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Odorous Compounds in Water Systems and their Analysis using Solid-Phase Microextraction

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number HRSMEC20060039.

Signature:

Hanna Driessen

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Abstract

Odours in water systems are a very challenging issue for water utilities to overcome. The management of odours is one of the most important, and often most difficult, requirements for water utilities worldwide. The control of odour is a problem faced by both drinking water distribution and wastewater systems. Distribution systems need to be able to provide water for drinking water purposes that is not only safe, but also aesthetically acceptable, while wastewater systems need to minimise odour at treatment plants, both sets of requirements being driven by consumer expectations. The importance of odour control for drinking water distribution is emphasised by the common consensus among the public that, if the water tastes or smells bad, it must not be safe. The fact that many countries also have sensory regulations highlights this importance. As an example, the Australian Drinking Water Guidelines stipulate that the taste and odour of drinking water should be acceptable to most people.

The biggest difficulties in managing odours are identifying the cause of the odour and measuring the odour. Customers may complain about an objectionable odour from a water system, and the way in which a water utility addresses the issue depends on the description they receive from the customer. If their description is not accurate or is not very specific, it can make identifying the cause of the odour even more difficult. Odour sensing can be subjective, where different people respond to odours differently: one person may find an odour offensive, whereas another person may find it acceptable. Even after the identification of an odorous off-flavour, actual analysis of the causative compound can be challenging. Due to the very low concentrations present in water, prior to separation it is generally necessary to pre-concentrate the sample and/or extract the analyte from the sample. Traditionally, for the analysis of odorous water samples, closed loop stripping analysis has been used for this step. The purge and trap technique, based on similar properties, has also been widely applied. However, more recently, newer methods, such as solid-phase microextraction (SPME) and stir bar sorptive extraction, are being employed as pre-concentration and extraction techniques for the analysis of odorous compounds in water.

SPME was developed in the early 1990s, for sampling and sample preparation, as an alternative, solvent-free method to solid-phase extraction. SPME traditionally involves use of a small diameter fused silica fibre, coated with a stationary phase, which is then introduced into an environment (*e.g.,* the headspace of a water sample), where the analytes partition into the stationary phase, and the fibre is then transferred into the injector of a gas chromatograph where the analytes are desorbed from the fibre for measurement. SPME has been widely applied to sampling and analysis of environmental, food, aroma, forensic, and pharmaceutical samples, including the analysis of odorous compounds in these samples.

Accordingly, this Thesis focused on the analysis of odorous compounds utilising headspace SPME as an analytical technique. The Thesis is divided into two parts, representing two different water systems and their odorous compounds. Chapters 2, 3 and 4 focus on chlorine related odorous compounds in drinking water, while Chapters 5, 6 and 7 focus on sulfur based compounds in wastewater systems.

In Chapter 2, a review of causative chlorinous off-flavour compounds is presented, with focus on chlorinous compounds related to amino acids. Chlorinous off-flavours are one of the most widely reported taste and odour complaints in drinking waters and are directly associated with the use of chlorine as a disinfectant. Worldwide, chlorine is the most commonly used disinfectant, with hypochlorous acid and the hypochlorite ion being the most prevalent species. While disinfectant residuals present in treated water are often the cause of objectionable tastes and odours, it can also be their reactions to form related compounds and subsequent disinfection by-products which produce causative chlorinous odour compounds. The focus of Chapters 3 and 4 is on the chlorination of four amino acids (valine, leucine, isoleucine and phenylalanine) and the formation of their corresponding aldehyde and nitrile by-products (isobutyraldehyde, isobutyronitrile, 2-methylbutyraldehyde, 2-methylbutyronitrile, 3-methylbutyraldehyde, 3-methylbutyronitrile, phenylacetaldehyde and phenylacetonitrile) as potential causative compounds for chlorinous off-flavour incidents.

In Chapter 3, an analytical method for the analysis of the four aldehyde and four nitrile compounds was developed. Headspace solid-phase microextraction (HS

SPME) followed by gas chromatography with mass spectrometric (GC-MS) detection was used for their analysis. A simple method for their simultaneous analysis, without the requirement for derivatisation, was developed and validated. The development of a combined HS SPME/GC-MS analytical method for the analysis of amino acid chlorination products has not been previously reported. Utilisation of this analytical method to further investigate the production of such compounds upon chlorination of amino acids in the presence of bromide ion and at different pH values was also novel (Chapter 4).

In Chapter 4, the chlorination of four amino acids (valine, isoleucine, leucine and phenylalanine) was studied at pH 7 and pH 9, and in the presence of bromide ion, after one hour reaction time by measuring the free and total chlorine equivalent concentrations and by-product formation of the corresponding aldehyde and nitrile species. Varying chlorine to amino acid ratios (0.2, 0.8, 1.6, 2.4 and 4 molar ratios) were investigated . The formation of odorous aldehyde and nitrile species upon chlorination was found. The concentration of nitrile species formed in all cases was less than the corresponding aldehyde and formation of the nitrile required a chlorine to amino acid ratio of one or more. At pH 9, greater free and total chlorine equivalent concentrations were observed for all amino acids in comparison to pH 7. This was also consistent with the lower by-product formation at pH 9, indicating less chlorine had been consumed for the formation of the aldehyde and nitrile compounds. Analysis of the reaction mixtures by an Odour Panel confirmed that the by-products were odorous with a range of descriptors, including chlorinous (as well as chemical). A kinetic study of the chlorination reactions of three of the amino acids (valine, isoleucine, and leucine) was carried out by monitoring the rate of aldehyde formation, and the rate of degradation of the intermediate monochloramino acid species. This study indicated that another intermediate by-product (potentially the dichloramino acid species) was being formed, with this by-product also having the potential to be odorous.

The second part of this Thesis focused mainly on the analysis of sulfur based odour compounds from wastewater systems. In Chapter 5, a review of the causative compounds and their formation in wastewater processes is discussed. Odorous emissions from sewer networks and wastewater treatment plants (WWTPs) can result

in public concern and complaint. The odorants released from WWTPs vary, depending upon the raw wastewater entering the system, and the operation of the treatment processes. The main odours that arise from wastewater treatment works are due to decomposition of organic matter. Typically, these odorous compounds are reduced sulfur or nitrogen compounds, organic acids, aldehydes or ketones. Odour management of emissions from sewer systems and WWTPs has traditionally been maintained by a number of different processes, including the use of buffer zones to separate odorous sources from population centres, the dispersion of emissions through ventilation stacks and the use of chemical dosing to prevent anaerobic conditions and control odour production. However, due to increasing numbers of complaints and the pressure on the land surrounding sewer ventilation points, pumping stations and WWTPs, the installation of odour abatement processes (including activated carbon, biofilters, biotrickling filters and chemical scrubbers) has become necessary in order to manage the production of odours to a limit which is acceptable.

In Chapter 6, the establishment of an analytical method for the analysis of several volatile sulfur compounds (*i.e.,* ethanethiol, dimethyl sulfide, ethyl methyl sulfide, dimethyl disulfide, diethyl disulfide and dimethyl trisulfide) associated with wastewater treatment processes was investigated. A HS SPME method utilising GC-MS detection was developed and validated for aqueous samples for dimethyl sulfide, ethyl methyl sulfide, dimethyl disulfide, diethyl disulfide and dimethyl trisulfide. The adaptation of the method for the analysis of gaseous samples was then attempted. Preparation of adequate and reproducible gas standards was not possible. Two different preparation methods were investigated (both previously published) and found to be unsuitable. Calibration curves constructed from these standards were not linear, and not reproducible. Evidence of analyte interaction became apparent, where the detection of ethanethiol was not possible. Ethanethiol is likely to have reacted with either other analytes present in the mixture or sampling or analysis equipment to undergo dimerisation to form diethyl disulfide. As standard generation was unsuccessful, gas and air samples were qualitatively analysed. The aqueous HS SPME method was also found to be suitable for analysis of a number of other volatile organic compounds associated with wastewater odours (*i.e.,* toluene, ethylbenzene, styrene, *p*-cresol, indole and skatole).

In Chapter 7, analysis of air samples from two sampling points at a wastewater treatment plant, specifically before and after a biotrickling filter, was undertaken utilising the HS SPME/GC-MS method developed in Chapter 6. Identification of a range of odorous sulfur compounds in both the inlet and outlet areas of the biotrickling filter included sulfur dioxide, methanethiol, carbon disulfide, dimethyl sulfide, propenethiol, propanethiol, dimethyl disulfide and dimethyl trisulfide. A range of other non-sulfur based compounds were also identified including, but not limited to, limonene, eucalyptol, *a*-pinene and various alkyl benzenes and straight chain hydrocarbons. A suite of disinfection by-products were also identified, such as chloroform and other trihalomethanes. While the analytical method was unable to be developed quantitatively, using abundance as an indication of concentration showed that these compounds were present in lower concentrations in the biotrickling filter outlet compared to the inlet, indicating that reduction in the concentration of odour compounds present in the sewer system was achieved by the biofilter. This outcome is an important confirmation of the usefulness of biofilters for wastewater odour reduction, particularly necessary when wastewater treatment plants are located in suburban areas with nearby housing.

This Thesis highlights the utility of the HS SPME technique for extraction and pre-concentration of odorous analytes in water samples. HS SPME has been successfully combined with GC-MS for analysis of odorous compounds in both drinking waters and wastewaters. The drinking water related study demonstrated that the presence of amino acids in drinking water has the potential to lead to odorous by-products, which could be described as chlorinous, upon chlorination. The impact of bromide ion, if present in drinking water source waters, would be to increase potential formation of such odorous by-products. Consideration of removal of the amino acid precursors in the treatment of drinking water may aid in the reduction of formation of chlorinous odorous in the distribution system. The HS SPME/GC-MS technique was successfully used for the identification of volatile sulfur compounds and other volatile compounds associated with off-flavours at a wastewater treatment plant. The technique shows promise for demonstrating odour reduction in odour abatement processes implemented in wastewater treatment plants and potentially offers a more simple sampling device for odour screening.

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List of Abbreviations

2-MBA	2-methylbutyraldehyde
2-MBN	2-methylbutyronitrile
3-MBA	3-methylbutyraldehyde
3-MBN	3-methylbutyronitrile
AWWA	American Water Works Association
BTEX	benzene, toluene, ethylbenzene and xylene
CAR	carboxen
CLSA	closed loop stripping analysis
CW	carbowax
DBP	disinfection by-product
DEDS	diethyl disulfide
DMDS	dimethyl disulfide
DMS	dimethyl sulfide
DMTS	dimethyl trisulfide
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
DVB	divinylbenzene
ECD	electron capture detection
EMS	ethyl methyl sulfide
EPA	Environmental Protection Agency
EtSH	ethanethiol
FID	flame ionisation detector
FPA	flavour profile analysis
FRA	flavour rating assessment
FTN	flavour threshold number
FTT	flavour threshold test
GC	gas chromatography
HAN	halonitrile
HOBr	hypobromous acid
HOCl	hypochlorous acid
HOI	hypoiodous acid

HPLC	high performance liquid chromatography
HS	headspace
I-THMs	iodinated trihalomethanes
IBA	isobutyraldehyde
IBN	isobutyronitrile
LLE	liquid liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MeSH	methanethiol
MIB	2-methyl isoborneol
MS	mass spectrometry
NIOSH	National Institute for Occupational Safety and Health
NOM	natural organic matter
O	olfactometry
OCl^-	hypochlorite ion
OTC	odour threshold concentration
P&T	purge and trap
PA	phenylacetaldehyde
PAH	polyaromatic hydrocarbon
PDMS	polydimethylsiloxane
PFBHA	o-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride
PFPH	pentafluorophenylhydrazine
PN	phenylacetonitrile
RSD	relative standard deviation
SBSE	stir bar sorptive extraction
SIM	single ion monitoring
SPE	solid-phase extraction
SPME	solid-phase microextraction
TDS	total dissolved solids
THM	trihalomethane
TIC	total ion chromatogram
TON	threshold odour number
TTC	taste threshold concentration

UV	ultra violet
VOC	volatile organic compound
WA	Western Australia
WHO	World Health Organisation
WWTP	waste water treatment plant

List of Publications

Publications arising from the present study:

Conference Presentations

Oral Presentations

Driessen, H., Joll, C. and Heitz, A. 2011. Odorous aldehydes and nitriles from chlorination of amino acids: effect of pH and bromide ion. *9th International Water Association Off-Flavours in the Aquatic Environment Symposium*, Aberdeen, Scotland, 14-19 August.

Poster Presentations

Driessen, H., Joll, C., von Gunten, U. and Heitz, A. 2010. A kinetic study of the chlorination of amino acids associated with the formation of chlorinous off-flavours. *5th IWA International Young Water Professionals Conference,* Sydney, Australia 5-7 July.

Driessen, H., Joll, C. and Heitz, A. 2008. Development of a simple method for the analysis of aldehydes potentially associated with chlorinous off-flavours. *8th IWA Symposium on Off-flavours in the Aquatic Environment*, Deajeon, Korea, 5-9 October.

Driessen, H. 2006. Causes and prevention of chlorinous off-flavours in drinking water. *CRC for Water Quality and Technology Postgraduate Students Conference*, Melbourne, Australia, 10-13 July.

Chapter 1

Introduction

1.1 Water Systems

There are two main types of water systems: drinking water systems and wastewater systems. Drinking water systems collect water from a variety of locations, including groundwater, surface water and seawater, to provide drinking water for human consumption. Drinking water is generally purified and disinfected prior to distribution to customers. Upon generation, wastewater is usually discharged into a sewer system and treated at a wastewater treatment plant.

1.1.1 Water Systems in Perth, Western Australia

Water in Perth, Western Australia (WA) is primarily provided by the Water Corporation. Drinking water in Perth comes from three major sources: groundwater, desalinated seawater and surface water reservoirs. The drying climate in the South West of WA has led to the development of new and more sustainable sources of drinking water, with the implementation of two large scale reverse osmosis desalination plants (Water Corporation, 2011). Groundwater is sourced from shallow subsurface aquifers or deeper aquifers in the sands of the Swan Coastal Plain (Davidson, 1995). Most of the 180 bores are located in Perth's northern suburbs. Surface water is obtained from eight dams in the Darling Range, situated in largely uninhabited Jarrah forests growing on low-nutrient lateritic sediment- South and North Dandalup, Serpentine, Wungong, Churchman Brook, Canning, Victoria and Mundaring Weir. Water is also supplied from Stirling and Samson Dams in the South West region (Water Corporation, 2013a). The Perth Seawater Desalination Plant, commissioned in 2006, produces up to 45 gigalitres per day of potable water, which is supplied into Perth's Integrated Water Supply Scheme. The integrated water supply scheme is responsible for providing fresh drinking water, with 31% being supplied from surface water, 46% from groundwater sources and the remaining 23% from desalination sources (Water Corporation, 2013b). The local shallow groundwater resources are usually highly coloured and contain relatively high concentrations of dissolved organic carbon (10 – 50 mg L^{-1}), sulfide (0.5 – 5 mg L^{-1}) and dissolved iron (1 – 4 mg L^{-1}) (Davidson, 1995), such that these resources require substantial treatment to produce drinking water which complies with the Australian Drinking Water Guidelines (NHMRC, 2011). Where water comes from protected

catchment areas (such as surface waters), very little treatment is required, and the water is usually only disinfected (Water Corporation, 2013a).

The wastewater system in Perth, WA is a reticulated pipe system that transports wastewater from households, businesses and industry for treatment prior to reuse or discharge. The network, consisting of approximately 10,000 km of pipe and over 500 pumping stations, treats more than 300 million litres of wastewater daily. The three largest metropolitan plants, Beenyup, Subiaco and Woodman Point, treat approximately 80% of the state's wastewater. The system itself is fairly new, with about 85% of sewer pipes being laid after 1970 (Water Corporation, 2006).

1.2 Odours in Drinking Water Systems

The importance of odour control for drinking water distribution is emphasised by the common consensus among the public that, if the water tastes or smells bad, it must not be safe (McGuire, 1995). The fact that many countries also have sensory regulations highlights this importance. The Australian Drinking Water Guidelines (NHMRC, 2011) stipulate that the taste and odour of drinking water should be acceptable to most people. The 1984 World Health Organisation (WHO) guidelines require that water should not be objectionable to most consumers, while their 2011 guidelines require taste and odour be acceptable to avoid consumer complaints. They go on to state that the presence of taste and/or odour could be an indication of some form of pollution or of a malfunction during water treatment and therefore may be an indication of the presence of potentially harmful substances (WHO, 2011). The European Economic Community Standards and the United States Environmental Protection Agency (USEPA) require that the threshold odour number of water not exceed 3 (USEPA). Odour, once again, is recognised as an indicator of the effectiveness of different types of treatment.

Odours from drinking water can arise from a number of different sources that can be classified as either man-made or natural. Natural sources include biological activity, while man-made sources arise from treatment of drinking water and within the distribution system (Khiari *et al.*, 2002). A summary of the different odour types and their likely source is shown in Table 1.1.

Table 1.1: The sources of odour problems in drinking water, Suffet and Rosenfeld (2007).

Odour Type	Source of Odour
Natural products	Grassy/say/straw/woody odours Fishy/rancid odours Fragrant/vegetable.fruity/flowery odours
Industrial products	Chemical/hydrocarbon/miscellaneous odours Medicinal/phenolic odours Chlorinous odours
Aerobic oxidation products	Earthy/musty.moldy odours
Anaerobic degradation products	Marshy/swampy/septic/sulphurous odours

1.2.1 Odours from Biological Activity

For a long time, offensive taste and odour from drinking water has been related to the growth of specific microorganisms. The first publication on this subject dates back to 1929 (Adams, 1929), where actinomycetes were associated with musty and earthy odours in water. In the period 1967-1970, two earthy smelling metabolites of Streptomyces strains and blue-green algae were identified as geosmin and 2-methyl isoborneol (MIB) (Gerber and LeChevalier, 1968). Since then, many taste and odour episodes have been linked to the presence of these metabolites (Zaitlin and Watson, 2006).

One of the more studied cases of biological activity in distribution systems relates to the formation of trichloroanisoles from the biomethylation of trichlorophenols (Montiel *et al.*, 1999; Nystroem *et al.*, 1992). Bruchet (1999) reported a number of instances where a mouldy/earthy odour was caused by the presence of 2,4,6-trichloroanisole. In water systems where there is low flow and no disinfectant residual, swampy or rotten vegetable odours can arise. Such an incident in California was attributed to the presence of organic sulfides believed to have been formed due to biological activity in an anoxic reservoir (Krasner *et al.*, 1989). Over a period of time from the early 1980s to late 1990s, complaints of swampy odours in distributed waters in Perth, WA, were received by local authorities (Franzmann *et al.*, 2001; Wajon *et al.*, 1986; Wajon *et al.*, 1985a). A range of sulfurous compounds were

identified in complaint samples and it was proposed microbial activity was responsible for the production of such odorous compounds (Franzmann *et al.*, 2001).

1.2.2 Odours from Disinfection Processes

One of the most widely reported taste and odour complaints in drinking waters is directly associated with the use of chlorine as a disinfectant. Worldwide, chlorine is the most commonly used disinfectant, with hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-) being the most prevalent species. When ammonia is used in conjunction with chlorine, this is known as chloramination and several chloramine species are formed. All of these species have similar descriptors of "bleach, swimming pool and chlorinous" (Khiari *et al.*, 2002). Chlorinous odours and off-flavours in drinking waters are a key focus of this research and will be discussed in more detail in Chapter 2. While disinfectant residuals present in treated water are often the cause of objectionable tastes and odours, it is also their reactions to form related compounds and subsequent disinfection by-products which produce presumed causative chlorinous odour compounds. This will be discussed in Section 2.4.3.

1.2.2.1 Consumer Perceptions related to Chlorinous Off-Flavours

Due to the widespread nature of taste and odour complaints by consumers to water utilities, and the importance of customer satisfaction, many studies and surveys have been carried out in order to determine what people think of their water, and to provide a gauge for utilities on their performance. McGuire (1995) reported that consumers believe if their water smells and tastes "bad", it is probably not safe. Consumers judge the quality of their drinking water by its aesthetic properties. While the water may meet all the required health regulations, utilities also need to ensure they provide water which is free from off-flavour problems. These off-flavour problems are reported to have led US consumers to increasingly purchase bottled water and point-of-use treatment devices (Mackey *et al.*, 2004). A survey carried out in Western Australia in 1998, found that 25% of the respondents used consumer water filters primarily to improve taste and remove impurities and chlorine. This same survey found that the main reason for consumption of bottled water was also improved taste (Market Equity Pty Ltd, 1998).

