enhance the reliability of the optimized method.

Since little is known about BFB with respect to SPME, BFB recovery needed to be determined as a function of extraction temperature and extraction time. With the increasing extraction time (Figure 4.1), the extraction efficiency improved. The highest response was observed when the extraction time was increased to 50 min. The most efficiency extraction condition was obtained at 50°C after 50 min extraction. The extraction efficiency of BFB declined after 60°C for all three extraction time profiles. The poorest BFB response was found to be at 80 °C regardless of extraction time. The decrease is most likely due to the volatility of BFB forcing partitioning to the gas phase over the fiber.

Figure 4.1 BFB raw peak area as a function of extraction time and temperature. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $T_{\text{room}} = 22 \text{ } ^\circ\text{C}$, $\text{Stir} = 500 \text{ rpm}$, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

4.1.2 Extraction temperature optimization for Geosmin and 2-MIB

Extraction temperature is an important parameter when analyzing compounds via headspace-SPME (HS-SPME) because it influences the mass transfer rates and partitioning of analytes between the aqueous and gas phases. Higher temperatures increase the vapor pressure of volatile analytes in the headspace. Ömür-Özbek and Dietrich (2005) measured Henry's law constants of geosmin, 2-MIB and nonadienal and found Henry's law constants and enthalpies increased as a function of the extraction temperature. However, higher temperatures may also negatively impact extraction efficiency due to different SPME fiber coating (Zhang and Pawliszyn, 1993, 1995; Jia et al., 1998). In Figure 4.2., temperature 30, 40, 50, 60, 70 and 80°C were chosen to optimize the SPME analysis for taste and odor compounds at 40 minute extraction time. Figure 4.2 shows 2-MIB, geosmin and BFB raw peak area as a function of extraction temperature. The extraction efficiency of 2-MIB and geosmin increased with increasing of the temperature where as the raw peak area of the internal standard BFB decreased after 40°C. The raw peak areas of 2-MIB and geosmin areas at 60°C were nearly 10% lower than those at 70°C, and there is no significant difference between geosmin and 2-MIB raw peak areas at 70°C and 80°C.

The ratios of 2-MIB/BFB and geosmin/BFB need to be examined as a function of extraction time and temperature. Here ratio is defined as the raw peak area divided by internal standard peak area. The ratios of 2-MIB/BFB and geosmin/BFB increased with increasing temperature (Figure 4.3). Compare to Figure 4.2, the ratios of 2-MIB/BFB and geosmin/BFB increased dramatically after 50°C due to the decreased recovery of BFB. The ratios of geosmin and 2-MIB at 60°C were nearly 25% lower than those at 70°C and

about 20% lower at 70 °C than 80 °C. After careful consideration, 70 °C was selected as the optimal extraction temperature of geosmin and 2-MIB to avoid potential headspace partitioning into the gas phase.

Figure 4.2. 2-MIB, gesomin and BFB raw peak area as a function of extraction temperature. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $T_{\text{room}} = 22 \text{ }^{\circ}\text{C}$, Extraction time = 40 min, Stir = 500 rpm, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

Figure 4.3. 2-MIB/IS and geosmin/IS ratio as a function of extraction temperature. $C_{2\text{-MIB}} = 100\text{ng/L}$, $C_{\text{Geosmin}} = 100\text{ng/L}$, $V_{\text{Sample}} = 20\text{ ml}$, $C_{\text{BFB}} = 0.25\text{ }\mu\text{g/L}$, $\text{NaCl} = 7\text{ g}$, $T_{\text{room}} = 22\text{ }^{\circ}\text{C}$, Extraction time = 40min, Stir = 500 rpm, Fiber type: PDMS/DVB, Ratio = SD area/ IS area. Error bars represent 95% confidence intervals.