1.2.2.1.1 Local Consumer Surveys

Several local studies in Western Australia were carried out over a period in the late 1990s (Market Equity Pty Ltd, 1998; Market Equity Pty Ltd, 1997). In a customer satisfaction survey in 1997, of the respondents who indicated that the quality of the tap water ranged from 'quite good' to 'terrible', a large number found the taste of the water to be a problem, while another group found the 'chlorine smell' to be a problem. The taste problems included 'too much chlorine', 'too metallic' and 'too many chemicals'. A later telephone survey conducted in 1998 found that 70% of all respondents were dissatisfied with the taste of their water, and 39% were dissatisfied with chlorine in their water. To reduce chlorine or chemicals in their water was the highest priority for 36% of respondents.

In another series of studies carried out on drinking water aesthetics during 1999 in Western Australia, participants in tasting experiments had a distinct preference for the water sample with no chlorine, and least preferred the samples with the highest levels of chlorine, while the survey respondents had concerns with the smell and taste of chlorine in their drinking water supply (Australian Research Centre for Water in Society, 2000).

1.2.2.1.2 International Consumer Surveys

A large number of international studies and surveys have also been conducted. During 1989, the American Water Works Association (AWWA) Taste and Odor Committee conducted a survey of water utilities. The survey was sent to 826 utilities and focused on how the utilities perceived the taste and odour problems in their water supplies. Chlorine was the most frequently reported odour problem and the third most frequently reported taste problem. It was also hypothesised, that as chlorine is one of the most widely used treatment methods for other off-flavour problems, the utilities may be substituting one type of taste and odour problem for another by using chlorine (Suffet *et al.*, 1996).

Neden *et al.* (1992) conducted a survey in the Great Vancouver Water district in Canada on public responses comparing chlorination and chloramination as secondary disinfectants. It was reported that chloraminated water was more acceptable from a taste and odour standpoint than chlorinated water. On the basis of public response,

measured by customer complaints, chloramine produced more negative reaction at the beginning of the treatment program than chlorine, however this may have been misleading due to a more widespread chloramine residual in comparison to chlorine, which was consequently detected by more people. A questionnaire was also carried out that showed while both treatments imparted a chlorinous taste to water, the taste from chloramination was generally less noticeable and offensive than that of chlorine (Neden *et al.*, 1992).

Another Canadian study (Levallois *et al.*, 1999) evaluated consumers attitudes towards tap water in the Quebec metropolitan area. In this case, more than half of the consumers rejected tap water due to organoleptic reasons, not health concerns. However, the main reason for these complaints was unclear. The use of chlorine had been known to cause problems in the area, so Levallois *et al.* (1999) reported that the use of chlorine may have contributed to the dissatisfaction of the customers.

An extensive research project carried out in the US focused on identifying public perceptions about chlorinous tastes and how these perceptions affected peoples' choices to use tap water alternatives, and then developing recommendations for water utilities to address this issue (Mackey *et al.*, 2004). The key findings from this extensive study highlighted that most consumers were satisfied with their tap water however a perception of an off-flavour (whether it be chlorinous, or another off-flavour) decreased satisfaction, and this dissatisfaction was associated with a perception of tap water 'unhealthiness' and 'lack of safety'. It was also found that untrained consumers were able to distinguish different flavour types (chlorine, medicinal, chemical) but couldn't describe them correctly. Consumers appeared to often misidentify chlorinous tastes and-or odours, and it was thought that this mistake was made as chlorine was the only chemical which consumers knew was in their drinking water (Mackey *et al.*, 2004). A summary of the wide range of compounds that are often present in drinking water (either naturally or as a disinfection by-product) that are associated with chlorinous off-flavours but do not have the descriptor chlorinous, and their odour threshold concentration (OTC) and taste threshold concentration (TTC), was presented in this study and is shown in Table 1.2.

Table 1.2: Perception thresholds of compounds that are associated with chlorinous flavours (reproduced from Mackey 2004).

Compound	Descriptor	OTC (mg L^{-1})	TTC (mg L^{-1})
Naphth-1-ol	Medicinal	1.29	
4-Chloro-2-methylphenol	Chemical Medicinal	0.062	0.025
Benzene	Phenol	0.19	
Chlorobenzene	Medicinal Chemical	0.19	0.19
1,3-Dichloro-benzene	Medicinal Disinfectant	0.077	0.19
Trichloroacetic acid	Perfume, chlorine	0.05	
2-Methylphenol (*o*-Cresol)	Medicinal	0.65	
3-Methylphenol (*m*-Cresol)		0.68	
Iodocresol	Medicinal, burnt	0.01	

Recommendations provided from the work of Mackey *et al.* (2004) proposed that it would be difficult to identify a consumer's taste and/or odour problem based solely on their description of the taste and that customer satisfaction could be increased by two methods: 1) improve customers' confidence in tap water quality and the safety of chlorinated tap water; 2) reduce chlorinous off-flavours and thereby reduce their detection.

All of these surveys and studies emphasise the importance of consumer perception on the taste and odour of their drinking water. Consumers tend to relate the safety of their water with its taste and odour and appearance. The presence of chlorinous off-flavours is also a widespread issue that might often be misidentified, but remains a key issue for water utilities to address.

1.3 Odours in Wastewater Systems

Many odorous substances originate in wastewaters or form and are emitted during wastewater collection and treatment. These substances can be a combination of both inorganic and organic gases and vapours. Inorganic gases can include hydrogen sulfide and ammonia. While both inorganic and organic gases can form as a result of biological activity, the organic gases can result from direct discharge of chemical waste. Within wastewater treatment, odorous compounds tend to form due to the

anaerobic conditions present (Frechen, 1988). Once the compounds are present, they then pass into the air and can be detected. Frechen (1988) identified the sources of odorous emissions after carrying out a survey of more than one hundred German wastewater treatment plants (WWTPs). The operators were asked for the particular parts of their plants which were determined as sources of odorous emissions. The results depicted in Figure 1.1 identify the major sources of emission and the percentage of respondents who identified that source (Gostelow *et al.*, 2001). This survey also found that operators were of the opinion that the main reasons for the emission of odorous substances were odorous substances coming in with the influent and odorous substances being formed by decomposition (Frechen, 1988).

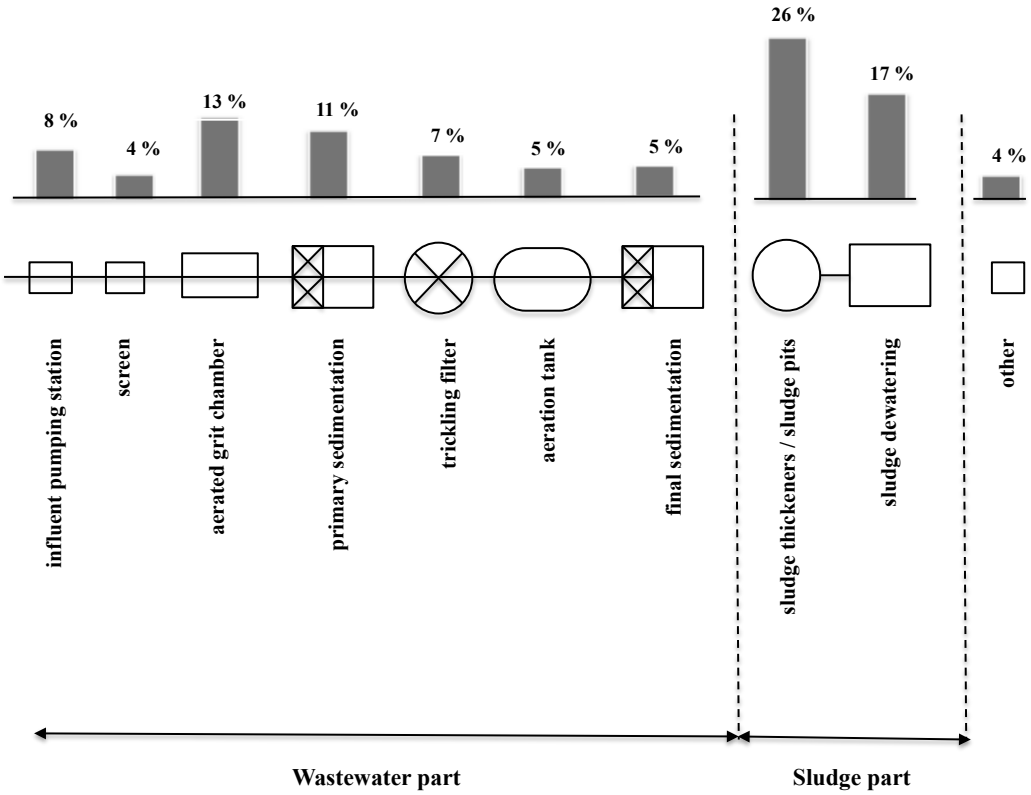


Figure 1.1: Sources of odour emission identified from Frechen's (1988) survey in wastewater treatment plants. Reprinted with permission from Gostelow *et al.* (2001) Copyright Elsevier, 2001.

While public concern over WWTP odours is not new, in recent times odour complaints have increased and this is thought to be due to several reasons: 1) increased housing near WWTPs; 2) environmental legislation requiring new or upgraded plants; 3) increased awareness of, and expectations of, the local environment and water companies by consumers; and 4) increased awareness of

consumer rights. Accordingly, the minimisation of odour emissions from WWTPs is one of the most significant challenges of the water industry.

A large number of compounds have been associated with WWTP odours. This will be further discussed in Chapter 5. A review paper by Gostelow *et al.* (2001) organises the large number of compounds identified in wastewaters into the following key classes: sulfurous, nitrogenous, organic acids and aldehydes or ketones.

1.4 Odour Measurement of Water

It is thought that, in order for water utilities to be able to control odour problems, an understanding of several criteria is required: known chemical and biological causes of odours, specific odour types, treatment mechanisms for removal of known chemical and biological causes and types, and case studies of odour problems from different water resources (Suffet *et al.*, 1995a). With this knowledge, a water utility should be able to effectively manage odour problems. It is also important for odours to be able to be measured (Gostelow *et al.*, 2001).

It is important to distinguish between odorants and odours. An odorant is the compound responsible for imparting an odour, and an odour is the perceived effect of the odorant interpreted by the olfactometry system. The link between odorant properties and perception is still quite unclear. Accordingly, two classes of measurement exist: sensory measurement and analytical measurement (Gostelow *et al.*, 2001).

1.4.1 Sensory Odour Measurement of Water

Sensory measurements utilise the nose as the odour detector; they relate directly to the properties of odours as experienced by humans. This is problematic as complex mixtures with interactions between compounds and varying concentration levels become irrelevant as the 'total effect' of the odour is measured (Gostelow *et al.*, 2001). In order to make odour management possible, odours need to be identified and also measured. Identification can be very difficult based on the complex

mixtures that create odours, however specific tools have been developed in order to streamline identification.

The latest edition of Standard Methods for the Examination of Water and Wastewater (APHA, 2012) presents four methods for the sensory evaluation of odours and tastes in water: 1) threshold odour test; 2) flavour threshold test (FTT); 3) flavour rating assessment (FRA); and 4) flavour profile analysis (FPA). In the first three methods a numerical value is assigned to a particular taste or odour (based on the strength of the off-flavour), while the last method, a description is assigned to the off-flavour.

The threshold odour test determines a threshold odour number (TON). The TON is determined by diluting a sample with odour free water until the least definitely perceptible odour is achieved *i.e,.* it is the dilution ratio at which odour is just detectable (APHA, 2012). Due to the variation of a person's capability to detect an odour, a TON can vary from day to day, and panellist to panellist.

The FTT is similar to the threshold odour test, except in this case deals with flavour, not odour. Flavour refers to a complex mixture of sensations that result from stimulation of nerve endings in the tongue, mouth and nasal cavity. The FTT results in determination of a flavour threshold number (FTN). The FTN is described as the greatest dilution of sample with reference water yielding a perceptible difference (APHA, 2012). In this test, observers are asked to describe the flavour of the most concentrated sample. Like a TON, a FTN is not a precise value

FRA is used when the purpose of testing is to estimate the suitability and acceptability of water for daily consumption (APHA, 2012). A tester is presented with nine statements about the water ranging on a scale from very favourable (1) to very unfavourable (9). The individual rating is the scale number of the statement chosen. The panel rating for a particular sample is generally the mean of individual ratings (APHA, 2012).

1.4.1.1 Flavour Profile Analysis for Sensory Analysis of Water

FPA is designed to evaluate the tastes and odours important in drinking water (APHA, 2012). It is not used to judge a sample, determine preferences between samples, or to determine the acceptance of a sample for public consumption. FPA is used to "define a water sample's taste and odour attributes, which could then be related to, or associated with, consumer perceptions" (APHA, 2012). A trained panel evaluates the taste and odour characteristics of a water sample. The FPA recognises the different flavours, alongside the flavour intensity. This sensory testing method was originally developed within the food industry so that a set of flavour and aroma characteristics of a sample could be obtained (Cairncross and Sjöstrom, 1950). It was based on the opinion of trained panellists, and employed reference standards periodically to retrain panellists. In this method, flavour represented all the sensations experienced while eating or drinking. Not only does it include taste bud sensations, but also incorporates the response of the nose, mouth and throat. Four tastes exist: salt, sweet, bitter and sour. The number of flavours and aromas or smells is in contrast unlimited, due to the large number of olfactory senses combining with feelings and senses. Amoore (1977) hypothesised that, while primary tastes exist, so do primary odours, however rather than there just being four, that the total number of human primary odours is at least 32.

FPA was first adapted by the Metropolitan Water District of Southern California for water treatment purposes (Krasner *et al.*, 1985). It has since been modified by different water utilities, however the philosophy is universally the same (Bartels *et al.*, 1986). A sample is presented to a group of panellists at the same time. A standardised procedure for smelling or tasting is used and the resulting descriptors and intensities are noted by each panellist. An open discussion is then held by the panel leader where the findings are compiled and developed into a flavour profile. The samples may be re-examined as necessary until at least 50% of the panellists agree on the note or flavour of the sample. The intensity for this sample is calculated by averaging the individual intensity scores. The FPA provides a set of descriptions and sometime notes each with a specific intensity score for each sample and this is called the flavour profile of the sample (Bartels *et al.*, 1986).

To facilitate the adaptation of FPA for drinking water analysis, a set of eight categories was suggested to aid in classifying odours in drinking water. These categories have since been extended and adapted to fit across not only the drinking water industry, but also the wastewater industry.

The development of a drinking water taste and odour wheel in 1986 based on those initial categories aimed to provide a tool that water utilities could use to try to identify odours and provide a common language that could be used. The wheel (Figure 1.2) also provided an up-to-date summary of the state of knowledge on specific chemicals that had been identified and caused problems in drinking water. The wheel consists of an inner circle with primary taste and odour categories. These categories have then been further defined by trained sensory panels in the outer circle. Finally, specific chemicals that have been attributed to the descriptors are given in the outside circle. Chemicals which have been identified in actual taste and odour episodes are marked with an asterisk.

The drinking water wheel is a powerful tool used by sensory panels for FPA. It has provided a set of common descriptors and odour standards in order to enhance reproducibility and sensitivity. Over time, it has also been used to reflect new research and identifications during taste and odour incidents (Suffet and Rosenfeld, 2007).

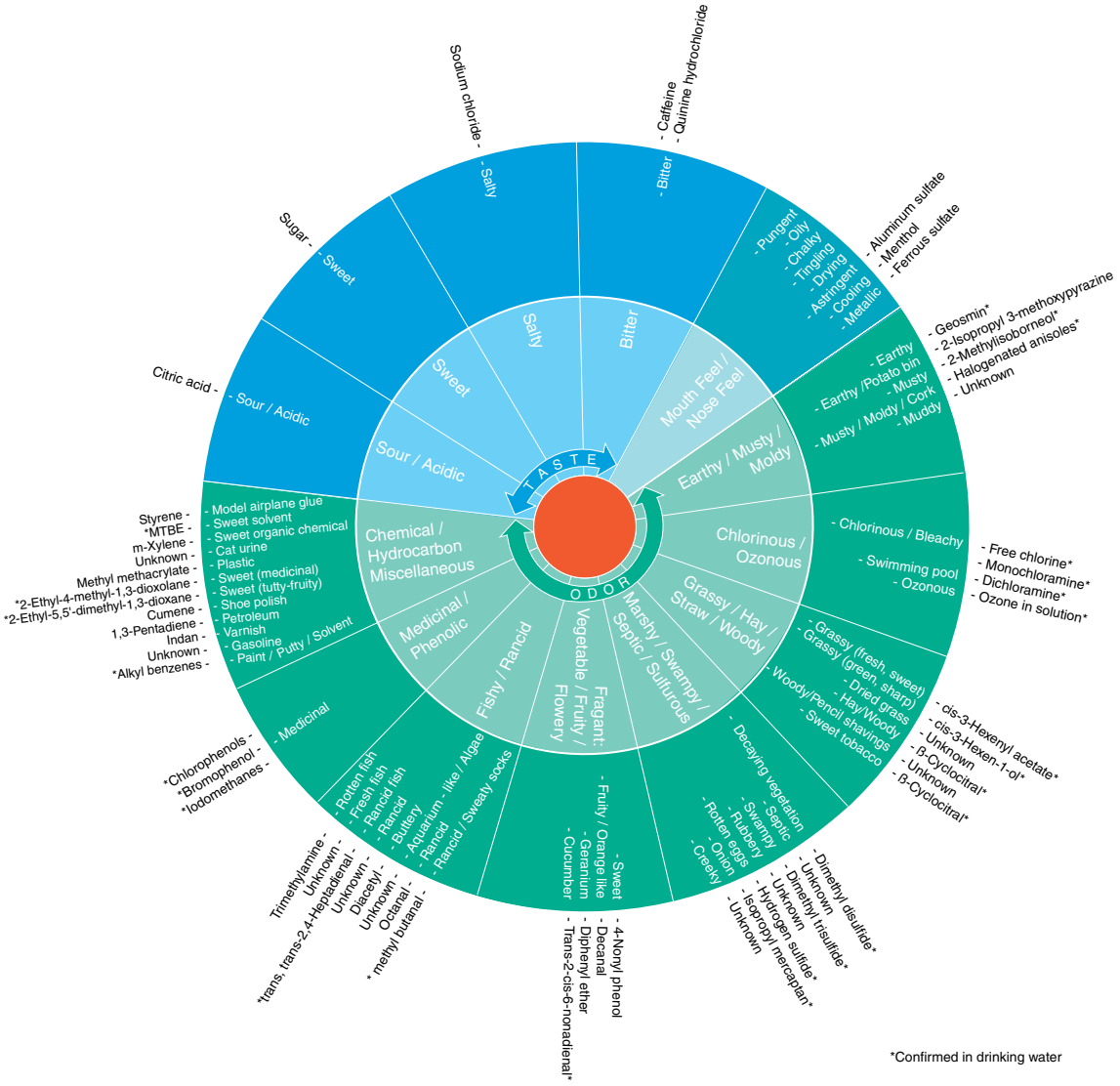


Figure 1.2: Drinking Water Taste and Odour Wheel. Reprinted from Suffet and Rosenfeld (2007), Copyright IWA Publishing, 2007.

Due to the success of the drinking water wheel in providing a standard set of descriptors to a sensory panel, the concept has since been extended to other water types where nuisance odours may be present and need to be controlled, *e.g.,* wastewater. Burlingame *et al.* (2004) presented the first wastewater odour classification scheme and wheel (Figure 1.3) based on the drinking water odour wheel. They presented 11 odour categories, including nose feel. While the list is not exhaustive, it provides the most commonly reported chemicals and odours relating to wastewater treatment processes.

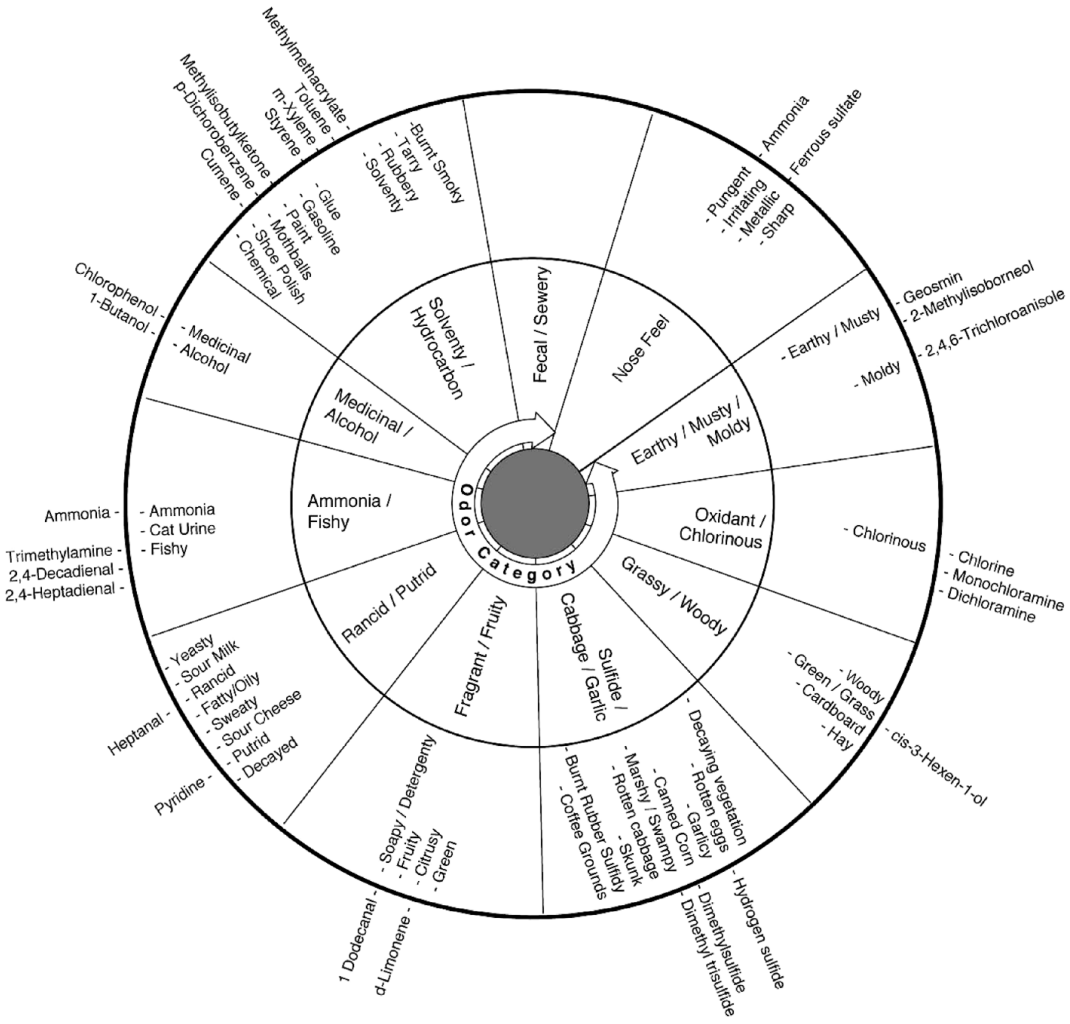


Figure 1.3: Wastewater odour wheel. Reprinted from Burlingame *et al.* (2004) Copyright Elsevier, 2004.

1.4.2 Analytical Measurement of Odours in Water

The use of sensory panels to give a description of an odorant tends to guide the analytical measurement of a sample. Analysis of the chemical composition of an odour requires a separation technique followed by a detection technique. Gas chromatography coupled with various detectors is one of the most widely used techniques employed to confirm the presence of a particular odorant. In some cases, gas chromatography is coupled to a sniffing port where trained analysts record characteristic odours against a retention time.

Due to the very low concentrations present, prior to separation it is generally necessary to pre-concentrate/extract the sample. Traditionally, for the analysis of odorous water samples, closed loop stripping analysis (CLSA) has been used for this

step. Purge and trap (P&T), based on similar properties, has also been widely applied. However, more recently, newer methods, such as solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE), are being employed as pre-concentration/extraction techniques for the analysis of odorous compounds in water (Khiari and Watson, 2007). Other pre-concentration techniques include liquid-liquid extraction and solid phase extraction.

1.4.2.1 Pre-Concentration/Extraction Techniques for the Measurement of Odours in Water

CLSA has traditionally been the pre-concentration/extraction method of choice for the determination of very low level volatile organic compounds. It is suitable for analysing a wide range of organic compounds. CLSA is recommended in Standard Methods (APHA, 2012) for the analysis of taste and odour causing compounds such as geranyl acetone, 6-methyl-hept-5-en-2-one, β-ionone and β-cyclocitral (APHA, 2012).

Specifically, CLSA has been used in the determination of sub-nanogram per litre concentrations of the algal metabolites, MIB, and geosmin (APHA, 2012; Romero *et al.*, 2007; Suffet *et al.*, 2006; Malleret *et al.*, 2001; McCallum *et al.*, 1998; Izaguirre *et al.*, 1982; McGuire *et al.*, 1981), which yield earthy-musty odours at 2 – 5 ng L^{-1} in water (Bruchet, 2006), and a range of sulfides, namely dimethyl trisulfide, which yields a swampy odour at 10 ng L^{-1} (Heitz, 2002; Franzmann *et al.*, 2001; Wajon *et al.*, 1988). Other specific compounds detected include the musk compounds, acetyl cedrene and amberonne (Mitjans and Ventura, 2005; Mitjans and Ventura, 2004), and a variety of toluene based compounds (Marti *et al.*, 2005).

CLSA has also been used in a range of studies into the investigation of volatile compounds responsible for off-flavour incidents in different water systems, identifying a range of compounds including, but not limited to, terpenoids, alkyl methoxypyrazines and a range of volatile organic compounds (VOCs) (Joll *et al.*, 2010; Wiesenthal and Suffet, 2007; Escalas *et al.*, 2003b; Kampioti and Stephanou, 2002; Kampioti and Stephanou, 1999; Aramendia *et al.*, 1998; Wilkesmann *et al.*, 1995; Bruchet *et al.*, 1991; Guardiola *et al.*, 1991; Gomez-Belinchon and Albaiges, 1987; Westendorf, 1982; Melton *et al.*, 1981).

The original CLSA design by Grob (1974) has since been modified to a variety of commercially available pieces of apparatus. In the closed loop design, a sample bottle is placed in a temperature controlled water bath and connected to a closed loop. An air pump circulates the air contained in the loop, generating fine bubbles through a glass frit in the sample bottle in order to strip volatile compounds. A filter containing an adsorbent material is inserted into the loop to collect the volatiles. The filter is contained within a heater to prevent condensation on the trap. The system is run for a prescribed amount of time, after which the trapped analytes are eluted from the carbon using solvents. The very low detection limits are achieved due to the high concentration factor implemented (40,000 times) and the absence of a solvent concentration step (Bruchet, 2006).

P&T has been defined as "a headspace gas analysis in which volatiles are stripped from the sample with an inert gas, trapped into a solid sorbent, and thermally desorbed" (Falcó and Moya, 2013). Initially described by Swinnerton and Linneborn (1967) as a method for the determination of small amounts of gaseous hydrocarbons in aqueous solution and then further developed by Bellar and Lichtenberg (1974), for the quantitative determination of a wide range of water-insoluble VOCs at detection limits of 0.5 $\mu g\ L^{-1}$, P&T has become a widely used technique for the analysis of VOCs in aqueous samples (Falcó and Moya (2013). P&T is recommended as an extraction technique in Standard Methods (APHA, 2012) for the analysis of volatile organic compounds, trihalomethanes and chlorinated organic solvents, and 1,2-dibromomethane and 1,2-dibromo-3-chloropropane. The USEPA also recommends P&T for a range of its standard protocols for the analysis of volatile compounds in waters.

P&T consists of three processes: 1) an aliquot of sample is stripped with a purge gas, 2) the analytes stripped by the purge gas are trapped into a solid sorbent, and 3) analytes are thermally desorbed for detection and identification. Automated systems are widely available, generally with gas chromatography (GC) coupled to a mass selective detector (MSD) (Falcó and Moya, 2013).

More recently, sorptive extraction techniques, which are solvent-free, have been employed to pre-concentrate and extract analytes from the sample into a sorbent.

SPME and SBSE are the most implemented sorptive techniques in water laboratories (Falcó and Moya, 2013). Since SPME is the focus analytical method used in research reported in this Thesis, it will be discussed in detail in Section 1.6.

In the SBSE technique, a magnetic stir bar, which is covered in a layer of polydimethylsiloxane (PDMS), is exposed to a sample and the compounds sorb to this layer. The compounds are then either thermally desorbed and analysed by GC-mass-spectrometry (MS) or desorbed by means of a liquid to allow analysis by liquid chromatography. The technique was developed in 1999 (Baltussen *et al.*, 1999) and is commercially known with the trademark name Twister® (Nogueira, 2012). The off-flavour compounds; MIB, geosmin. 2,4,6-trichloroanisole, 2,3,6-trichloroanisole, 2,3,4-trichloroanisole, and 2,4,6-tribromoanisole have been reported in water samples, where SBSE was used as the extraction technique (Benanou *et al.*, 2004; Benanou *et al.*, 2003; Frank *et al.*, 2003). An on-tap device containing a stir-bar has also been marketed for the detection of off-flavour compounds in water (Benali *et al.*, 2008; Tondelier *et al.*, 2008).

1.4.2.2 Separation and Detection Techniques for the Measurement of Odours in Water

Generally, gas or liquid chromatographic techniques coupled with appropriate detectors are used for the separation and detection of odorous compounds in water. High-pressure (or performance) liquid chromatography (HPLC) and GC are extensively used. A wide range of detectors are used for GC analysis and include, but are not limited to: flame ionisation detection (FID), photoionisation detection (PID), electron capture detection (ECD), electrolytic conductivity detection (ELCD), MS and MSD, and Fourier transform infrared detection (FTIR) (Falcó and Moya, 2013). MSDs allow low detection limits in single/selection ion monitoring (SIM) mode, and accordingly is the detector used in research reported in this Thesis.

1.5 Odour Measurement of Air

1.5.1 Sensory Odour Measurement of Air

The most commonly used sensory odour measurement techniques in air involve dilution. An odorous sample is diluted to its threshold odour concentration with

odour-free air. Dynamic olfactometry is the favoured technique for odour measurements, due mostly to its capacity to provide rapid dilution for evaluation by odour panellists (Koe, 1989). This involves the mixing of dynamic flow-rates of the sample with odour-free air to achieve threshold dilution. The resultant air is presented at a controlled flow-rate to a panellist. Commercially available olfactometers from different manufacturers differ in their design and consequently the manner in which dilutions of samples are obtained. Odour measurement results obtained by different olfactometers, even on the same sample can vary considerably (Koe, 1989). These variations are caused by differences in flow systems, differences in presentation of mixtures to panellists, and also variation in sensitivity by different panellists.

1.5.2 Analytical Measurements of Odours in Air

Analysis of air, whether indoor or outdoor air, primarily consists of three steps: (1) sampling of the air, (2) chemical analysis of the sampled air, and (3) quality assurance to ensure the precision and accuracy of the measurement. Before performing the sampling, it is important to know the physicochemical properties of the target contaminants. In addition, sampling for outdoor air requires a proper sampling plan that includes the selection of proper sites for sampling based on the weather conditions, the topography, and other factors.

In order to analyse air, a measured volume of air must be sampled. This volume of air can be collected in two ways: either collecting the air from the site in a container, or by trapping, or pre-concentration of, the analytes by passing a measured volume of air through a filter, or an adsorbent or absorbing solution.

Direct collection of air samples can be achieved either in a bag (*e.g.,* Tedlar®) or a canister (*e.g.,* SUMMA passivated canister) or a glass bulb. Traditional methods for trapping analytes include the use of adsorbent tubes (these are commonly used for sampling air for organic analysis). Activated charcoal is one of the most widely used substances for trapping organic compounds. Tenax and many other porous polymeric materials may be used for the same purpose. Silica gel is a common adsorbent for the adsorption of polar compounds such as alcohols. Adsorbent tubes for air sampling are commercially available or may be prepared (Patnaik, 2010).

The Standard Method described by the USEPA, Method TO17 (USEPA, 1999), describes the analysis of volatile compounds after trapping onto a sorbent followed by thermal desorption and GC-MS analysis. A large variety of sorbents are commercially available, with selection depending upon the target compounds analysis. TO17 details the range of sorbents, with recommended usage. Similarly, the National Institute for Occupational Safety and Health (NIOSH) has published a NIOSH Manual of Analytical Methods (NIOSH, 2006), which is a collection of methods for sampling and analysis of contaminants in air. The majority of these methods involve collection of the analytes on a solid sorbent, depending upon the analyte of interest (the range of sorbent materials is listed in Table 1.3). The compounds range from (but are not limited to) organotins, polyaromatic hydrocarbons, herbicides, pesticides and volatile organic carbon compounds.

Table 1.3: Sampling media recommended by the NIOSH Analytical Methods (NIOSH, 2006).

Sampling Media
Anasorb
Silver membrane
Coconut shell charcoal
Chromosorb
Dinitrophenylhydrazine
Mixed cellulose ester
Molecular sieve
Petroleum charcoal
Poropak
Polytetrafluoroethylene (Teflon)
Polyurethane
Polyvinyl chloride
Quartz fiber
Silica gel
Soda lime
Triethanolamine
XAD Polymeric resin

The detection techniques required to complete these methods are quite similar to those used in water analysis as described in Section 1.4.2.2.

Other pre-concentration techniques being used in air analyses also include the use of SPME. The analytes are collected either by direct exposure of the fibre or by the use of the headspace method (which is discussed in Section 1.6). The application of

SPME for analysis of air samples is widely spread across many different fields and is discussed in Section 1.6.6.

1.6 Solid-Phase Microextraction as a Pre-Concentration/Extraction Technique for Analysis of Odours

1.6.1 History of Solid-Phase Microextraction

SPME was developed in the early 1990s as a sampling and sample preparation method (Arthur and Pawliszyn, 1990) adapted from laser desorption from fused silica fibres. The technique eliminated the problems encountered with solid-phase extraction (SPE), such as high blank values and also the construction of cartridges from plastic which can potentially adsorb analytes, while maintaining a solvent-free system. SPME traditionally involves the use of a small diameter fused silica fibre, coated with a thin polymer layer, referred to as the stationary phase, which is then introduced into an environment (*e.g.,* the headspace of a water sample) where the analytes partition into the stationary phase and are then desorbed into the injector of a gas chromatograph for measurement. The amount of analyte absorbed or adsorbed depends on the magnitude of the partition coefficient of the analyte between the sample matrix and the fibre coating. Figure 1.4 depicts a commercial SPME fibre device.

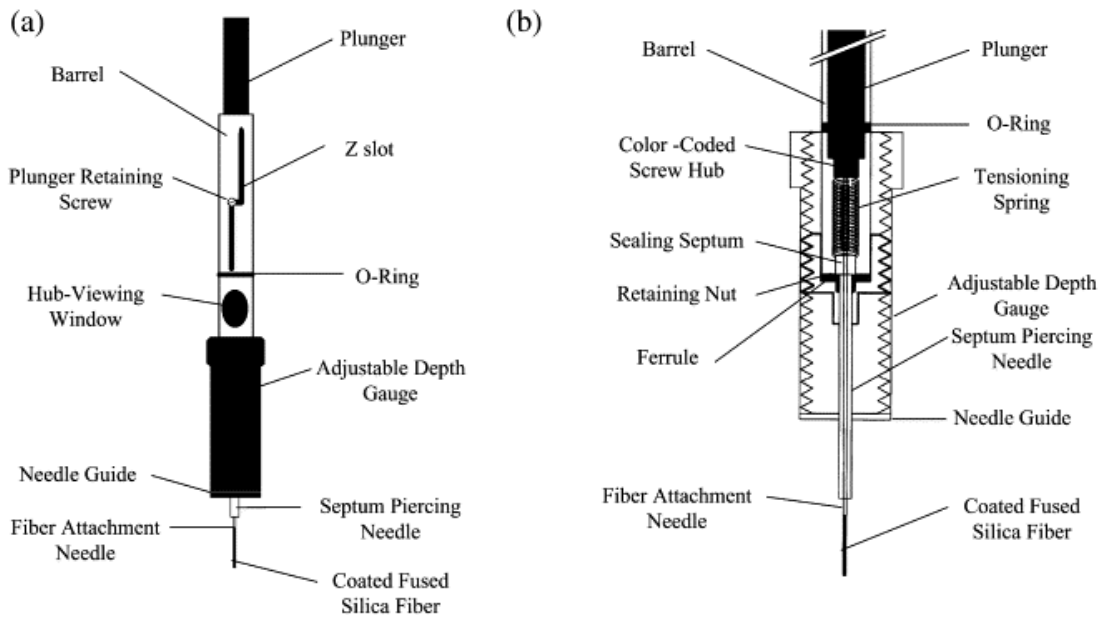


Figure 1.4: Typical SPME fibre sampling device. Reprinted with permission from Mester *et al.* (2001) Copyright Elsevier 2001.

1.6.2 Theory of Solid-Phase Microextraction

SPME is a multiphase equilibration process, as the extraction system is generally complex. For example, a sample may comprise an aqueous phase with suspended solid particles. In this instance, the analytes would have adsorption interactions with the solid particles, with the aqueous phase, as well as with the gaseous headspace (Pawliszyn, 2000). Most widely used for quantification of SPME is the equilibrium extraction method (Lord and Pawliszyn, 2000). In this case, SPME is considered to be complete when the analyte concentration has reached equilibrium between the sample matrix and the fibre coating (Pawliszyn, 2000) This means that once equilibrium has been reached, the extracted amount is constant within experimental error and is independent of increases in extraction time. Exposing the fibre for longer periods of time will not accumulate more analytes. The following Equation 1.1 describes the equilibrium conditions, assuming that the sample matrix is represented as a single homogenous phase and that no headspace is present in the system:

$$n = \left(\frac{K_{fs} V_f V_s}{K_{fs} V_f + V_s}\right) C_0$$

Equation 1.1

where n is the mass of the analyte extracted by the fibre coating, K_{fs} is a fibre coating-sample matrix distribution constant, V_f is the fibre coating volume, V_s is the sample volume and C_0 is the initial concentration of a given analyte in the sample.

This equation implies that once equilibrium has been reached, there is a direct proportional relationship between the sample concentration and the amount of analyte extracted. This relationship allows for analyte quantification. This equation is however limited to partitioning equilibria involving liquid polymeric phases such as PDMS (Lord and Pawliszyn, 2000).

While equilibrium extraction is most common, other extraction quantification approaches exist, *e.g.,* exhaustive extraction and pre-equilibrium extraction. In the case where sample volumes are small and the distribution coefficient of the analyte between the fibre coating and sample matrix is very large, Equation 1.1 can be simplified to:

$$n = V_s C_0$$

Equation 1.2

Equation 1.2 (Ouyang and Pawliszyn, 2006) implies that all the analytes present in the sample are extracted onto the fibre and the concentration of the target analyte can be easily calculated from the amount of analyte extracted by the fibre and the sample volume. This process is described as exhaustive extraction.

There are three different extraction methods that can be utilised with SPME: direct extraction, headspace extraction and a membrane protection approach, referred to as the in-tube method. Direct extraction and headspace extraction are more widely used and are discussed here. In direct extraction, the coated fibre is submersed in the sample matrix and the analytes transfer directly onto the phase of the fibre. In order for transfer to be rapid, sample agitation is required. In the headspace mode, the analytes need to be transferred through the barrier of air before they reach the fibre coating. This mode serves as a protection for the fibre coating from damage by the sample matrix, and allows for a longer fibre lifetime. The amounts of analyte extracted onto the fibre coating at equilibrium from the same sample in headspace or direct extraction are identical, as long as the sample and gaseous headspace volumes are the same (Lord and Pawliszyn, 2000) This is because the equilibrium concentration is independent of the fibre location in the sample/headspace system. If the volumes differ, a sensitivity difference between the direct and headspace methods exists only for very volatile analytes.