4.1.3 Extraction time optimization

The duration of fiber exposure to the headspace strongly influences the analyte adsorption to the stationary phase of the fiber coating. Previous work has shown extraction yield increase over relatively long exposure times (Watson et al., 2000). Extraction time is rarely set at equilibrium for geosmin and 2-MIB for HS-SPME. Waston et al. (2000) found 2-MIB and geosmin reached an apparent equilibrium after 1-2 hours. In drinking water samples, maximum extraction/recovery is far more important than high throughput (Waston et al., 2000). However to balance sample extraction efficiency with sample throughput, extraction times of 30 min, 40 min, and 50 min were investigate at 70 °C to optimize 2-MIB and geosmin recoveries. Extraction efficiency of 2-MIB increased with increasing the extraction temperature and time till 70 °C (Figure 4.4). At this temperature, there was no statistical significance between 50 min and 40 min extraction time.

The ratio of 2-MIB/BFB displayed a slightly different profile as a function of extraction time. The ratio of 2-MIB/BFB increased with increasing the temperature (Figure 4.5). After 50°C , 2-MIB/BFB ratios at 50 min extraction time were lower than 40 min extraction time where the raw peaks were very similar. This is more than likely due to the partitioning of the internal standard favoring the vapor phase over the fiber as temperature increases. Due to optimal extraction for both internal standard and 2-MIB at 70 °C, extraction time of 40 min appears to give the most reliable response for 2-MIB quantification.

Figure 4.4. 2-MIB raw peak area as a function of extraction time and temperature. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

Figure 4.5. 2-MIB/IS ratio as a function of extraction time and temperature. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, Fiber type: PDMS/DVB, Ratio = SD area/ IS area. Error bars represent 95% confidence intervals.

The extraction efficiency of geosmin increased with increasing the extraction temperature. After 50°C in Figure 4.6, the most efficient recovery was found to occur at 40 min extraction time. Comparing the raw peak areas for geosmin at 40 min and 50 min extraction time, there was no significant difference until 80 °C. A very similar trend was observed in Figure 4.7, the geosmin/BFB ratios. The extraction efficiency of geosmin increased with increasing the extraction temperature. After 50 °C , the most efficient recovery was found to occur at 40 min extraction.

Figure 4.6. Geosmin raw peak area as a function of extraction time and temperature. $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

Figure 4.7. Geosmin/IS ratio as a function of extraction time and temperature. $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, Fiber type: PDMS/DVB, Ratio = SD area/ IS area. Error bars represent 95% confidence intervals.

It can be concluded that 40 min is the best extraction time for extract 2-MIB and geosmin. The extraction condition of 40 min at 70°C provides sufficient extraction efficiency and allows the headspace SPME procedure to be performed approximately in the same time for analysis by GC/MS. The optimized method sought to balance sample extraction efficiency, sample throughput and also to improved quantification through the optimization of internal standard recovery as well.

4.1.4 Analyte desorption from the fiber in the GC inlet.

Another parameter that will impact analyte response is desorption from the fiber in the GC inlet. After optimizing extraction time and temperature, Izaguirre and Taylor (1995) and Ligor and Buszewski (2006) evaluated SPME fiber desorption for 2-MIB and geosmin and conclude that 220°C is the optimal temperature without destruction of analytes on the fiber coating. However, desorption time in the GC inlet has yet to be verified. Therefore, 0, 0.5, 1.0, 1.5 and 2.0 min were investigated in order to determine the optimal desorption time in the GC inlet. Figure 4.8 shows geosmin, 2-MIB, and BFB raw peak areas as a function of desorption time. The raw peak area of those three compounds increased with increasing desorption time. For geosmin, the highest response was at 1 min desorption but was statistical similar when compared to 2 min desorption time. The response of 2-MIB reached the maximum raw peak area response after 2 min desorption time, which was a slight advantage compared to 1 min and 1.5 min desorption time.

When comparing the analyte to internal standard ratio, geosmin/IS and 2-MIB/IS ratio, desorption time did not appear to be very significant. The desorption efficiency of the three compounds slightly increased with increasing desorption time (Figure 4.9).