The type of extraction mode has a significant effect on the extraction kinetics (*i.e.,* time required to reach equilibrium). In headspace mode, the analytes are removed from the headspace first, followed by indirect extraction from the (water) matrix. The overall mass transfer rate is limited by the mass transfer rates from the sample matrix to the headspace. Accordingly, volatile analytes are extracted faster than semi-volatile analytes (Lord and Pawliszyn, 2000). Temperature can also affect the kinetics of the process: increasing the extraction temperature can aid the mass transfer of semi-volatiles into the headspace. In addition, agitation using a magnetic stirrer or shaker can be used to increase the rate of mass transfer. Equilibration times for volatiles are actually shorter in the headspace mode than for direct extraction

under the same agitation conditions (Lord and Pawliszyn, 2000). This is because a substantial proportion of the analyte is in the headspace prior to extraction, and diffusion coefficients in the gaseous phase are generally orders of magnitude greater than in aqueous media.

1.6.3 Fibre Coatings for Solid-Phase Microextraction

Currently, there are seven fibre stationary phase combinations commercially available. Within these combinations, several film thicknesses are also available (Table 1.4).

Table 1.4: Fibre coatings commercially available for SPME from Supelco (Falcó and Moya (2013).

Fibre Coating	**Film Thickness (µm)**
Nonpolar Fibres	
Polydimethylsiloxane (PDMS)	100
	30
	7
Polar Fibres	
Polyacrylate (PA)	85
Carbowax-divinylbenzene (CW-DVB)	65
Carbowax-templated resin (CW-TPR)	50
Bi-Polar Fibres	
Polydimethylsiloxane-divinylbenzene (PDMS-DVB)	65
	60
Carboxen-polydimethylsiloxane (CAR-PDMS)	75
Divinylbenzene-Carboxen-PDMS (DVB-CAR-PDMS)	30
	55
Other Polar Fibre	
Carbowax (CW)	60

1.6.4 Application of Solid-Phase Microextraction

SPME has been widely applied to sampling and analysis of environmental, food, aroma, forensic, and pharmaceutical samples. A review carried out by Ouyang and Pawliszyn (2006) highlighted the large number of applications of SPME to environmental analyses, noting that of the thousands of articles published on SPME, approximately 40% focus on its environmental applications. The SPME Applications Guide is a bibliographic resource of published technical articles about SPME prepared by Supelco. While it was last updated in 2001, the large number of resources at that stage emphasise the wide-ranging applications of SPME (Supelco, 2001). Specific fibre coatings have been developed to extend the applications of

SPME for environmental analyses (Sigma-Aldrich). On-site sampling and analysis of environmental samples has focused on the development of new sampling devices and calibration methods for both grab sampling and long-term monitoring. The development of new calibration methods for SPME has allowed for the acceleration of applications of SPME to on-site sampling and analysis. The list of analytes in various phases (aqueous, soil and sediments and air/gas) detected by SPME is continuing to expand.

As mentioned in Section 1.4.2, sampling of analytes in aqueous media by SPME can be performed by direct extraction by immersing the fibre into the sample, by the headspace method, or by the in-tube method. Traditional calibration methods, *i.e.,* the use of an external standard (calibration plot) and internal standard, are most often used for quantification of analytes in aqueous samples. Analysis of soil and sediment samples is generally carried out by headspace or direct extraction SPME, aided by sonication, heating or the use of microwaves. Applications of SPME in air sampling are advantageous as the technique can be performed on-site, or in the laboratory if the air sample is collected in a bag. The analytes are collected either by direct exposure of the fibre to the sample, or by the use of the headspace method after an air sample is collected. Quantification of analytes from air samples can be achieved by equilibrium extraction and application of Equation 1.1 to calculate the amount of analyte extracted. Calibration plots, which can be constructed after use of either a standard gas generating system or standard gas mixtures can also be used (Ouyang and Pawliszyn, 2006).

Equilibrium extraction is used for most applications for the quantification of SPME however the exhaustive method has been reported for the analysis of the volatile organic compounds, benzene, toluene, ethylbenzene and xylenes (BTEX) in soil samples (Ouyang and Pawliszyn, 2006).

1.6.5 Solid-Phase Microextraction for Water Analysis

Highly advanced scientific equipment has been used and developed for the identification and quantification of organic compounds in aqueous samples. However, less progress had been made in the development of advanced and automated methods for sample extraction and clean-up (Grote and Levesn, 1999).

The introduction of SPME into water analysis was a step in the direction of advancement and automation. SPME was first applied to a water matrix and even today most SPME applications are reported on water analysis.

As detailed in an extensive review by Ouyang and Pawliszyn (2006), SPME has been used to analyse a wide range of analytes within environmental water samples, *e.g.,* BTEX (Moliner-Martinez *et al.*, 2013; Arambarri *et al.*, 2004; Cho *et al.*, 2003), polyaromatic hydrocarbons (PAHs) (de Perre *et al.*, 2014; Chen, 2004), pesticides (Beceiro-González *et al.*, 2007; Sakamoto and Tsutsumi, 2004), herbicides (Lin *et al.*, 2003; R. Carabias-Martınez *et al.*, 2003), organometallic compounds, phenols and aromatic amines (Ouyang and Pawliszyn (2006) and references therein). A variety of different commercially available fibre phases (as detailed in Table 1.4) have been utilised in these analyses. SPME has been applied to a diverse range of environmental samples and some of these examples are discussed in further detail below. The use of SPME for the analysis of chlorinous odour compounds will be discussed in Chapter 3; and its use for the analysis of sulfur and nitrogen compounds will be discussed in Chapter 6.

Arambarri *et al.* (2004) developed a HS SPME method for the analysis of methyl tert-butyl ether (MTBE), ethyl *tert*-butyl ether, ethyl butyl ether, *tert*-amyl methyl ether and BTEX in water. The analysis was performed using GC with a FID detector. A PDMS-DVB fibre was chosen. The developed method was successfully applied to the analysis of the rivers, marinas and fishing harbors surface waters from Gipuzkoa (North Spain) (Arambarri *et al.*, 2004). Cho *et al.* (2003) developed a HS SPME method for the analysis of a similar set of compounds to assess contaminated groundwater. They investigated the use of a CAR-PDMS fibre. While the response of the CAR-PDMS fibre was higher than the conventional PDMS fibre, competition amongst the analytes resulted in a reduction of the extracted amount for some of the analytes. The extracted amounts of benzene and toluene did not show a consistent tendency as the concentration of each component increased (Cho *et al.*, 2003). Moliner-Martinez *et al.* (2013) used a HS SPME method followed by GC-FID to analyse BTEX levels in water in order to extract the parameters that are most important in assessing seasonal variations of water quality. A PDMS-DVB fibre was chosen and the method was optimised and validated (Moliner-Martinez *et al.*, 2013).

Chen (2004) studied the determination of PAHs in water using HPLC coupled with fluorescence detection using a PDMS fibre. The technique provided good linearity and detection limits at low nanogram per litre concentrations. The method was intended to be used for real-time monitoring of PAHs in drinking water samples (Chen, 2004). de Perre *et al.* (2014) used SPME coupled with GC‑MS to study interactions between PAHs and dissolved organic matter. The method was optimised by determination of the most suitable fibre phase. Three kinds of fibres were tested- PDMS (100 μm thickness), PDMS-DVB and a PDMS fibre with a thinner phase. For non-polar compounds PDMS is most suitable, however for more polar compounds a PDMS-DVB can improve the fibre capacity of retention (de Perre *et al.*, 2014). In this study, however, the classic PDMS 100 μm fibre was chosen as it provided good sensitivity and reproducibility for each of the PAHs. The fibre was also immersed in the sample (de Perre *et al.*, 2014).

Sakamoto and Tsutsumi (2004) investigated the applicability of HS SPME to pesticide determination in water samples. They chose 174 pesticides and, of these, 90% were extracted with a PA fibre. Five fibre phases were evaluated- PA, PDMS, PDMS-DVB, CW-DVB and CAR-PDMS. To determine the quantitative nature of the developed method, 45 pesticides were chosen that are among the pesticides that are typically monitored in waters. Recoveries of spiked samples of tested pesticides in actual matrices were essentially in agreement with those obtained by SPME (Sakamoto and Tsutsumi, 2004). In a separate study, Beceiro-González *et al.* (2007) developed a SPME method for the simultaneous determination of pesticides with a wide range of polarities and chemical structures (organochlorine, organophosphorous, triazines, pyrethroids and others) in water samples by GC–MS. Three different fibres and parameters that influence the extraction and desorption efficiency were studied. The selected fibre was a PDMS-DVB phase. Good detection limits, linearity and repeatability were obtained with this method for the 46 pesticides studied (Beceiro-González *et al.*, 2007).

Lin *et al.* (2003) successfully applied direct immersion SPME coupled with HPLC for the analysis of nine phenylurea herbicides. PDMS and CW fibres were chosen for

further study, and either were found to be suitable for the analysis of lake waters for the compounds of interest (Lin *et al.*, 2003).

Other environmental applications of SPME exist beyond the scope of those discussed above. Standard Methods (APHA, 2012) describes a HS SPME procedure for the analysis of three taste and odour compounds (MIB, geosmin and isopropyl methoxy pyrazine), while also stating it may be applicable for other compounds. The method uses a DVB-CAR-PDMS fibre that is introduced into the headspace of the water sample. Determination of the compounds is carried out by GC-MS. Researchers also use SPME for the analysis of disinfection by-products, occurring in treated waters, *e.g.,* trihalomethanes (Allard *et al.*, 2012), halonitriles (Kristiana *et al.*, 2012) and a range of VOCs listed in an EPA method for analysis of disinfection by-products (DBPs) (Niri *et al.*, 2008).

In work conducted in our laboratory at Curtin University, Allard *et al.* (2012) successfully developed a HS SPME method for the analysis of ten trihalomethanes (THMs) in water. The analysis of ten THMs in water samples in a single run is challenging due to the differeing concentrations present of each different type of THM- the iodinated THMs are found at very low concentrations (ng L^{-1} range), while the Australian regulated chlorinated and brominated trihalomethanes are present at much higher concentrations (above μg L^{-1}). The DVB-CAR-PDMS fibre was found to be the most suitable (Allard *et al.*, 2012). Kristiana *et al.* (2012) reported the development of a simple HS SPME method for the analysis of haloacetonitriles (HANs), a class of DBP that while it is not regulated, may be more toxic than Australian regulated DBPs. The analysis was carried out by GC-MS, and the validated method was successfully used to study the stability of the analytes in aqueous systems (Kristiana *et al.*, 2012).

Niri *et al.* (2008) developed an SPME method for the analysis of a wide range of DBPs. The majority of these analytes were typical VOCs. Five fibre phases were investigated, including PDMS (7 μm and 100 μm), CAR-PDMS, PDMS-DVB, and DVB-CAR-PDMS. DVB-CAR-PDMS was chosen as the most suitable fibre. Following the evaluation of the method using spiked standard samples, the method was used to analyse water samples subjected to 11 disinfection regimes. The results

of the analyses were compared to those obtained from the more traditional pre-concentration technique for these analytes, liquid-liquid extraction. It was found that the results were comparable, and that the use of SPME was shown to be as a quick, sensitive screen for analysing VOCs, including DBPs (Niri *et al.*, 2008).

The application of SPME of aqueous samples is not limited to environmental applications. Both direct and headspace mode SPME has been used in the food and beverage industry for the direct analysis of, and also during the production stages of beer, wine, other alcoholic beverages, tea, coffee and fruit juices. Analytes vary from flavour enhancers to off-flavours and contaminants.

1.6.6 Solid-Phase Microextraction for Air Analysis

The application of SPME for analysis of air samples is used across many different fields. Indoor air has been analysed for a large variety of analytes, most commonly VOCs (Larroque *et al.*, 2006; Godoi *et al.*, 2005; Koziel and Novak, 2002), and some recent examples of different applications are discussed below.

Larroque *et al.* (2006) compared two different quantification methods for the analysis of VOCs in indoor air. They investigated equilibrium versus non-equilibrium extraction, where in the latter, the fibre was analysed after a short extraction time, where the system had not been allowed to come to equilibrium. The limit of detection achieved for nine of the VOCs by the equilibrium method was approximately ten times lower than for the non-equilibrium method. Humidity was found to impact only one analyte. The methods were also applied to indoor air samples containing different VOCs and it was concluded that the non-equilibrium method involving a short extraction time was suitable for the detection of pollution peaks, while the equilibrium extraction was preferable for trace concentration levels (Larroque *et al.*, 2006).

Mocho *et al.* (2007) examined the adsorption kinetics of toluene on SPME fibres by producing a theoretical model to predict toluene adsorption onto the CAR-PDMS SPME fibre. They suggested that modelling may provide a useful alternative to time consuming experimental data. Comparison of the model with experimental data was

promising, with only an 11% difference for calibration curves, and 30% for the estimation of the limit of detection (Mocho *et al.*, 2007).

Synthetic musks are extensively used as fragrance components in a wide number of consumer products. Regueiro *et al.* (2006) developed an SPME method for the analysis of musks in air. External calibration was performed, and found to be suitable for all musk compounds. Low limits of detection were achieved for the range of musks studied (Regueiro *et al.*, 2006). Lamas *et al.* (2010) reported the analysis of 24 volatile fragrance allergens in indoor air by headspace SPME. External calibration using spiked sorbent samples was found to be suitable for quantification. Recoveries of analytes were in excess of 85%. The method was then applied to studies of household air quality, to detect 18 of the 24 compounds, with benzyl alcohol, linalool, citronellol, ionone and lilial being among the most common (Lamas *et al.*, 2010).

SPME has been investigated within the livestock industry (Bulliner *et al.*, 2006; Koziel *et al.*, 2006). Air sampling and the characterisation of odorous livestock gases has proved to be one of the most challenging analytical tasks. Livestock operations emit complex mixtures of volatile organic compounds and other gases (Koziel *et al.*, 2006). Koziel *et al*. (2006) used SPME for field air sampling of odours downwind from swine and beef operations, with sampling times ranging from 20 minutes to 1 hour. The analysis was coupled with a GC-MS olfactometry (O) unit, facilitating simultaneous chemical and sensory analyses. Bulliner *et al*. (2006) also utilised SPME coupled with GC-MS-O for identification of odorants present in swine environments. VOCs, as well as a number of sulfur compounds, were found to be present (Bulliner *et al.*, 2006).

1.7 Scope of Work in this Study

In this study, two different classes of odorous compounds were studied: one set arising from water samples, one set arising from air samples, both utilising HS SPME as the pre-concentration/extraction technique. Hence, HS SPME is the key technique used in this Thesis study. The remainder of this Thesis is divided into two different parts. Chapters 2, 3 and 4 focus on chlorinous odour compounds from

drinking water, while Chapters 5, 6 and 7 focus on sulfur based compounds from wastewater systems, all with the use of HS SPME as an analytical technique.

Chapter 2 provides an introduction to chlorinous off-flavours and analytical techniques for their detection. Chapter 3 details the development of a novel HS SPME/GC-MS method for the analysis of chlorinous off-flavour compounds. In Chapter 4, the formation of chlorine related off-flavour compounds is investigated, utilising the method developed in Chapter 3.

Chapter 5 provides an introduction to wastewater odour compounds and analytical techniques for their detection. Chapter 6 describes the development of a HS SPME/GC-MS method for the analysis of sulfur-based and other volatile odorous compounds, and adaptation of the method for the analysis of air samples. Chapter 7 utilises this method to investigate odour compounds present in wastewater systems and the treatment processes utilised for their reduction and/or removal.

Chapter 2
Chlorinous Odours and Off-Flavours in Drinking Water: A Review

2.1 Introduction

The first part of this Thesis (Chapters 2, 3 and 4) focuses on drinking water and the identification and analysis of causative compounds for chlorinous odours by SPME/GC-MS. This Chapter provides an overview of chlorinous compounds associated with drinking waters and techniques for their analysis.

Identifying compounds responsible for taste or odour incidents can be challenging. Often the causative compounds are present at very low concentrations and may be present in a complex mixture of other organic compounds, which can make detection and identification difficult. In order to standardise the assessment of taste and odour incidents and to provide a solution for such issues, a drinking water 'Taste and Odor Wheel' (Figure 1.2) was proposed by the Taste and Odor Committee of the International Association on Water Pollution Research and Control (Mallevialle and Suffet, 1987). As discussed in Chapter 1, the current wheel comprises the four basic taste groups, one group for mouth feel and eight odour groups.

The chlorinous/ozonous odour group is of particular interest as chlorinous off-flavours are a major taste and odour related complaint to water utilities in Australia (McDonald *et al.*, 2013; Joll *et al.*, 2007). Only four different sensations exist for taste (sour/acidic, sweet, salty and bitter). All other sensations are actually smell and are sensed by the receptors in the nose and sinuses, rather than the taste buds. Accordingly, a chlorinous sensation is actually an odour, as it cannot be detected by the tastebuds. However, many consumers perceive chlorinous sensations as tastes, as well as odours. Accordingly, a more broad term of chlorinous off-flavours can be used, to encompass both tastes and odours.

In Western Australia, a significant number of taste and odour complaints are received by the Water Corporation (the water utility supplying the majority of drinking water in the state), which are typically described as 'chlorinous' odours (McDonald *et al.*, 2009). This is not only a local issue; international survey data from both France and the United States (Mackey *et al.*, 2004) has shown high levels of consumer dissatisfaction with tap water quality due to chlorinous tastes and odours.

As it has been shown (Welte and Montiel, 1999; Bruchet *et al.*, 1992) that chlorinous odours can also occur in cases where the measured concentration of free chlorine or free chlorine equivalents is lower than the reported free chlorine odour threshold concentration of 0.1 – 1.1 mg L^{-1} (McDonald *et al.*, 2009; Piriou *et al.*, 2004), it must be considered that chlorinous off-flavours may arise in the absence of either free or combined chlorine and that they may be caused by one or more other compounds. A range of such compounds have been identified in actual taste and odour events as being the likely cause of chlorinous odours including: organic chloramines (Kajino *et al.*, 1999), trichloramine , aldehydes and nitriles (Hrudey *et al.*, 1989) and by-products from the reaction of urea and chlorine (Welte and Montiel, 1999; Samples, 1959). These compounds, however, do not account for all instances and, in some cases, the causative compounds remain unknown (Bruchet, 1999).

2.2 Factors Affecting Chlorinous Off-Flavours in Drinking Water Supplies

Water taste is affected by mineralisation, and the form that mineralisation takes, such as through the presence of chloride, sulphate and carbonate. Chlorination alters this balance and alters the taste imparted by these ions. The role of solution temperature and presence of chlorine on sensory responses for eight mineralised ($CaCl_2$, $MgCl_2$, NaCl, $NaHCO_3$, $CaSO_4$, $MgSO_4$, Na_2SO_4 and Na_2CO_3) waters was examined in a study conducted by Pangborn *et al.* (1970). It was reported that the characteristic taste of chlorine in water can be modified considerably by the specific dissolved minerals in the water. Chlorine was found to be much more difficult to perceive in aqueous solutions of Na_2CO_3 and $NaHCO_3$ at both temperatures studied (1 °C and 24 °C), than in the other, less basic solutions. At the lower temperature, significantly lower intensities of all the taste qualities, especially bitterness, were perceived (Pangborn *et al.*, 1970).

Mackey *et al.* (2001) conducted a survey on the public's perception of chlorinous flavours in tap water and different factors which influence this. Water temperature was found to affect chlorinous taste thresholds: a 5 °C increase in temperature lowered the threshold by more than half. Since taste is partially influenced by odours emitted from the sample, it is likely that the lower taste threshold concentration was

in part caused by an increase in gaseous chlorine at the higher temperature (Mackey *et al.*, 2001). While the contribution of gaseous chlorine to the decreased taste threshold cannot be quantified, the taste threshold was measured and shown to have a definite temperature dependency. Total dissolved solids (TDS) were also shown to have an effect on chlorinous taste threshold values. Two bottled waters, with differing concentrations of TDS, had chlorine added at levels previously detected, but trained panellists could not detect the chlorine in the presence of high TDS. It took nearly ten times the amount of chlorine in water with high TDS (1400 mg L^{-1}) before the chlorinous taste could be detected (Mackey *et al.*, 2001).

The effect of temperature on drinking water odorants was investigated by Whelton and Dietrich (2001). Chlorinous odours were found to be greater at 45 °C than at 25 °C. This suggests that utilities should conduct sensory analyses at temperatures greater than ambient, and that testing warm or hot water from a consumer may be more representative of an odour problem than tests on cold water (Whelton and Dietrich, 2001). This is in accordance with published methods for determination of tastes and odours (APHA, 2012).

Another study, conducted by Piriou *et al.* (2004), examined water characteristics which may influence the chlorinous flavour perception in drinking water. The findings in this study were somewhat contradictory to other studies, in that temperature was found to induce no significant change on chlorine threshold concentrations in water. The influence of TDS on chlorine perception remained unclear and, as reported by Zhang *et al.* (1992), musty background tastes may significantly impact chlorine flavour threshold concentrations (Piriou *et al.*, 2004).

2.3 Chlorine as a Source of Chlorinous Off-Flavours

Chlorine is the most commonly used disinfectant world-wide for drinking water disinfection. The process of using chlorine to disinfect water is called chlorination. In water, molecular chlorine undergoes rapid and complete hydrolysis to form chloride and HOCl (White, 1986). Chlorine dissolved in water exists primarily as HOCl between pH 3.4 and 7.5, and as OCl^-, at higher pH values (Larson and Rockwell, 1979).

$$Cl_2 + H_2O \leftrightarrows HOCl + H^+ + Cl^- \quad \textbf{Equation 2.1}$$

$$HOCl \leftrightarrows OCl^- + H^+ \quad \textbf{Equation 2.2}$$

As a consequence of the widespread usage of chlorine as a disinfectant, a range of disinfection by-products can form, which, in addition to potential health concerns, can produce taste and odour issues. The cause of chlorinous taste and odour issues reported to water utilities can generally be attributed to elevated concentrations of chlorine (White, 1986).

A variety of chlorine species are present in aqueous chlorine, including HOCl and OCl^-, both with an odour descriptor of bleach, falling within the chlorinous odour category on the Taste and Odor Wheel (Suffet *et al.*, 1995a). When ammonia is also present, the solution can also contain several pH-dependent chloramine species, as discussed in Section 2.4.1.1.

Table 2.1 shows the reported sensory threshold values for chlorine (as hypochlorous acid and hypochlorite ion) and the inorganic chloramines determined by Krasner and Barrett (1984), where aroma refers to odour, and flavour to taste. All of these species appear to produce an off-flavour that can be described as chlorinous (except trichloramine).

Table 2.1: Sensory threshold values (reproduced from Krasner and Barrett 1984).

Compound	Descriptor	Aroma ($mg\ L^{-1}$)	Flavour ($mg\ L^{-1}$)
Hypochlorous Acid	Chlorinous	0.28	0.24
Hypochlorite Ion	Chlorinous	0.36	0.30
Monochloramine	Swimming Pool	0.65	0.48
Dichloramine	Swimming Pool	0.15	0.13
Trichloramine	Geranium	0.02	

Over time, a variety of threshold concentrations for chlorine and related compounds have been reported. As sensitivity to taste and odour varies, so too will the reported

threshold concentrations. This sensitivity can vary for a number of different reasons: age, environment, and exposure are just a few factors that can influence a person's ability to detect a taste or odour present in a water sample. Sensitivity can vary quite widely, and also can vary amongst geographical locations.

For example, in a study carried out by Piriou *et al.* (2004), French panelists had a flavour threshold concentration for chlorine of 0.2 mg L^{-1} (Piriou *et al.*, 2004) while an American panel reported 1.1 mg L^{-1}. The higher threshold concentration from the US was attributed to higher residuals in their distribution system acclimatising people to the flavour of chlorine (Piriou *et al.*, 2004). A recent study carried out in our laboratory at Curtin University determined an odour threshold concentration of 0.1 mg L^{-1} for free chlorine equivalents (McDonald *et al.*, 2009). This concentration is consistent with the background exposure Perth residents would be subject to since, in the Perth distribution system, chlorine equivalent residuals range from less than 0.1 to 0.9 mg L^{-1} (McDonald *et al.*, 2009).

Chlorine itself is not always the causative agent of chlorinous off-flavours. Odours in drinking water samples have been reported as being chlorinous, despite the reactive oxidants (such as free chlorine) being quenched prior to testing. Additionally, it has been shown both locally in Australia and internationally (Welte and Montiel, 1999; Bruchet *et al.*, 1992) that chlorinous tastes and odours can also occur in cases where the concentration of free chlorine equivalents is lower than the reported odour threshold concentrations ranging 0.1 – 1.1 mg L^{-1} (McDonald *et al.*, 2009; Piriou *et al.*, 2004).

2.4 Causes of Chlorinous Off-Flavours Attributed to Compounds Other than Chlorine

It has been established that chlorinous off-flavours may be caused by;

a. excessive concentrations of free chlorine;
b. other compounds, which are not free chlorine, with tastes and odours perceived as 'chlorinous'.

These other compounds are the focus of the discussion below.

2.4.1 Alternative Disinfectants as a Source of Chlorinous Off-Flavours

The chlorinous/ozonous odour group on the Taste and Odor wheel gives a number of descriptors, including swimming pool and bleach, and the specific chemicals listed to cause these odours are free chlorine, monochloramine, dichloramine and ozone in solution. Each of these chemicals has also been identified as the specific cause of a chlorinous related taste and odour incident in drinking water, and the presence of the chemical was found to be a direct result of the disinfection process.

2.4.1.1 Chloramination

The reaction of chlorine with ammonia produces chloramines. This reaction is also described by the breakpoint chlorination curve, where different ratios of chlorine and ammonia are mixed (White, 2010). This will be further discussed in Chapter 4. Prior to breakpoint chlorination, inorganic chloramines are the dominant species present.

Inorganic chloramines are often referred to as combined chlorine. In dilute solutions, chlorine reacts with ammonia to form three species of chloramines: monochloramine, dichloramine and trichloramine. Dichloramine is the preferred disinfectant species due to its biocidal properties and its stability. The optimum conditions for formation of dichloramine are pH 8.3 at 25 °C, and a 4:1 and 5:1 weight ratio of chlorine to nitrogen (Kirmeyer *et al.*, 2004).

Table 2.1 lists the taste and odour threshold concentrations for each of the chloramine species, and it can be seen that trichloramine has the lowest threshold concentration, but it is not generally described as chlorinous. Dichloramine has a much lower taste and odour threshold than monochloramine and is therefore more likely to be responsible for odour complaints.

Discrepancies appear in the reported sensory threshold values of monochloramine, where Krasner and Barrett (1984) reported an aroma threshold of 0.65 mg L^{-1} and a flavour threshold of 0.48 mg L^{-1}, while White (1986) reported that the smell and taste of monochloramine would only be obvious at levels in excess of 5 mg L^{-1}, while another group reported the taste threshold of monochloramine in potable water to be in excess of 9.3 mg L^{-1} (O'Halloran and Veres, 1989).

2.4.1.2 Ozonation

Ozone is commercially produced from dry air or oxygen and is formed by the corona discharge of high voltage electricity (White, 2010). Due to its strong oxidising properties, ozone decomposes to give free radicals, which also have strong oxidising capacity. These radicals are considered to be the principal species reacting in ozonated water (White, 2010). As ozone is highly unstable in water it cannot be used as a terminal disinfectant and needs to be used with a persistent secondary disinfectant such as chloramine (Singer, 1994). Ozone reacts with organic matter to produce by-products which have been described as chlorinous and is discussed in Section 2.4.3.3.

2.4.1.3 Chlorine Dioxide

Chlorine dioxide has been proposed as an alternative disinfectant to chlorine treatment in cases where chlorine generates offensive tastes and odours, since chlorine dioxide generates far fewer volatile and non-volatile chlorinated products (Schmidt, 2004; Hoehn *et al.*, 2003; Gates, 1993). This is due to the fact that chlorine dioxide oxidises rather than chlorinates organic matter and does not form trihalomethanes or other chlorination by-products (Singer and Reckhow, 1999). However, in some cases, chlorine dioxide treatment, including the use of chlorine dioxide with chlorine, can result in strong chlorine odours (Hoehn *et al.* 1990). Other odorous compounds, with descriptors of 'bleachy', 'kerosene-like' and 'akin to kitty litter' (Hoehn *et al.*, 1990) have also been generated from the use of chlorine dioxide. These odours were attributed to transient volatile compounds, suspected to be oxidation products of organic compounds (Hoehn *et al.*, 1990).

2.4.2 Leaching from Distribution Systems as a Source of Chlorinous Off-Flavours

Schweitzer *et al.* (2004) reported that tastes and odours originating from compounds leaching from materials in distribution systems and/or degradation/transformation products of the original compounds have not been well studied, even though most materials have a high potential to impact the organoleptic quality of water. The following examples are all cases where the off-flavour incident resulted from the reaction of leached phenolic compounds with chlorine: styrene and chlorophenols leaching from polyester resins and an additive in water tank refurbishment (Rigal and

Danjou, 1999), phenolic compounds leaching from coating materials (Bruchet, 2001), and leaching of phenol from an acrylic coating with subsequent reaction in the distribution system to produce bromophenols and bromodichloroiodomethane (Khiari *et al.*, 1999b). Yorkshire Water services identified a suite of phenols derived from the leaching of hosing in washing machines and dishwashers into the household plumbing system as the most common cause of chlorine complaints (Willmore, 2005). Such incidents led to the development of a utility quick test method for testing drinking water system components for their potential to contribute to taste and odour problems (Schweitzer *et al.*, 2004).

2.4.3 Disinfection By-Products as a Source of Chlorinous Off-Flavours

Disinfection of water supplies is paramount, yet all chemical disinfectants are known to form various types of DBPs. Precursors to such products include natural organic matter (NOM) and naturally occurring bromide and iodide ions.

NOM consists of a heterogeneous mixture of humic substances, hydrophilic acids, proteins, lipids, carbohydrates, carboxylic acids, amino acids and hydrocarbons (Thurman, 1985). The bulk of NOM is comprised of humic substances (Korshin *et al.*, 1996). Aquatic humic substances tend to be defined based on their solubility properties in aqueous solutions (Schwarzenbach *et al.*, 2003). NOM fractions are commonly referred to as humic substances if they are soluble in aqueous base (and insoluble in organic solvents), and kerogen if they are not. The humic substances can then be further subdivided into fulvic acids if they are soluble in both acidic and basic solutions, and humic acids if they are not soluble at pH 2 (Schwarzenbach *et al.*, 2003).

There have been numerous attempts to determine the structure of NOM, including work from within our laboratory at Curtin University (Couton, 2010) but it is generally concluded that NOM cannot be defined as a particular chemical structure, but rather broadly characterized in terms of its properties. The character of NOM is dependent upon the source from which it was derived and the chemical and biological degradation to which it has been subjected (Drikas, 2003).

The presence of NOM influences the reactions of chlorine in drinking water treatment. The reaction between the disinfectant and NOM produces a large number of halogenated and non-halogenated organic by-products, which are commonly referred to as DBPs. These DBPs can be associated with off-flavour incidents, and a number have been linked to chlorinous off-flavour complaints. Two important reaction mechanisms are involved in the by-product formation: oxidation reactions and substitution with chlorine.

Bromide and iodide exist in many natural waters, and the chlorine used in water treatment oxidises these readily to form hypobromous acid (HOBr) and hypoiodous acid (HOI) during disinfection:

$$Br^- + HOCl \leftrightarrows HOBr + Cl^- \qquad \textbf{Equation 2.3}.$$

$$I^- + HOCl \leftrightarrows HOI + Cl^- \qquad \textbf{Equation 2.4}$$

Therefore, as well as chlorine, bromine and iodine species may be present in chlorinated waters. These oxidants can then react with NOM in a similar way as chlorine to form brominated and iodinated DBPs. A wide range of compounds can arise as DBPs, from which the following have been identified as causing objectionable off-flavours which could be described as chlorinous: halophenols, halomethanes, and aldehyde and nitrile species. These are discussed in later subsections.

In general, chloramines are less effective as oxidation agents than chlorine, however the concentration of chloramination by-products is generally less than that of chlorination by-products, therefore chloramination may provide an alternative disinfectant with less odour complaints than chlorine (Duguet *et al.*, 1995).

However, it is the formation of inorganic chloramine species during the disinfection process that can result in chlorinous off-flavour complaints. Such chloramine species also have the potential to form aldehyde and nitrile species which are odorous (Hrudey *et al.*, 1989; Hrudey *et al.*, 1988) and will be further discussed in Section

2.4.4. Like chlorine, monochloramine and dichloramine have the potential to react with NOM to produce DBPs (Suffet *et al.*, 1995b).

2.4.3.1 Phenols and Halophenols

Phenol and phenolic species are very important taste and odour compounds that react with chlorine to produce chlorophenols (Burttschell *et al.*, 1959). The chlorination of phenol proceeds by progressive electrophilic aromatic substitution of available *ortho* and *para* positions (Figure 2.1).

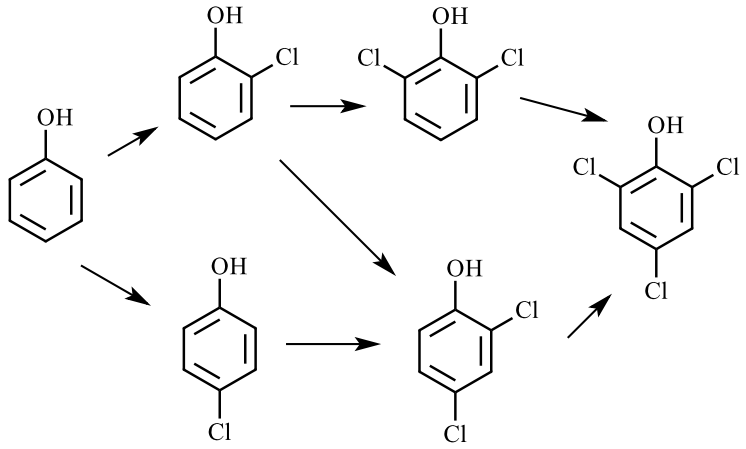


Figure 2.1: The course of phenol chlorination adapted from Burttschell *et al.* (1959).

The presence of bromide or iodide in water can also result in the production of bromo, iodo and mixed halophenols. These compounds generally have a medicinal descriptor associated with them, but since consumers can easily confuse medicinal and chlorinous as descriptors (Mackey *et al.*, 2004), the phenols also require consideration as causes of chlorinous off-flavours.

Several incidents in the Yorkshire Water Services area in the UK saw consumers make large numbers of chlorine related complaints in their service area. These complaints were linked to the presence of a suite of phenols and chlorophenols (Willmore, 2005). Taste and odour testing of standard compounds confirmed the strong off-flavour properties of these phenols at very low concentrations. 2,3-Dimethylphenol has a chlorine odour, and mixtures of other chlorinated phenols also have odours with descriptors of swimming pool, chlorinous and disinfectant (Willmore, 2005).

2.4.3.2 Iodinated Trihalomethanes

The formation of THMs from the chlorination of drinking water was first reported in 1974 (Rook, 1974). THMs are the most extensively studied class of DBP. Studies have shown that chlorination of humic acids in aqueous solutions results in the formation of chloroform (Croue *et al.*, 2000; Norwood *et al.*, 1987). The corresponding Br and I-substituted products are thought to result from the parallel halogenation reactions initiated by the action of chlorine on the background concentrations of bromide and iodide ions dissolved in most natural waters.

Iodide can be present in drinking water source locations, especially in coastal regions in elevated concentrations (up to 1 mg L^{-1}) (Kristiana *et al.*, 2010a), and is rapidly oxidised to hypoiodous acid during oxidation and disinfection with chlorine, monochloramine, and ozone. HOI can be further oxidised to iodate (IO_3^-) or react with NOM to form iodo-organic compounds as DBPs. Iodinated trihalomethane species have been found in chloraminated drinking water, and the presence of iodoform was associated with an objectionable medicinal taste in the water (Hansson *et al.*, 1987).

When a groundwater-fed reservoir was added to the Philadelphia Suburban Water system in 1969, chloramination was chosen as the final disinfectant (Gittelman and Yohe, 1989). After commencement of chloramination, an odour described as medicinal-chlorinous was found to be present in a treated reservoir water. This odour was generated during the treatment process, as it was not evident in the raw water. I-THMs, formed as a result of the chloramination, were believed to be the cause of the odour as they were identified in the water by GC- MS and an odour panel agreed that the complaint sample odour was identical to that of iodoform (Gittelman and Yohe, 1989). Another study showed the potential for formation of iodoform and bromoform in treated waters under different chlorine residual conditions where chloramine concentration was a critical factor in the formation of such species (Bruchet *et al.*, 1989). Another study comparing different disinfectants highlighted that iodinated halomethane species were the main products from chloramination in waters with iodide present (Bichsel and von Gunten, 2000).

2.4.3.3 Aldehydes and Nitriles

Reactions of ozone with organic matter present in natural waters form aldehydes as intermediate products and, upon further oxidation, these aldehydes can produce aldo-acids and carboxylic acids (Froese *et al.*, 1999). Aldehydes are commonly recognised as odorous by-products resulting from ozonation (Froese *et al.*, 1999) and have been found to have a swampy, swimming pool type odour, which may be described as chlorinous based on the association of chlorine and swimming pools (Hrudey *et al.*, 1989). The reactions of chlorine with amino acids also result in the formation of aldehyde and nitrile species which have been related to chlorinous off-flavours. These are discussed in detail in the next Section.

2.4.4 Nitrogenous Organic Precursors as a Source of Chlorinous Off-Flavours

Organic nitrogen compounds have been identified in many studies as being associated with the generation of chlorinous off-flavours upon the chlorination of natural waters (Bruchet, 1999; Kajino *et al.*, 1999; Bruchet *et al.*, 1992; Hrudey *et al.*, 1989; Hrudey *et al.*, 1988). Free and combined amino acids are ubiquitous components of natural waters, and, according to Bruchet *et al*. (1992), these probably represent major precursors to taste and odour compounds in chlorinated natural waters. Combined amino acids are four to five times more abundant than free amino acids (Hureiki *et al.*, 1994). Structurally they may be part of the humic fraction of natural organic matter. Accordingly, as the dissolved organic carbon concentration increases, so does the amino acid concentration. As they are present during all stages of water treatment, they tend to be of concern as they can exert a high chlorine demand (Hureiki *et al.*, 1994).

Depending upon the dose rate, chlorination of amino acids initially leads to the formation of organic mono- and di-chloramines, then to the production of specific aldehydes and nitriles (Hureiki *et al.*, 1994). This is the focus of Chapter 4 and is discussed in detail there. These aldehyde and nitrile species have been reported to be responsible for a number of taste and odour incidents recorded as chlorinous by complainants (Hrudey *et al.*, 1989; Hrudey *et al.*, 1988).

Odour incidents occurring in Edmonton, Canada, during the spring of 1985 and 1986 caused consumer complaints, with consumers describing the odours as organic,

swampy or swimming pool character (Hrudey *et al.*, 1989). A series of low molecular weight branched and aromatic aldehydes were detected (Hrudey *et al.*, 1989). Little work at that time had identified such compounds in drinking water, and only one report existed that linked any of these aldehydes to the occurrence of odour in water (Kikuchi *et al.*, 1983), which identified phenylacetaldehyde as one of several odorous metabolites produced. Further work (Conyers *et al.*, 1993; Bruchet *et al.*, 1992; Nweke and Scully Jnr, 1989) established that such aldehydes could be produced by the chlorination or chloramination of specific amino acids present in waters. An investigation was also conducted to establish whether such aldehydes could be found in swimming pools, due to the combined aldehyde odour being described as swimming pool. Such aldehydes were confirmed to be present in swimming pools, and it was proposed that these aldehydes contribute to the character of the commonly recognised swimming pool odour (Hrudey *et al.*, 1989).

An incident during the start-up of a new water treatment plant in France during 1991 saw consumers complain of a very strong and transient chlorinous odour, while, at the same time, only combined chlorine could be measured in the water, with no detectable level of free chlorine (Bruchet *et al.*, 1992). Due to earlier incidents where a chlorinous odour was reported where there was no free chlorine equivalent present (Hrudey *et al.*, 1989; Hrudey *et al.*, 1988), this episode prompted an investigation of the chlorination of amino acids and the potential by-products. It was established that upon chlorination, specific odorous aldehyde and nitrile species were formed, and it was proposed that these species could perhaps explain the incidents in the treatment plant (Bruchet *et al.*, 1992) .