However, the geosmin/BFB and 2-MIB/BFB ratios show no real significant difference as a function of desorption time. For geosmin/BFB, the highest response was after 1 min desorption. The 2-MIB/BFB ratio after 1.5 min desorption time was only slightly more statistically significant than at 1 min. Therefore, a desorption time of 1 min was found to be optimal for 2-MIB and geosmin, which is consistent with Ligor and Buszewski (2006).

Figure 4.8. 2-MIB and geosmin raw peak areas as a function of desorption time in GC/MS inlet. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, Fiber type: DVB /PDMS. Error bars represent 95% confidence intervals.

Figure 4.9. 2-MIB/IS and geosmin/IS ratio as a function of desorption time in GC/MS inlet. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

4.1.5 Ultra-sonic validation test.

Since geosmin and 2-MIB may not be in the aqueous phase, cell bound concentrations may need to be determined in order for total geosmin and 2-MIB concentrations to be reported. In 1975, it was shown that brief exposure to ultrasonic energy causes a thinning of cell walls attributed to the freeing/lysing of the cytoplasm membrane. Some studies recently reported ultrasound can degrade the toxin microcystin (Song et al., 2005; Hudder et al., 2007). Song and O'Shea (2007) reported about 90% geosmin and 2-MIB can be removal after 40 min sonication. Therefore, sonication can significantly affect the analysis of taste and odor compounds. The influence of sonication was evaluated as a function of time (10, 20 and 30 min) and temperature (4 °C and 25 °C) in a temperature controlled water bath sonicator (Figure 4.10). The response of geosmin and 2-MIB decreased with increasing sonication time at 25 °C water bath. The raw peak area response of both compounds showed only a slight decline after sonication in 4 °C ice bath as sonication time increased to 30 min, while there was not real statistical difference between 0 and 10 minutes. In order to increase the extraction efficiency, raw water samples will be sonicated for 10 min at 4 °C for maximum analyte recovery.

Figure 4.10. 2-MIB/IS and geosmin/IS ratio as a function of sonication time at 4 °C and 25 °C water bath. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40\text{min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, $\text{Fiber type: PDMS/DVB}$. Error bars represent 95% confidence intervals.

4.1.6 GC/MS method optimization

Gas chromatography-mass spectrometry (GC/MS) is a widely used analytical technique for the identification and quantification of trace chemicals in complex matrixes. When samples contain multiple analytes, it is common to observe co-elution of two or more components, resulting in an overlap of signal peaks observed in the GC/MS total ion chromatogram. In Figure 4.11, the peak of decanal and 2-MIB overlapped at approximately 9 min, which decreases the accuracy of the quantification. Therefore, the GC oven temperature was modified as well as the addition of a 15-m medium polarity guard column to minimize co-elution of other constituents that would interfere with 2-MIB analysis. After optimizing the physical apparatus and GC oven temperature profile resulted in prolonging elution time, for decanal and 2-MIB to 16.34 min and 16.51 respectively (Figure 4.12).

Figure 4.11 Total ion chromatograph of a sample containing 2-MIB, camphor, and decanal before method optimization. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $C_{\text{Camphor}} = 100 \text{ ng/L}$, $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 60 \text{ } ^\circ\text{C}$, Fiber type: PDMS/DVB.

Figure 4.12 Total ion chromatograph of a sample containing 2-MIB, camphor, and decanal after method optimization. $C_{2\text{-MIB}} = 100 \text{ ng/L}$, $C_{\text{Geosmin}} = 100 \text{ ng/L}$, $C_{\text{Camphor}} = 100 \text{ ng/L}$ $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, Fiber type: PDMS/DVB.