The identification of specific aldehyde and nitriles species in odour incidents prompted research into the influence of chlorination by-products of naturally occurring nitrogenous organic compounds, specifically amino acids, on tastes and odours in drinking water (Bruchet *et al.*, 1992). This study demonstrated that upon chlorination, amino acids produced by-products that were odorous and sometimes described as chlorinous.

Other studies into the chlorination of amino acids reported the formation of another intermediate species of unexpected stability (Nweke and Scully Jnr, 1989). By

varying chlorine to nitrogen ratios and analysing the reaction mixtures by a variety of techniques, Nweke and Scully (1989) were able to identify a *N*-chloroaldimine species. Later work by Freuze and coworkers further established the formation of such intermediate products and reported their odour properties (Freuze *et al.*, 2005a; Freuze *et al.*, 2005b; Freuze *et al.*, 2004). This is the focus of Chapter 4 and further discussion is provided there.

In summary, in the above mentioned chlorinous off-flavour incidents, speculation still exists as to which compounds were specifically responsible. Initially, it was thought that the off-flavours were a result of the presence of the aldehyde and nitrile species, but it was then postulated that the odour was caused by the presence of intermediate species. Later work proposed a chloroaldimine species as the responsible compound.

2.5 Analysis of Odorous Compounds related to Chlorinous Off-Flavours

In order to deal with taste and odour incidents, it is necessary to be able to identify the compounds responsible for causing the objectionable taste and/or odour. This analysis can be either of a sensory or chemical nature. As technology has improved over time, so too have analytical methods. Sample preparation methods (such as pre-concentration) have advanced. Due to the volatility of odorous compounds, and their presence in often very low concentrations, precise procedures are required. A variety of different techniques have been applied for the analysis of compounds which have been related to chlorinous off-flavours, such as halophenols, I-THMs and chlorinated amino acid mixtures.

A variety of analytical techniques have been applied to the determination of phenols and halophenols in water, including pre-concentration/extraction with direct liquid–liquid extraction (LLE) (Malleret and Bruchet, 2002; Allonier *et al.*, 1999; Whitfield *et al.*, 1988; Sithole *et al.*, 1986; Ventura and Rivera, 1986), solid-phase extraction (SPE) (Reitzel and Ledin, 2002; Kim and Kim, 2000; Davì and Gnudi, 1999), SPME (Malleret *et al.*, 2003; Adams *et al.*, 1999) and simultaneous steam distillation extraction (Chung *et al.*, 2003; Jensen and Whitfield, 2003; Whitfield *et al.*, 1988).

The phenols are either extracted from the water sample and analysed directly by GC or liquid chromatography (LC) techniques, or are extracted and derivatised, or derivatised and then extracted, prior to analysis.

The analysis techniques for compounds related to the chlorination of amino acids, such as aldehydes, chloroaldimines, and the amino acids themselves, have also developed over time. Initial aldehyde analysis used purge-and-trap techniques followed by derivatisation for analyte isolation, followed by GC analysis (Daignault and Hrudey, 1988). The use of HPLC-UV also afforded the identification of aldehyde and nitrile species produced after chlorination (Bruchet *et al.*, 1992). Some of the other by-products from chlorination of amino acids have also been identified by HPLC, such as the chloroaldimine species (Freuze *et al.*, 2004). However, the most recent studies have utilised a headspace apparatus with a sorbent trap coupled to a GC-MS for analysis of chloroaldimines (Brosillon *et al.*, 2009). The development of an analytical method for the analysis of such compounds is the focus of Chapter 3.

2.6 Scope of Work in Chapters 3 and 4 of this Study

Chlorinous odours are the cause of a significant proportion of consumer complaints to drinking water utilities. The causes of these odours are generally attributed to elevated concentrations of chlorine. However, it has been shown that chlorinous odours occur even when the concentration of free chlorine is considerably lower than the odour threshold concentration of free chlorine. The odour in these situations has been attributed to the presence of chloramines, but may also be caused by a variety of other compounds. The main focus of work reported in Chapter 3 was to develop an analytical method (utilising HS SPME, the focus technique in this Thesis) for the quantification of a range of aldehyde and nitrile species, which have been previously found to be responsible for chlorine related taste and odour events. The focus of Chapter 4 was to investigate possible causes of chlorinous odours, specifically chlorination of organic nitrogen compounds (amino acids were studied), using the analytical method developed in Chapter 3.

Chapter 3

Solid-Phase Microextraction Method Development for the Analysis of Some Classes of Compounds Associated with Chlorinous Odours

3.1 Introduction

Chlorinous tastes and odours (off-flavours) are a significant cause of consumer complaints to water utilities in localities where chlorine-based disinfectants are commonly used. Organic nitrogen compounds, namely amino acids, have been identified in many studies as being associated with the production of chlorinous off-flavours after chlorination of natural waters, as discussed in Section 2.4.4. Free and combined amino acids exist in all natural waters, and, according to Bruchet *et al.* (1992), these probably represent major precursors to taste and odour compounds in all chlorinated natural waters. The chlorination of amino acids results in the production of aldehydes and nitriles, depending upon the dose rate. Initially, chlorination leads to the formation of organic mono- and di-chloroamines, then onto the production of specific aldehydes and nitriles (Hureiki *et al.*, 1994). These reaction pathways will be further discussed and investigated in Chapter 4.

After a series of taste and odour episodes in both Europe and the United States, several studies were undertaken (Freuze *et al.*, 2005b; Hrudey *et al.*, 1989; Hrudey *et al.*, 1988), from which a series of odorous low molecular weight aldehydes (including isobutyraldehyde, 2-methylbutyraldehyde, 3-methylbutyraldehyde and phenylacetaldehyde) were identified and found to be produced from the reactions of amino acids with various oxidants, including chlorine, chloramines and chlorine dioxide. While the causative compounds were believed to be the aldehyde species, upon further chlorination, it was found that the production of nitriles (which were also odorous) could also occur. Another study found nitriles present in water after chloramination (Khiari *et al.*, 1999a).

A variety of analytical methods were utilised in these studies (Freuze *et al.*, 2005b; Hrudey *et al.*, 1989; Hrudey *et al.*, 1988) to identify the odorous compounds including: HPLC with ultraviolet (UV) detection, with derivatisation (Freuze *et al.*, 2004; Nweke and Scully Jnr, 1989) and without derivatisation (Bruchet *et al.*, 1992); and purge-and-trap GC-MS with SIM (Hrudey *et al.*, 1989). Not only were these analyses able to identify the aldehyde and nitrile by-products, they also provided a screening process as they could also potentially monitor other intermediates present,

such as chloramines and chloraldimines. These studies will be discussed further in Chapter 4.

3.1.1 Analysis of Aldehydes

Trace analysis of low molecular weight aldehydes in various matrices, including water, is usually carried out using a gas chromatography method. The compounds are generally derivatised prior to analysis due to their high volatility and reactivity. The most commonly used derivatisation agent for the analysis of low molecular weight aldehydes is o-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) (Glaze *et al.*, 1989; Yamada and Somiya, 1989). PFBHA reacts with low molecular weight carbonyl compounds to form the corresponding oximes, with two geometric isomers of the oxime derivatives being formed (Glaze *et al.*, 1989). These are extractable with organic solvents and analysed by GC-MS or GC-ECD.

Aldehydes are by-products commonly associated with drinking water ozonation. but not generally as arising from chlorine-based disinfection (Froese *et al.*, 1999). The reaction of ozone with organic matter present in natural waters forms aldehydes as intermediate products and, upon further oxidation, these aldehydes can produce aldo-acids and carboxylic acids. Accordingly, a routine method exists for the analysis of aldehydes within the Standard Methods for the Examination of Water and Wastewater (APHA, 2012; APHA, 1998). The method is a PFBHA liquid-liquid extraction, followed by GC-MS which is able to measure straight-chain, low-molecular weight aldehydes in raw and treated drinking water. Aldehydes, while not considered a serious health hazard to consumers at a microgram per litre concentration, could react with other compounds, therefore can be a potential threat (APHA, 2012; APHA, 1998). The detection levels from this method vary from 0.082 – 0.228 μg L^{-1} depending on the aldehyde, while the reporting level is 0.5 μg L^{-1} for all analytes (APHA, 2012; APHA, 1998). A similar standard method from the USEPA also uses PFBHA derivatisation with detection levels (LODs) ranging from 0.21 to 1.0 μg L^{-1} (USEPA, 1998). Both methods use 1,2-dibromopropane as an internal standard to achieve quantitative results.

However, both the APHA and USEPA Standard Methods are time-consuming and have high detection limits, requiring substantial sample preparation to isolate the

analytes prior to GC-MS or GC-ECD. Once samples have been collected, they are required to be derivatised, and left for 1 hour and 45 minutes. They are then extracted, and the extract cleaned up prior to GC-MS analysis. While time consuming, this also limits the number of samples that can be prepared at once, due to the manual labour component required.

SPME has been used in conjunction with derivatisation, allowing for automation and faster sample preparation. A similar method to the USEPA Method (USEPA, 1998) but using SPME as the isolation technique, instead of LLE, was reported to have detection limits ranging from 0.04 to 0.16 μg L^{-1} (Cancho *et al.*, 2002).

3.1.2 Analysis of Aldehydes using Solid-Phase Microextraction

Addition of PFBHA to aqueous samples followed by extraction of the derivatised aldehydes onto an SPME fibre has been reported for the extraction and pre-concentration of aldehydes by several researchers (Li *et al.*, 2006; Sowinski *et al.*, 2005; Wardencki *et al.*, 2003; Cancho *et al.*, 2002; Bao *et al.*, 1998). Coating of the fibre with the derivatisation agent prior to extraction has also been reported (Schmarr *et al.*, 2008; Stashenko *et al.*, 2006; Wang *et al.*, 2005; Tsai and Chang, 2003; Martos and Pawliszyn, 1998). Once sorbed onto the fibre, the derivatised aldehydes are measured by GC-MS or GC-ECD.

The analysis of aldehydes without derivatisation is not widely practiced. Methods for the direct analysis of aldehydes *via* SPME/GC-MS/ECD without derivatisation, generally from solid samples, for example, food stuffs, have been reported (Duflos *et al.*, 2006; Romeo *et al.*, 2006). Some liquid matrices have also been analysed (Keszler and Heberger, 1999; Keszler *et al.*, 1998a), without the use of a derivatising agent.

3.1.2.1 Analysis of Aldehydes using Derivatisation followed by Solid-Phase Microextraction

Following the development of SPME and its use across a wide range of compound classes, Bao *et al.* (1998) reported a method using SPME for the determination of carbonyl compounds in aqueous samples. The method uses derivatisation with PFBHA in the water samples, followed by extraction with SPME and GC-ECD

analysis. The range of compounds investigated was 23 carbonyl compounds, including C_1-C_{10} saturated aliphatic aldehydes, a range of unsaturated aldehydes, ketones and some glyoxal compounds. Isobutyraldehyde and isovaleraldehyde were also included. The method of extraction of the derivatised analytes from the headspace or from the liquid were compared and method detection limits of 0.006 – 0.2 μg L^{-1} were reported for both methods of extraction, except for the glyoxal compounds where the detection limits were higher in the HS extraction (Bao *et al.*, 1998).

Cancho *et al.* (2002) developed an HS SPME procedure followed by GC-ECD detection for the determination of aldehydes in drinking water samples at μg L^{-1} concentrations. Prior to extraction, the samples were derivatised with PFBHA. A variety of fibre phases were examined and it was found that the DVB-PDMS phase was most suitable for these derivatised analytes. LODs between 0.04 and 0.16 μg L^{-1} were reported for the compounds investigated which included a range of C_2-C_{10} aldehydes (including isobutyraldehyde, 2-methylbutyraldehyde and isovaleraldehyde) and glyoxal and methyl glyoxal. Repeatability and reproducibility based on addition of the analytes to ultrapure water and multiple analyses were 3 to 16 % relative standard deviation (%RSD) and 5 to 15 %RSD respectively. These values are of the same order to those reported by Bao *et al.* (1998). The method was also compared to EPA Method 556, which uses LLE as a pre-concentration technique for the determination of carbonyl compounds, and no significant differences in recovery were found when carrying out each of the extraction techniques for the lower molecular weight aldehydes, suggesting that HS SPME may be a good alternative to LLE for these compounds (Cancho *et al.*, 2002).

Coating of the fibre with the derivatisation agent prior to extraction has also been reported. Martos and Pawliszyn (1998) developed an original method consisting of on-fibre derivatisation with PFBHA onto a PDMS fibre for gaseous formaldehyde followed by GC with FID detection. Stashenko *et al.* (2000) adapted this method using pentafluorophenylhydrazine (PFPH) as a derivatising agent for the analysis of volatile carbonyl compounds in sunflower oils. In this method, PFPH was absorbed onto a DVB-PDMS fibre and then exposed to the vapours of aldehyde containing matrices. The hydrazones formed on the fibre were then analysed by GC-ECD. The

range of carbonyl compounds investigated included C_2 to C_{10} straight chain aldehydes and some heavier molecular weight aldehydes, all associated with the degradation of sunflower oil. Detection limits ranging from 10 to 90 fmol were reported (Stashenko *et al.*, 2000).

Tsai and Chang (2003) reported the use of on-fibre derivatisation to determine aldehydes in water. In contrast to the method described by Bao *et al.* (1998), where aldehydes derivatised with PFBHA to form oximes were determined following extraction of the headspace or liquid, in this study PFBHA was first loaded onto the fibre. The HS of the sample was then extracted, with the derivatisation of the aldehydes occurring on-fibre. GC-MS was then used for identification of the derivatised analytes. Method detection limits of 0.12 – 0.34 μg L^{-1} were achieved for the range of compounds investigated which included C_1-C_5 straight chain aldehydes.

While these methods are much faster than those utilising the conventional derivatisation procedures, such as derivatisation followed by LLE, they still require either addition of the derivatisation agent, or coating of the fibre with the derivatising agent.

3.1.2.2 Analysis of Aldehydes using Solid-Phase Microextraction without Derivatisation

The analysis of aldehydes without derivatisation is not widely reported. Daignault and Hrudey (1988) developed a simple and sensitive purge-and-trap method coupled with GC-FID, with a linear range of 0.5 – 100 μg L^{-1}, for the analysis of isobutyraldehyde, isovaleraldehyde and 2-methylbutyraldehyde from water samples. Methods for the direct analysis of aldehydes *via* SPME/GC-MS/ECD without derivatisation have been reported, but generally are for analysis of solid samples, for example, food stuffs.

Romeo *et al.* (2006) reported the first investigation of the flavour profile of capers using direct analysis of the headspace of pickled capers where low molecular weight aldehydes (including isovaleraldehyde and phenylacetaldehyde, amongst other compounds) were identified. Quantitative results were achieved by using 1-butanol as the internal standard, which was added to the aqueous slurry of the caper samples

prior to extraction. A DVB-CAR-PDMS fibre was used, and was introduced into the headspace of the sample vial for extraction for 40 minutes at 35 °C prior to introduction into the port of the GC for identification (Romeo *et al.*, 2006).

Cubes of fish were placed into vials and the headspace analysed directly *via* SPME to measure alcohols, aldehydes, ketones and pyrazines as a means for determining food spoilage (Duflos *et al.*, 2006). The method was qualitative only, but did identify the presence of isobutyraldehyde, 2-methylbutyraldehyde and isovaleraldehyde. In this method, a CAR-PDMS fibre was introduced into the headspace of a sample vial containing an amount of fish muscle tissue, and the fibre was then introduced into the GC for MS identification (Duflos *et al.*, 2006).

Liquid matrices have also been analysed by direct SPME (without derivatisation) for aldehydes. C_5 and higher aldehydes were quantified from sunflower oil in a series of studies (Keszler and Heberger, 1999; Keszler *et al.*, 1998a; Keszler *et al.*, 1998b). Keszler *et al.* (1998a) reported the development of a rapid, simple method to quantify short-chain aliphatic aldehydes in sunflower oil. The HS of the sample was extracted using a PDMS fibre at 40 °C for 40 minutes, prior to introduction into the GC for identification. The detection limits for the range of compounds studied ranged from 0.1 to 1 mg L^{-1} and these were C_5 to C_9 aldehydes. This method was applied to later studies to quantify these aldehydes from sunflower oil (Keszler and Heberger, 1999; Keszler *et al.*, 1998a; Keszler *et al.*, 1998b).

3.1.3 Analysis of Nitriles

The analysis of alkyl nitriles in relation to DBPs has been predominantly by GC-MS (Freuze *et al.*, 2004; Khiari *et al.*, 1999a). Most of these methods have been used to capture a variety of analytes, not just nitriles (Khiari *et al.*, 1999a). The more commonly associated nitrile species with disinfection by-products however are the HANs. Nine brominated and/or chlorinated HANs can form in drinking waters, and some of these compounds are thought to be more toxic and carcinogenic than regulated DBPs (Bull, 2003). Recent analytical methods for the analysis of HANs have been by SPME/GC-MS (Kristiana *et al.*, 2012).

Previous studies have utilised one analytical method to examine both the aldehyde and nitrile species (Freuze *et al.*, 2004; Nweke and Scully Jnr, 1989). These studies confirmed that it was possible for these analytes to be measured using the same technique, and as it had been reported that SPME had been used for the analysis of aldehydes and for halonitriles, it was concluded that SPME could be used for the analysis of the nitriles that were of interest for this study.

3.1.4 Scope of Work in Chapter 3 of this Study

As outlined in Section 1.7, the first part of this study focused on the establishment of an analytical method for the analysis of several chlorination by-products: isobutyraldehyde (IBA), isobutyronitrile (IBN), 2-methylbutyraldehyde (2-MBA), 2-methylbutyronitrile (2-MBN), 3-methylbutyraldehyde (3-MBA), 3-methylbutyronitrile (3-MBN), phenylacetaldehyde (PA) and phenylacetonitrile (PN). These analytes had been previously identified in conjunction and/or associated with chlorinous off-flavour incidents (Bruchet *et al.*, 1992; Hrudey *et al.*, 1988). Accordingly, the aim of this Chapter was to develop a simple method for the simultaneous analysis of these four specific aldehydes and their corresponding nitriles. The process of development and validation will be described in the following sections of this Chapter. SPME in conjunction with GC-MS without derivatisation for the analysis of both aldehydes and nitriles was chosen as the analytical method by considering the advantages and disadvantages of existing analytical methods as well as the availability of instruments and materials in the laboratory. These techniques are fast, solvent-free and sensitive.

3.2 Experimental

3.2.1 Chemicals and Reagents

Inorganic reagents, organic solvents, and organic compounds were of analytical grade purity (AR grade ≥ 90% pure) or better, and were used without further purification.

3.2.2 Purification of Laboratory Water

Laboratory water used in this study was purified through a stage of treatment steps, in order to obtain water of sufficiently high purity to meet the requirements of this

study. Tap water was first passed through an Ibis® reverse osmosis system which consisted of a 5 µm pre-filter followed by an activated charcoal filter and two mixed-bed ion exchange purification packs arranged in series. Water exiting the ion exchange packs was then passed through a reverse osmosis membrane and fed to an Elga Purelad Ultra purification system, as required, producing high purity water with a conductivity of 18.2 MΩ and a total organic carbon concentration of less than 1 µg L^{-1}. This high purity water is referred to in this Thesis as MilliQ (MQ) water.

3.2.3 Cleaning Procedures

All non-volumetric glassware was washed with Pyroneg® detergent, rinsed with deionised water and annealed overnight at 600 °C prior to use. All volumetric glassware was washed with Pyroneg® detergent, rinsed with deionised water and MilliQ water. All syringes were flushed with appropriate solvent prior to use.