4.2 Calibration and detection limits of target analytes

4.2.1 Geosmin and 2-Methylisoborneol (2-MIB)

Using the optimized extraction and analytical methods, calibration curves for 2-MIB and geosmin were created. Calibration standards were made by injecting a portion of the geosmin/2-MIB working solution into 20 mL DI water to obtain a concentration in the range of 1-300 ng/L. Then 7g NaCl, internal standard (BFB, 0.5 μ L, 1mg/L), and stir bar were added to the sample. The optimized HS-SPME method provides reproducible and linear analyte recovery even at trace concentrations (Figure 4.13), where both geosmin and MIB yielded very linear calibration curves over a range of 1-300 ng/L ($R^2=0.9974$ and $R^2=0.9971$, respectively).

Figure 4.13. Calibration curves for 2-MIB and geosmin. $C_{2\text{-MIB}} = C_{\text{Geosmin}} = 1\sim 300$ ng/L, $V_{\text{Sample}} = 20$ ml, $C_{\text{BFB}} = 0.25$ $\mu\text{g/L}$, NaCl = 7 g, Stir = 500 rpm, Extraction time = 40min, Extraction temp = 70 $^{\circ}\text{C}$, Desorption temp = 220 $^{\circ}\text{C}$, Desorption time = 1min, Fiber type: PDMS/DVB.

4.2.2 Interferents: Decanal and Camphor

Decanal and Camphor both were considered to be organic wastewater-related contaminants often found in drinking water sources due to poorly treated wastewater effluents. However, Johnson (2004) found the genes *luxC*, *luxD* and *luxE* in 10 different cyanobacteria that produce enzymes that make the molecule decanal in response to the algal circadian rhythm. Prior to method optimization, decanal co-eluted with 2-MIB prior and would completely obscure the 2-MIB peak if decanal concentrations were excessively high. Camphor has a similar structure with 2-MIB and many camphor-degrading bacteria that are able to transform 2-MIB have been identified. Eaton and Sandusky (2009, 2010) demonstrated camphor may affect biodegradation of 2-MIB and geosmin. Therefore, it was important to be able to separate them from 2-MIB and be able to quantify them.

After optimization, the combined extraction/analytical method provides a reproducible, linear analyte recovery, even at trace concentrations. With HSPME of standard analyte solutions, camphor showed a highly significant linear response over a range of 1-300 ng/L ($R^2=0.9932$, respectively). The calibration curve of decanal over a range of 50 -300 ng/L ($R^2=0.9732$, respectively). Since decanal presents in DI water samples, the calibration curve of decanal is shift in X axis.

Figure 4.14 Calibration curves for camphor and decanal. $C_{\text{camphor}} = 1\sim 300$ ng/L, $C_{\text{decanal}} = 50\sim 300$ ng/L, $V_{\text{Sample}} = 20$ ml, $C_{\text{BFB}} = 0.25$ $\mu\text{g/L}$, NaCl = 7 g, Stir = 500 rpm, Extraction time = 40min, Extraction temp = 70 °C, Desorption temp = 220 °C, Desorption time = 1min, Fiber type: PDMS/DVB. Error bars represent 95% confidence intervals.

The Method Detect Limits (MDLs) were calculated (based on the lowest level analyzed, 1 ng/L) where the raw peak is three times higher than base noise level and determined as three times the standard deviation of seven replicate runs. The MDLs 1ng/L 2-MIB, geosmin and Camphor spikes in deionized water were in the range of 0.42-0.84 ng/L/ (Table 4.1). The limit of quantification (LOQ) was determined at a higher concentration (10 ng/L) than the MDLs, which is calculated as 10 times the standard deviation above the mean blank value and mathematically defined as equal to 10 times the standard deviation of the results for a seven replicates runs. The LOQ of 2-MIB, geosmin and camphor at 10 ng/L were in the range of 6.93-8.42 ng/L (Table 4.1).

Table 4.1 The calibration data of for the analysis of earthy and musty odors compounds by SPME method.