3.2.4 Analysis of Aldehyde and Nitriles

The samples were analysed *via* automated HS SPME, using a 75 µm CAR-PDMS fibre, followed by GC-MS analysis. Automated HS SPME was performed on a Gerstel Multipurpose Sampler MPS2, which was interfaced to an Agilent Technologies Series II GC 6890N with an Agilent Technologies 5973N Mass Selective Detector. The sample volume used for SPME was 10 mL in a 20 mL amber glass vial. Sodium sulfate (3.00 g) and an aliquot of the internal standard (15 µL of a 2.5 ng μL^{-1} working solution of 1,2-dibromopropane) were added to the sample. The autosampling device transferred the vial into the agitator, where it was agitated at 500 rpm at 60 °C for 10 minutes. The SPME fibre was introduced into the headspace of the sample for 15 minutes and then transferred to the GC injector for thermal desorption of analytes.

The SPME fibre was desorbed at 300 °C for 3 minutes in the injector port of the GC. The analytes were introduced *via* a splitless injection (50 mL min^{-1} with a 0.05 min. purge time) onto a fused silica 5% phenyl – 95% dimethylpolysiloxane capillary column (30 m x 0.25 mm id, column phase thickness of 1 µm (ZB-5; Phenomonex)). Helium was used as the carrier gas operating at a flow of 0.7 mL min^{-1}. The oven temperature was initially set at 35 °C for 2 minutes prior to being increased at a rate of 5 °C min^{-1} until 105 °C, then 15 °C min^{-1} to a temperature of 300 °C with a final

hold time of 5 minutes. The samples were analysed by mass spectrometry in the SIM mode with the following ions monitored: m/z 27, 29, 39, 41, 42, 43, 44, 54, 55, 57, 58, 68, 71, 72, 86, 90, 91, 116, 117, 120, 121 and 123. A dwell time of 30 ms was used for all ions.

Calibration of aldehyde and nitrile concentrations was achieved by analysis of a series of external standards (2-methylbutyraldehyde (>90%), 2-methylbutyronitrile (98%), 3-methylbutyraldehyde (97%), 3-methylbutyronitrile (98%), isobutyraldehyde (99%), isobutyronitrile (99.6%), phenylacetaldehyde (>90%), phenylacetonitrile (98%); Sigma Aldrich). Individual stock solutions for each aldehyde, nitrile and 1,2-dibromopropane (97%, Sigma Aldrich) were prepared in HPLC grade methanol (Mallinckrodt Baker) at 5000 mg L^{-1} and stored at 4 °C. A working standard solution containing each aldehyde, and one containing each nitrile was prepared from the stock solutions using appropriate dilution with methanol and further standards were prepared by appropriate dilution of this solution as required into MilliQ water. 1,2-dibromopropane was used as an internal standard and was added at a concentration of 3.75 µg L^{-1} to each sample.

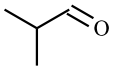
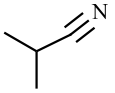
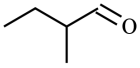
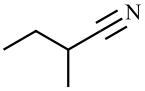
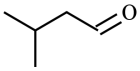
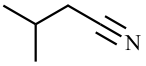
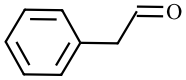
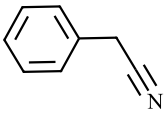
3.2.5 Validation Procedure

The method was validated under optimised conditions as described in Sections 3.2.4, by determination of linearity, detection and quantification limits, and repeatability and reproducibility. The effect of sample matrix on analysis of the aldehydes and nitriles was investigated. A real water sample was collected and analysed by the optimised method, and then spiked with known concentrations of each aldehyde and nitrile and their recoveries were calculated.

3.3 Results and Discussion

A HS SPME/GC-MS method for the determination of four aldehyde and four nitrile species (Table 3.1) in drinking waters was developed and optimised. For quantification purposes, the analyses were carried out in SIM mode to enhance selectivity and reduce noise.

Table 3.1: The aldehydes and nitriles used in this study, and their structures.

Compound	Acronym	Structure	Boiling Point *
Isobutyraldehyde	IBA		63 °C
Isobutyronitrile	IBN		107-108 °C
2-methylbutyraldehyde	2-MBA		90-92 °C
2-methylbutyronitrile	2-MBN		125-126 °C
3-methylbutyraldehyde	3-MBA		90 °C
3-methylbutyronitrile	3-MBN		128-130 °C
Phenylacetaldehyde	PA		195 °C
Phenylacetonitrile	PN		233-234 °C

* Boiling point data from Sigma Aldrich

3.3.1 Optimisation of the Method

An automated SPME sampler (Gerstel Multipurpose Sampler MPS2) was used for the SPME analysis. Each sample is transferred into a heating/agitator box, where the extraction (sample absorption/adsorption to the fibre) takes place. After a pre-determined amount of time, the fibre is transferred into the injector port of the GC, where the analytes are desorbed. The steps involved in sample transfer, sample extraction by the SPME fibre and analyte desorption from the fibre were all performed automatically and programmed through the MASter software interfaced to the MSD Chemstation software, which was connected to the GC-MS and the Gerstel Multipurpose Sampler.

Initial optimisation (fibre choice, sample volume and agitation) was carried out on the smaller molecular weight aldehydes alone (phenylalanine was not considered due to its structural differences). Subsequent factors were considered for all of the

aldehydes and nitriles, and it was found that results were consistent between aldehydes and their corresponding nitriles. This result was most likely due to the similarity in their structure and molecular weight so it was concluded that the earlier optimisation selections would have also suited the nitriles. The method was optimised for the similarly structured aldehydes and nitriles, and was found to be applicable also for phenylacetaldehyde and phenylacetonitrile, even with the difference in size and presence of the aromatic ring in these two analytes.

Previous reports of the analysis of aldehydes by SPME had utilised a variety of different fibre phases, so there was no real indication of which SPME fibre would be most suitable for the extraction of the aldehydes and also nitriles from drinking water. Mixed phase coatings are suitable for volatile compounds (Pawliszyn 1997). Four commercially available mixed phase SPME fibres were evaluated for their efficiency to extract the specific aldehydes from the HS of a water sample: a 75 µm CAR-PDMS fibre, a 50/30 µm 2cm DVB-CAR-PDMS fibre, a 65 µm PDMS-DVB fibre, a 65 µm and a 70 µm CW-DVB fibre. Their efficiency was evaluated based on achieving greatest peak area response from GC-MS analysis and the CAR-PDMS 75 µm phase fibre provided the best performance for the aldehydes and was chosen as the fibre phase for the rest of the study (results not shown).

SPME can be carried out in the direct or HS mode. The mode is determined by the volatility of the analytes, the sample matrix and the affinity of the analytes to the matrix (Pawliszyn, 1999). Since the aldehydes and nitriles being examined are reasonably volatile, (boiling points shown in Table 3.1) with the exception of the aromatic phenylacetaldehyde and nitrile, and HS mode results in longer fibre lifetimes, the HS mode of SPME was chosen for this study.

In order to develop a HS SPME method for the extraction of aldehydes and nitriles from water samples, a number of fibre extraction conditions need to be considered and optimised: sample and headspace volume, sample agitation, salt addition, temperature and time of extraction and fibre desorption conditions. The effect of sample matrix must also be taken into account. These factors were optimised using 20 mL Teflon-lined screw cap vials containing a mixture of 20 µg L^{-1} aqueous

solution of each of the aldehydes, and subsequently, the nitriles. One factor at a time was varied.

The sample volume and HS volume must be considered in HS SPME. During HS SPME, the volume of the gaseous phase should be minimised to achieve high sensitivity since volatile compounds, such as aldehydes and nitriles accumulate in the HS and when the HS volume is large, sensitivity decreases (Pawliszyn, 1999). However, practicalities mean that this is not always achievable, *e.g.*, the volume cannot be so great that the fibre is immersed or splashed with the sample. The vial size (20 mL) was constrained by the autosampling device, so a sample of 10 mL was chosen, being the greatest volume that the vial could hold prior to the fibre being immersed or splashed with sample.

Prior to introduction of the SPME fibre into the vial, the sample is agitated in order to promote volatilisation of the analytes. Traditionally samples were stirred with a magnetic stir-bar to agitate the sample, however due to automation of the SPME technique, the sample is instead placed in a box and shaken at a specified speed. Agitation of the sample influences the equilibration time, and consequently influences the extraction time. In aqueous samples, agitation is required to facilitate mass transport between the aqueous sample and the HS of the sample and then the fibre (Pawliszyn, 1999). The autosampling device was able to agitate the sample at speeds ranging from 250 to 750 rpm, accordingly, the optimum speed of agitation was determined by trialling three speeds in this range and the GC-MS peak area responses of the six analytes at the different agitation speeds are presented in Figure 3.1. Other parameters were kept constant. While the peak area responses at an agitation speed of 250 rpm were lower, there was little difference in the peak area responses between 500 and 750 rpm for each analyte. Since 500 rpm was in the middle of the range for the agitator, it was chosen as the optimum speed for agitation.

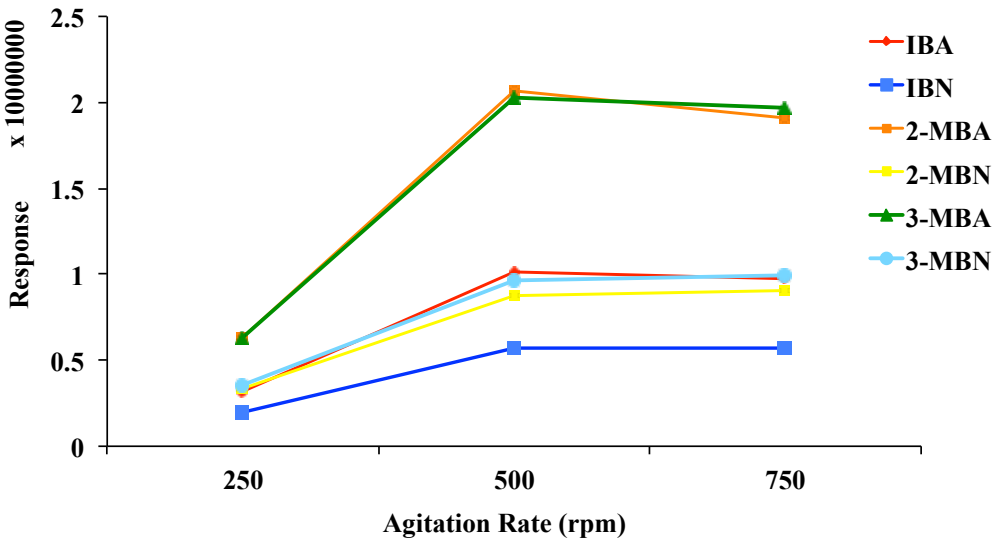


Figure 3.1: The effect of agitation rate on the extraction of aldehydes and nitriles by HS SPME.

The addition of salt (*e.g.,* sodium sulfate (Na_2SO_4)) to aqueous samples aids in volatilisation by 'salting out' of analytes, effectively decreasing their solubility in water and increasing the proportion recovered by the fibre (Pawliszyn, 1997). Based on this, the effect of salt addition was investigated. In this work, sodium sulfate was used. Various amounts of Na_2SO_4 (0, 1, 3 or 5 g) were added to the sample and the GC-MS peak area responses of the eight analytes with different salt concentrations are shown in Figure 3.2. Other parameters were kept constant. Based on the GC-MS peak area responses, 3 g of salt gave the highest response across the majority of analytes and was chosen as the optimum amount.

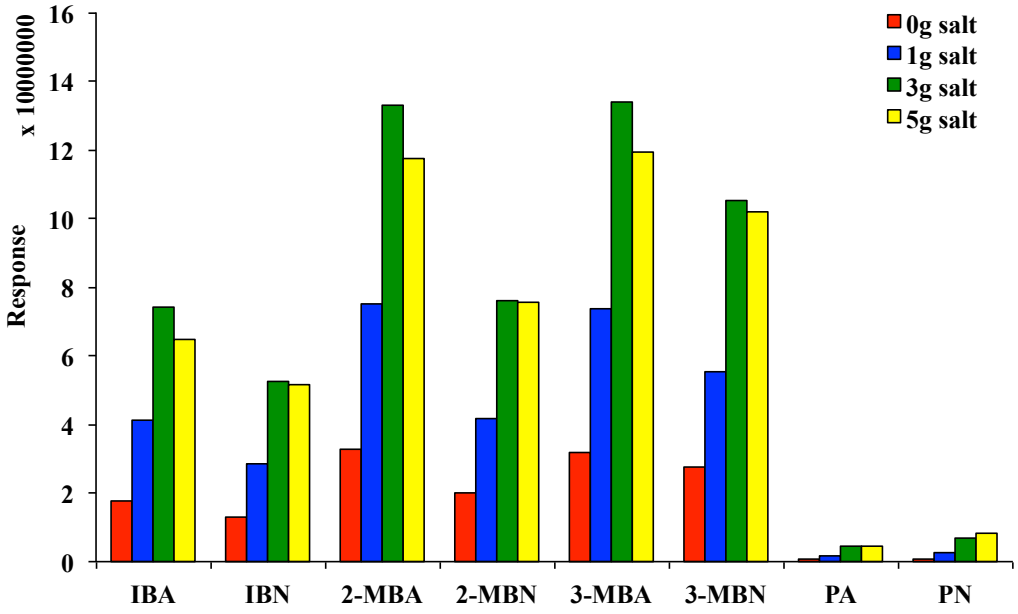


Figure 3.2: The effect of salt concentration on the extraction of aldehydes and nitriles by HS SPME.

In the case of HS SPME, extraction is an equilibrium process and, accordingly, extraction temperature affects both the sensitivity and extraction kinetics. The optimum extraction temperature for analysis was determined by observing the variation in GC-MS peak area response for each aldehyde and nitrile when only the extraction temperature was varied by heating the sample in the autosampling device (50 °C, 60 °C and 70 °C); other parameters were kept constant. Figure 3.3 displays the effect of extraction temperature on the amount of each analyte extracted by the fibre. There was no obvious trend in response based on extraction temperature. There was great variability in the response of each analyte with increasing temperature, most likely due to the volatility range of the analytes (shown by the variation in boiling point of analytes in Table 3.1). The smaller, more volatile IBA showed decreased response with increasing extraction temperature. The larger, less volatile 3-MBN showed increased response with increasing extraction temperature.

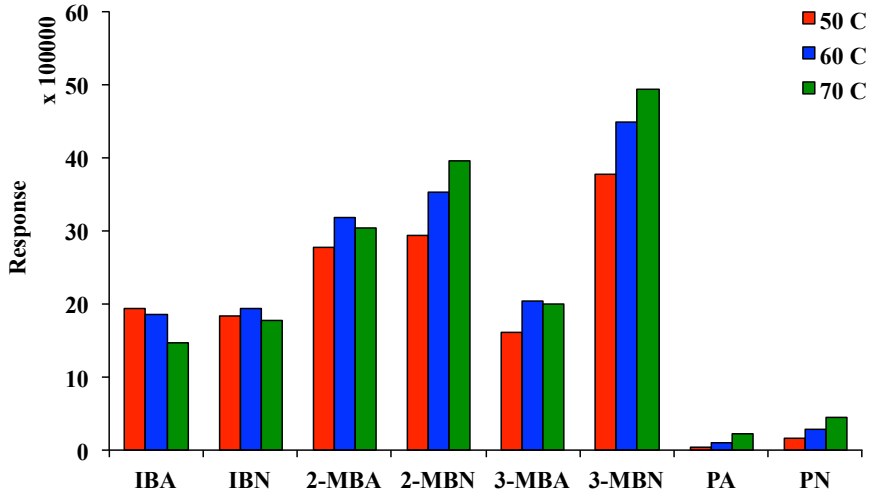


Figure 3.3: The effect of temperature on the extraction of aldehydes and nitriles by HS SPME.

The recoveries of both PA and PN were significantly lower than those of the other analytes, with the peak area response of both analytes much lower. This was most probably due to the higher molecular weights (*e.g.,* PA 120.15 g mol^{-1} and PN 117.15 g mol^{-1} in comparison to 2-MBA 86.13 g mol^{-1} and 2-MBN 83.13 g mol^{-1}) and the lower volatilities (boiling point (PA 195 °C and PN 233 °C) and Henry's Law constant of these compounds compared to the other analytes. The high boiling points of PA and PN make these compounds not sufficiently volatile for efficient volatilisation from the aqueous phase at the selected temperature ranges. Structural differences (the presence of an aromatic ring, while the other analytes are aliphatic) probably contribute to the decreased volatility and increased water solubility.

However, six out of the eight analytes were readily volatilised, and a temperature of 60 °C was chosen as the best compromise temperature. A higher extraction temperature may have resulted in a marginal increase in the extraction of PA and PN, but could have resulted in the potential degradation of the other analytes, such as IBA and IBN (which were of greater interest).

Unlike most extraction methods, SPME is an equilibrium extraction method, rather than an exhaustive one. Thus, the optimal approach is to allow the analyte to reach equilibrium between the sample and the fibre. The equilibration time refers to the time after which the amount of analyte extracted remains constant and corresponds to the extracted amount after infinite time, within limits of experimental error

(Pawliszyn, 1997). In order to determine the equilibration time, and hence extraction time of each analyte, analyses were carried out after different extraction times and the GC-MS peak area response was plotted against the extraction time in Figure 3.4, while other parameters remained constant. Figure 3.4 shows that the response of each analyte remains fairly constant after an extraction time of 15 minutes, so an extraction time of 15 minutes was chosen as the optimum for this analysis.

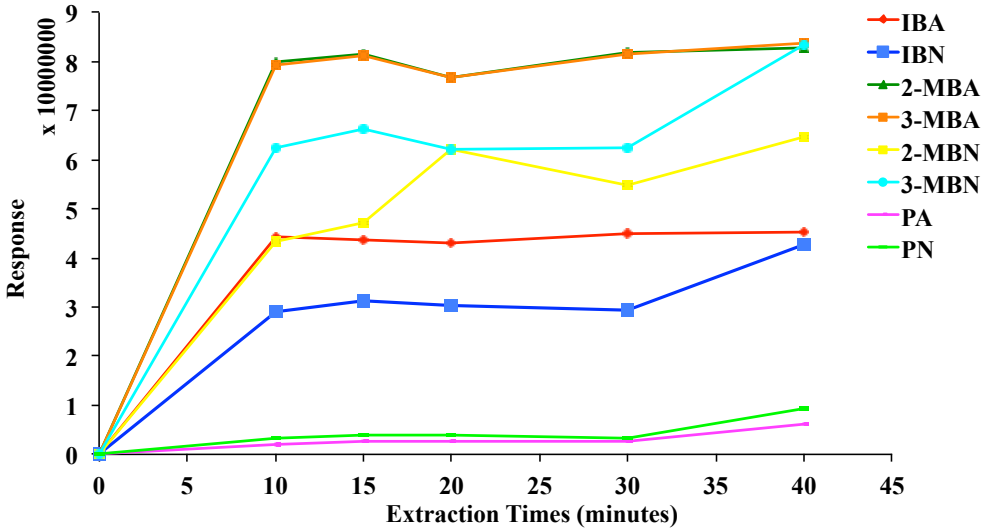


Figure 3.4: Extraction time profile for the extraction of aldehydes and nitriles by HS SPME.

In summary, the optimum HS SPME conditions for the extraction of the four aldehydes and four nitriles investigated in this Thesis using a 75 µm CAR-PDMS fibre and a 20 mL sample vial were agitation at 500 rpm at 60 °C for 10 minutes, followed by HS extraction (15 minutes) with 10 mL of sample and addition of 3 g of sodium sulfate.

3.3.2 Method Validation

3.3.2.1 Calibration of the Method

Calibration curves for the individual aldehydes and nitriles were established by spiking varying concentrations (in total) of each aldehyde and nitrile (1, 5, 10, 50 and 100 ng L^{-1}), together with a fixed concentration (3.75 µg L^{-1}) of internal standard (1,2-dibromopropane), into MilliQ water and analysing by the optimised HS SPME/GC-MS method. 1,2-Dibromopropane was chosen as an internal standard as it used in the APHA Standard Methods (APHA, 2012; 1998) and USEPA Standard Method (1998). Deuterated analogues could have been more suitable, however these were not readily available. Relative response factors for each standard were

determined by dividing the total area of the standard by the area of the internal standard and these were then plotted versus the concentration of each standard. Linear calibration curves with high correlation coefficients (Table 3.2) were achieved for each aldehyde and nitrile (typical curves shown in Figure 3.5).