Parameter	2-MIB	geosmin	Camphor	Decanal
Average of retention time	16.28 min	20.98 min	15.27 min	16.35 min
Calibration curve	$y = 68.886x - 5.468$	$y = 49.205x - 5.2703$	$y = 147.34x - 9.4646$	$y = 137.84x - 311.61$
Correlation coefficient (R^2)	0.9971	0.9974	0.9932	0.9732
Range of concentration of standard solution	1-300 ng/L	1-300ng/L	1-300 ng/L	50-300 ng/L
LOQ (ng/L)	7.43	6.93	8.42	
MDLs (ng/L)	0.81	0.42	0.84	/

4.3 Analysis of Raw and Finished Drinking Water Samples

4.3.1 Akron taste and odor water sample analysis:

T&O compounds were monitored in Akron's source water from May through December of 2011. Geosmin was found to be the only T&O compound produced by the cyanobacterial stains in the Upper Cuyahoga Watershed. Figure 4.15 shows

concentrations of geosmin in water samples taken from the three reservoirs: East Branch, LaDue, and Rockwell from May to July 2011. Figure 4.16 shows concentrations of geosmin from East Branch and Rockwell from August to December 2011. The average geosmin concentration in East Branch and Rockwell Reservoirs were 63.18 ng/L and 18.16 ng/L respectively over this time frame, May-December. The midsummer sample (Jun 19th) from Rockwell contained very high levels of geosmin ~300 ng/L; however, it is unknown what caused such a high aqueous geosmin concentration during midsummer opposed to winter. Figure 4.17 shows the concentration of geosmin in the samples taken from City of Akron Water Treatment Plant and distribution system from May to August 2011. For these samples, a dechlorinating agent (i.e., sodium sulfite) was added to the sample in order to quench residual chlorine prior to HS-SPME to avoid potential artifacts due to chlorination. The highest concentration was found in the tap water from August 20th sample, geosmin = 21.3 ng/L. The concentrations decanal for the three reservoirs in the Upper Cuyahoga Watershed are shown in the Appendix (Figure A.1), as well as the frequency of other wastewater contaminants observed in Akron water samples are also shown in Appendix Table A.1.

Figure 4.15 Geosmin analysis for the three reservoirs in the Upper Cuyahoga Watershed from May to July 2011. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, Stir = 500 rpm, Extraction time = 40min, Extraction temp = 70 °C, Desorption temp = 220 °C, Desorption time = 1min, Fiber type: PDMS/DVB.

Figure 4.16 Geosmin analysis for the East Branch and Rockwell reservoirs in the Upper Cuyahoga Watershed from August to December 2011. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ } ^\circ\text{C}$, $\text{Desorption temp} = 220 \text{ } ^\circ\text{C}$, $\text{Desorption time} = 1 \text{ min}$, Fiber type: PDMS/DVB.

Figure 4.17. Geosmin analysis in the City of Akron Water Treatment Plant and distribution system from May to August 2011. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40\text{min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, $\text{Desorption temp} = 220 \text{ }^{\circ}\text{C}$, $\text{Desorption time} = 1\text{min}$, Fiber type: PDMS/DVB.

4.3.2 Alliance taste and odor water analysis.

The City of Alliance needed to know the total concentrations of geosmin and 2-MIB (i.e., cell bound + aqueous concentrations); therefore, 40 mL samples were split into 2*20 mL aliquots where one aliquot was filtered, like the Akron samples, while the other was sonicated prior to extraction. Sonication was performed to lyse the cyanobacterial membranes releasing the cell bound fraction of T&O compounds into the aqueous phase. The Alliance water samples were obtained from the intake of the water treatment plant from December 2011 until February 2012.. In Figures 4.18 and 4.19, cell bound concentrations were calculated as the difference between the concentration of T&O compounds measured after sonication minus the concentration of T&O compounds measured in the filtered samples. Although, both 2-MIB and geosmin were detected in the Alliance samples, the concentration of 2-MIB was found to an order of magnitude greater than geosmin. The samples analyzed in this series contained high levels 2-MIB (111 ng/L) and relatively low concentrations of geosmin (6.10 ng/L) on Dec 19th (Figures 4.18 and 4.19). 2-MIB appeared to be mostly in the aqueous phase while geosmin was found to be mostly cell bound. This could be due to different strains of cyanobacteria becoming dominant in the bloom and dying off at different times.

Figure 4.18. 2-MIB analysis for the City of Alliance Water Treatment Plant. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ } ^\circ\text{C}$, $\text{Desorption temp} = 220 \text{ } ^\circ\text{C}$, $\text{Desorption time} = 1 \text{ min}$, Fiber type: PDMS/DVB.

Figure 4.19. Geosmin analysis for the City of Alliance Water Treatment Plant. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40\text{min}$, $\text{Extraction temp} = 70 \text{ } ^\circ\text{C}$, $\text{Desorption temp} = 220 \text{ } ^\circ\text{C}$, $\text{Desorption time} = 1\text{min}$, Fiber type: PDMS/DVB.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

5.1 Summary

The overall objective of this research was to optimize a solid phase micro extraction (SPME) method coupled with GC/MS detection in full scan mode to quantify low concentrations of 2-MIB and geosmin in the presence of potential interferents like camphor and decanal. The first objective of this study was to optimize the SPME method and desorption procedure. Therefore, parameters that would significantly influence SPME extraction efficiency such as extraction temperature, extraction time, GC/MS inlet desorption time, sonication time and temperature were varied in order to obtain the highest recovery of geosmin and 2-MIB. Raw peak areas of geosmin and 2-MIB as well as ratios to the internal standard, 4-bromofluorobenzene, were analyzed to determine the optimal extraction/desorption conditions. BFB was added to enhanced quantification of T&O compounds in water samples impacted by wastewater and other algal metabolites since most T&O methods do not employ an internal standard. The second objective was to optimized the GC configuration and oven temperature profile to create calibration curves and calculated the MDLs's and LOQ's for 2-MIB and geosmin and two potential 2-MIB interferents (i.e., camphor and decanal). The final objective was to apply the optimized method to quantify geosmin and 2-MIB in real raw water samples from two drinking water sources. In order to maximize target analyte response, the following extraction method parameters were identified and optimized:

- Headspace Absorption: extraction temperature and time were optimized to obtain sufficient recovery for both geosmin and 2-MIB at 70°C and 40 min extraction time.
- Desorption in GC Inlet: 2-MIB and geosmin adsorbed on the SPME fiber were found to completely desorbed at approximately 1min in the GC/MS inlet port at a constant 220°C .
- Sonication: degradation of geosmin and 2-MIB was evaluated as a function of time (10, 20 and 30 min) and temperature (4 °C and 25 °C) in a water immersion ultra-sonicator. Raw water samples sonicated for 10 min at 4°C was found to be sufficient in order to lyse cyanobacterial membranes while not degrading target analytes.
- The modified GC temperature profile with guard column attached to the capillary column minimized co-elution of other constituents that would interfere with 2-MIB analysis by prolonging the elution time creating definitive separation between decanal and 2-MIB peaks.
- The optimized method displayed good linearity over the concentration range 1-300 ng/L and with correlation coefficients greater than 0.9732. The method detection limits (i.e., signal/noise) for 2-MIB, geosmin and camphor were 0.81, 0.42, and 0.84, respectively, and the limit of quantification for these three compounds were 7.43, 6.39 and 8.42 respectively.

The optimized SPME method yielded highly sensitivity analysis of earthy and musty odor compounds in raw water samples in full scan MS mode. The method was then used to detect trace levels of geosmin, 2-MIB, and camphor in two source waters in

Northeast Ohio. By employing full scan mode, numerous other VOCs can be detected. Therefore, this method can also be used to identify potential wastewater contamination in the source waters or other algal derived volatile organic compounds (ADVOCs) not associated with T&O issues but relevant to cyanobacterial bloom dynamics. Most of the all, the method was found to yield reliable and reproducible results for T&O compounds in water samples that are either heavily impacted by wastewater contamination and/or under eutrophic conditions.

5.2 Recommendations for future work

The presence of musty and earthy odor compounds in water samples can be connected with factors like temperature, sunlight, pH and the concentration of micro-nutrients. In order to evaluate algal bloom growth dynamics, chlorophyll counts are generally perceived as the most reliable indicator of algal bloom density. However, cyanobacteria emit numerous volatile organic compounds that could provide some insight into algal bloom dynamics as function of environmental conditions. Therefore, future work will look to quantify additional algal derived volatile organic compounds (ADVOC) and identify correlations that may exist with cyanobacteria bloom dynamics. This may include:

- Determining SPME fiber absorption capability and lifetime in the presence of a variety of ADVOCs produced by cyanobacteria.
- Investigate the connection between nutrient loading and ADVOCs.
- Determine if correlations exist between ADVOCs and dominate cyanobacteria species during eutrophication events.
- Identify connections between ADVOCs and bloom die-off. Determine if

ADVOCs vary in concentration or proportions prior to die-off giving municipalities the ability to make treatment train modifications that reduce the presence of taste and odor compounds at the tap.

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APPENDICES

APPENDIX A

THE WWC AND GEOSMIN OBSERVED IN AKRON WATER SAMPLES

Figure A.1 Decanal analysis for the three reservoirs in the Upper Cuyahoga Watershed from June to December 2011. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40 \text{ min}$, $\text{Extraction temp} = 70 \text{ } ^\circ\text{C}$, $\text{Desorption temp} = 220 \text{ } ^\circ\text{C}$, $\text{Desorption time} = 1 \text{ min}$, Fiber type: PDMS/DVB.

Table A.1 The frequency of other waste water contaminants observed in Akron water samples from May to December 2011. (The total sample amount =15).

Compound Name	East Branch
Decane	13
Nonanal	15
Butanonic acid, butyl ester	15
2-octanol, 2-methyl-6-methylene-	3
Propane,2-chloro-2-nitro	3
Limonene	3
2-Propanol, 1-butoxy	3
Benzoic acid, 2-ethylhexyl ester	7
2-Hepten-2-one, 6-methyl-	2
Toluene	8
Octanal	12
Naphthalene	5
Dibutyl phthalate	3
Acetophenone	3
Lilial	3
Benzene, 1,3-dimethoxy-	5
Camphor	3
Benzophenone	2
Diphenyl, ether	3
Diethyltoluamide (DEET)	5
Borneol	3
Pheonl-2,4 bis(1,1-dimethylethyl)	5

Table A.2 Geosmin analysis in Akron water samples from May to December 2011.
(ng/L)

Date	East Branch	Rockwell	LaDue	Cleanwell	CT-Station	Pump-Station	Tap
5/17	9.71						
6/18		5.23	7.28		21.01	12.34	
6/19		301.53					
6/20		44.82					6.9
6/27		47.82			2.83	12.54	
6/29	15.95						
7/12		53.65		8.27	8.79		
7/15	7.05						
7/22	9.12						
7/26	48.22	24.5					
8/2		6.4		5.01			
8/19							
8/30		21.54					21.38
9/27							
10/6							
10/25	8.31						
11/2							
12/1	28.81						

APPENDIX B

TASET AND ODOR CONTAMINANTS ANALYSIS IN ALLIANCE SAMPLES

Figure B.1 2-MIB and geosmin analysis City of Alliance Water Treatment Plant from November 2011 March 2011. $V_{\text{Sample}} = 20 \text{ ml}$, $C_{\text{BFB}} = 0.25 \text{ } \mu\text{g/L}$, $\text{NaCl} = 7 \text{ g}$, $\text{Stir} = 500 \text{ rpm}$, $\text{Extraction time} = 40\text{min}$, $\text{Extraction temp} = 70 \text{ }^{\circ}\text{C}$, $\text{Desorption temp} = 220 \text{ }^{\circ}\text{C}$, $\text{Desorption time} = 1\text{min}$, Fiber type: PDMS/DVB.