3.3.2.2 Method Sensitivity

In order to determine the sensitivity of the method for each analyte, LODs, and limits of quantification (LOQs) were determined using a method described by Adams (2004). Six replicate blank analyses (MilliQ water and internal standard) were carried out. The spectrum noise over the retention time window of each analyte was integrated, the mean and standard deviation of these areas calculated, and then converted to equivalent concentrations of each respective analyte using the calibration curves. The LOD was the mean concentration of the blank analyses plus three times the standard deviation, and the LOQ was the mean concentration of the blank plus ten times the standard deviation.

LODs for all of the compounds were below 0.6 μg L^{-1}, which is lower than the odour threshold concentrations (OTC) for most of the compounds (Table 3.2). For 3-MBA, the LOD was 0.25 μg L^{-1}, which is slightly greater than the odour threshold concentration for this compound (0.2 μg L^{-1}). These LODs are also close in value to other methods using SPME but with on-fibre-derivatisation (0.12 – 0.34 μg L^{-1}) for similarly structured aldehydes (Tsai and Chang, 2003).

Table 3.2: Correlation coefficients (r^2), LODs and LOQs for analysis of aldehydes and nitriles using the optimised method. Odour threshold concentrations of the aldehydes and nitriles where reported from literature are also shown.

Compound	**Linearity** (r^2)	***Sensitivity (μg L^{-1})***		**OTC (μg L^{-1})**
		LOD	**LOQ**	
IBA	0.9907	0.30	0.76	0.90[a]
IBN	0.9947	0.24	0.45	204[b]
3-MBA	0.9857	0.25	0.70	0.20[a]
2-MBA	0.9856	0.10	0.25	12.5[c]
3-MBN	0.9992	0.17	0.40	2.3[b]
2-MBN	0.9996	0.26	0.70	
PA	0.9635	0.22	0.53	4
PN	0.9712	0.53	0.65	

a: (Guadagni *et al.*, 1972; Guadagni *et al.*, 1963),b: (Fabrellas *et al.*, 2004), c: (Buttery *et al.*, 1971).

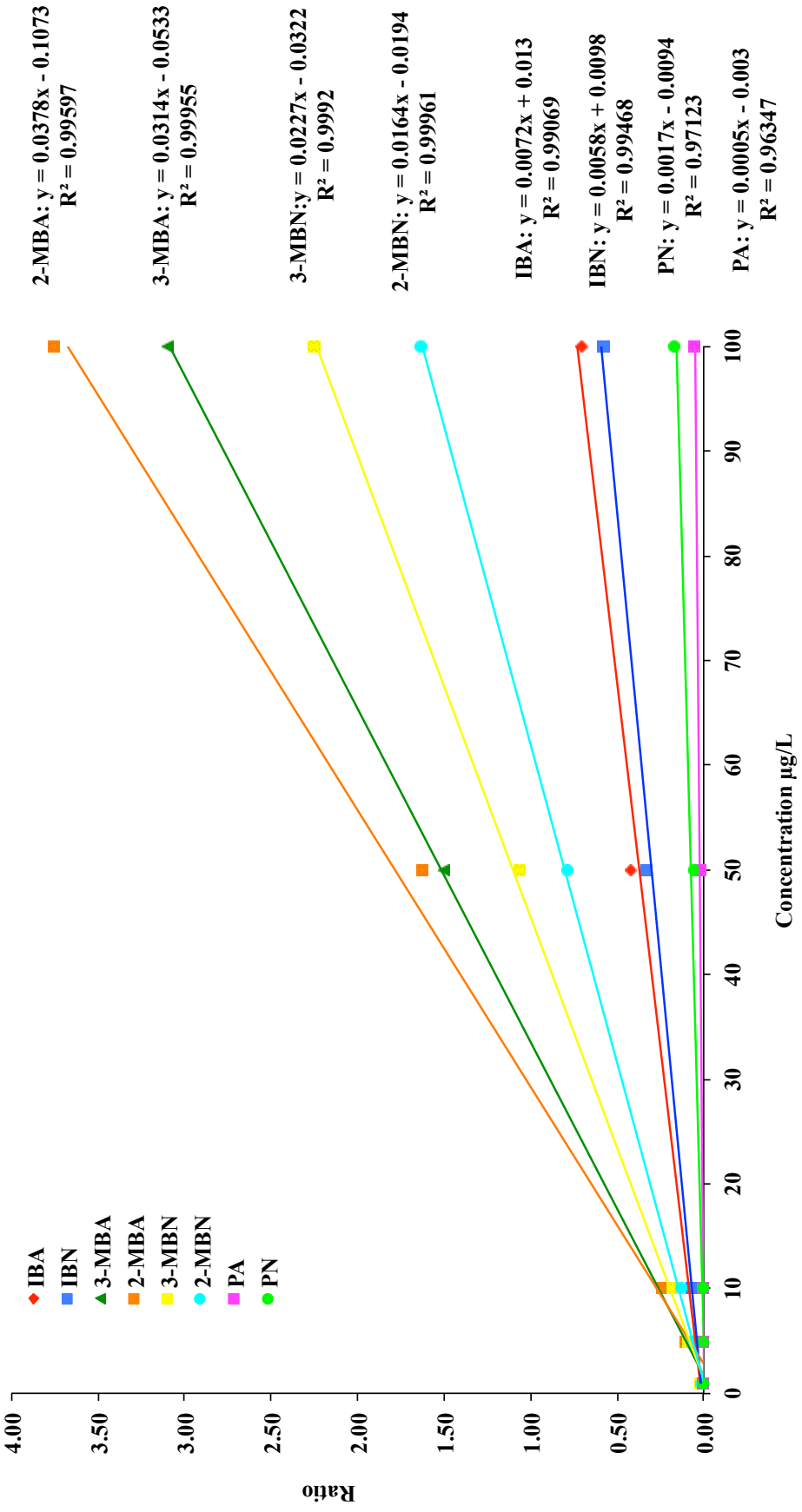


Figure 3.5: Calibration curves for the analysis of the aldehydes and nitriles by HS SPME/GC-MS.

3.3.2.3 Method Precision

The precision of the method was estimated by determining the repeatability and the reproducibility of the method. The repeatability refers to 'run to run' precision, while reproducibility refers to 'day to day' precision. Three concentrations (1, 10 and 50 µg L^{-1}) of each analyte were spiked into MilliQ water and analysed by the optimised HS SPME/GC-MS method. The relative standard deviation (RSD) of the analytical results for each concentration was calculated, with the %RSD being the standard deviation divided by the mean multiplied by 100 for each concentration sample set. Repeatability was calculated by analyses of the sample at three concentration levels on one GC-MS run, with the calculation of the associated RSD. Reproducibility was calculated by analyses of the same three concentration levels on three separate days, with the calculation of the associated RSD. The %RSD values are presented in Table 3.3.

Table 3.3: Repeatability and reproducibility (%RSD) for analysis of the aldehydes and nitriles using the optimised HS SPME/GC-MS method.

Compound	*Repeatability*			*Reproducibility*		
	%RSD 1 µg L^{-1}	**%RSD 10 µg L^{-1}**	**%RSD 50 µg L^{-1}**	**%RSD 1 µg L^{-1}**	**%RSD 10 µg L^{-1}**	**%RSD 50 µg L^{-1}**
IBA	19	12	12	11	13	10
IBN	14	16	14	9	20	14
3-MBA	28	14	11	15	13	6
2-MBA	20	15	11	10	12	6
3-MBN	18	16	13	7	16	9
2-MBN	20	16	13	7	15	8
PA	7	22	3	8	21	12
PN	28	25	14	15	20	26

The repeatability of the method was found to be very good, with %RSDs ranging from 7 to 28, with slightly higher values at the lowest concentration. In the case of PA and PN, the best repeatability was found at the highest concentration. This is once again most likely due to the much lower peak area responses in comparison to the other analytes as the analytical method was not optimised for these aromatic compounds.

Good reproducibility was achieved with %RSDs ranging from 3 to 26. PA and PN showed the lower precision, once again emphasising more suitable SPME extraction and desorption conditions might have improved their analysis.

3.3.3 Effect of Sample Matrix

In order to establish the impact of different aqueous matrices on the recovery of analytes, aldehydes and nitriles were spiked into a water sample and the recoveries (based on the concentration spiked) of these analytes were determined. The sample type was a water which had previously had chlorinous off-flavour issues. Dissolved organic carbon (DOC) in the water was 2.35 mg L^{-1}, while alkalinity (as $CaCO_3$) was 130 mg L^{-1} (Table 3.4).

Table 3.4: Water quality characteristics for water used in experiments to determine matrix effects.

Water characteristics	**DOC (mg L^{-1})**	**Total alkalinity (as $CaCO_3$ mg L^{-1})**	**Colour (Hazen units)**	**Conductivity (mS m^{-1})**
Water A	2.35	130	<1	77

The analytes were spiked into the samples at two concentrations (5 and 50 µg L^{-1}) and the analysis was performed in triplicate. A chromatogram is shown in Figure 3.6 a of the analysis of a 50 µg L^{-1} spike of the aldehydes and nitriles into the water sample. Blank analyses without the spiked analytes (but with the internal standard) were also carried out to ensure that the sample matrix was free from co-eluting species and other interferences. No interfering species were found in the blank analyses, as shown in the chromatogram in Figure 3.6 b.

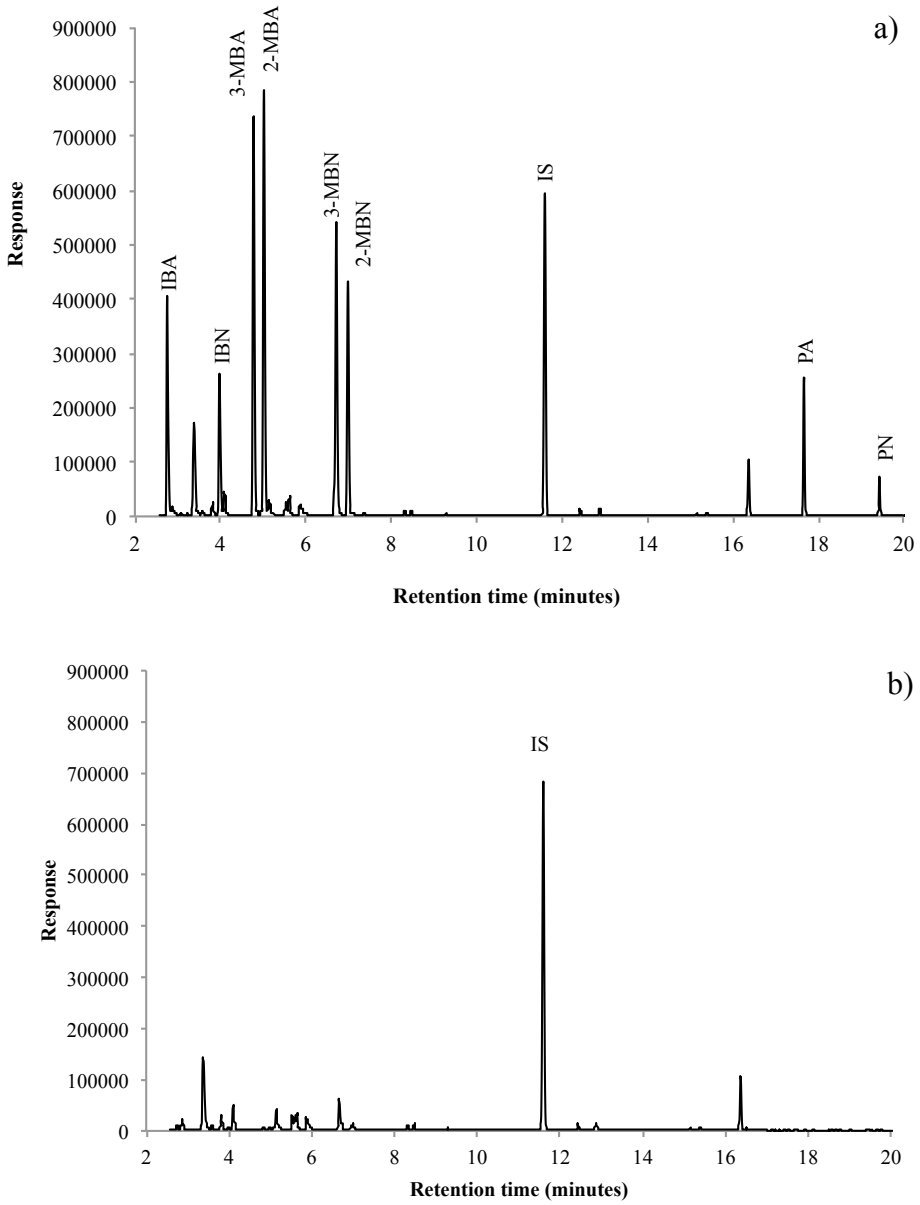


Figure 3.6: a) Chromatogram (GC-MS; SIM mode) of analysis of 50 µg L^{-1} aldehydes and nitriles added into Water A**. b)** Chromatogram (GC-MS; SIM mode) of IS added into Water A.

Recoveries (Table 3.5) for seven out of the eight analytes at both concentration values ranged from 80 – 109%, with %RSD values in the range of 4 – 13. In the case of PA, a low recovery was achieved at the low concentration standard, while a more acceptable recovery of 106% was achieved for the higher concentration value. Reproducibility was poor at the low concentration (31%) and somewhat improved for the high standard (24%), but still inferior to that of the other compounds. This suggests that there was potentially something within the sample that reacted with PA, reducing its recovery.

Table 3.5: Average percentage recoveries of aldehydes and nitriles from real water sample. Initial added concentrations were 5 and 50 µg L^{-1}. RSD values are shown in parentheses. Analyses were carried out in triplicate. N.D: not detected.

Spike	Recovery of aldehydes and nitriles %							
	IBA	**IBN**	**3-MBA**	**2-MBA**	**3-MBN**	**2-MBN**	**PA**	**PN**
Water A	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5 µg L^{-1}	80 (6)	88 (8)	85 (5)	87 (4)	86 (7)	82 (5)	38 (31)	97 (3)
50 µg L^{-1}	91 (11)	99 (13)	101 (11)	100 (10)	102 (12)	104 (12)	106 (24)	109 (13)

3.4 Conclusions

This is the first report of a HS SPME/GC-MS method for the analysis of the four aldehydes and their corresponding nitriles: isobutyraldehyde (isobutyronitrile), 2-methylbutyraldehyde (2-methylbutyronitrile), 3-methylbutyraldehyde (3-methylbutyronitrile), and phenylacetaldehyde (phenylacetonitrile). The method is particularly novel in that it does not involve the use of derivatising agents and therefore has only minimal sample preparation requirements. The method was developed, optimised and validated. The method is rapid and simple, with automated agitation and extraction, with a total analysis time of 45 minutes, free from interferences, with good reproducibility and linearity for the majority of analytes.

The precision of the method ranged from 3 to 26%, and detection limits near to, or less than, the odour threshold concentrations of the aldehydes and nitriles were achieved. The method is comparable in terms of precision and detection limits to methods previously reported using derivatisation steps for the same compounds or for compounds with similar structures.

This method was applied in Chapter 4 to examine the mechanisms of chlorination, and the effect of pH and bromide, on the production of these odorous, low molecular weight aldehydes and nitriles, in relation to chlorinous off-flavours in water samples.

Chapter 4
Formation of Chlorinous Off-Flavour Compounds from Amino Acids

4.1 Introduction

4.1.1 Occurrence of Amino Acids in Waters

Amino acids are the building blocks of life. They combine, usually through amide linkages, to create proteins, peptides and other biological macromolecules. All proteins are composed of only 20 amino acids, known as the standard amino acids (Voet and Voet, 2011). These standard amino acids are listed in Table 4.1, along with their molecular weights, percentage average occurrence in proteins and their pK values. These amino acids are known as α-amino acids because, with the exception of proline, they have a primary amine group and a carboxylic acid group substituent attached to the same carbon atom (Figure 4.1). Proline has a secondary amino group. Many other "non-standard" amino acids also exist (Dotson and Westerhoff, 2009). The "non standard" amino acids (those other than the 20 from which proteins are synthesised) and their derivatives also have important biological functions as neurotransmitters, metabolic intermediates, and poisons (Voet and Voet, 2011).

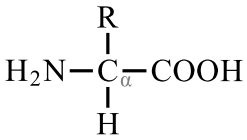


Figure 4.1: General structural formula for the α-amino acids. There are 20 different R groups in the standard amino acids.

The most common way of classifying the standard amino acids is according to the polarity of their side chains. According to this classification scheme, there are three major types of amino acids: those with nonpolar R groups, those with uncharged polar R groups and those with charged polar R groups (Voet and Voet, 2011).

Table 4.1: Standard amino acids, their molecular weight, their occurrence in proteins and the pK values of their ionisable groups (Data from Voet and Voet (2011) and references therein).

Name	Molecular weight (Da)	Average occurrence in proteins (%)	pKa*	pKb*	pKc *
Amino acids with non polar side chains					
Glycine	75.1	7.1	2.35	9.78	
Alanine	89.1	8.3	2.35	9.87	
Valine	117.2	6.9	2.29	9.74	
Isoleucine	131.2	9.7	2.33	9.74	
Leucine	131.2	6	2.32	9.76	
Methionine	149.2	2.4	2.13	9.28	
Proline	115.1	4.7	1.95	10.64	
Phenylalanine	165.2	3.9	2.2	9.31	
Tryptophan	204.2	1.1	2.46	9.41	
Amino acids with uncharged polar side chains					
Serine	105.1	6.5	2.19	9.21	
Threonine	119.1	5.3	2.09	9.1	
Asparagine	132.1	4	2.14	8.72	
Glutamine	146.2	3.9	2.14	9.13	
Tyrosine	181.2	2.9	2.2	9.21	10.46 (phenol)
Cysteine	121.2	1.4	1.92	10.7	8.37 (sulfhydryl)
Amino acids with charged polar side chains					
Lysine	146.2	5.9	2.16	9.06	10.54 (NH_3^+)
Arginine	174.2	5.5	1.82	8.99	12.48 (guanidine)
Histidine	155.2	2.3	1.8	9.33	6.04 (imidazole)
Aspartic acid	133.2	5.4	1.99	9.9	3.90 (COOH)
Glutamic acid	147.1	6.8	2.1	9.47	4.07 (COOH)

* a- COOH group; b- NH_2 group, c- additional sidechain

The study of aquatic NOM is important to researchers as they try to understand the formation of DBPs upon disinfection of water, but due to the complexity of NOM, it has been a difficult material to analyse (Chinn and Barrett, 2000). Studies have relied on general aggregate measurements such as dissolved organic carbon (DOC) concentration or UV absorbance at 254 nm, however specific information about the reactive functional groups present in NOM is also necessary. DOC is often measured to indicate the amount of organic material present in water systems and to indicate

the concentration of DBP precursors. Dissolved organic nitrogen (DON) is a subset of the DOC in water. DON includes any organic nitrogen containing species present in water. Amino acids have been identified as a large portion of the nitrogen component of DON in water (Thurman, 1985).

Amino acids exist in either the free (unbound) or combined (bound) form. Combined amino acids are those bound within biological molecules (*e.g.,* proteins and peptides). The source, occurrence and fate of amino acids have been widely studied within freshwater systems. Thurman (1985) reported that total amino acids accounted for 2.6% of the DOC and 35% of the DON in lakes. It was also reported that total amino acids occur at concentrations between 50 and 1000 μg L^{-1}, with a mean concentration of 100 to 300 μg L^{-1}, in rivers, streams and lakes (Thurman, 1985). Lytle and Perdue (1981) proposed that amino acids are associated with the humic substances in soil and are carried into the water by surface runoff into rivers. Their study also postulated that the surface runoff was the primary source of amino acids in the river they investigated (Lytle and Perdue, 1981).

Environmental amino acid levels have been related to algal blooms, which could also contribute to the level of other components of NOM. They also play a role in the microbial nutrient web and have a role in cycling processes occurring in biologically active aquatic systems (Chinn and Barrett, 2000). Between this and studies of the marine environment, there is little published information on naturally occurring levels of amino acids in various drinking water sources, reportedly due to the low concentrations present in the environment and difficulties with analytical methods (Chinn and Barrett, 2000).

A study of two drinking water sources identified serine (average 5.1 μg L^{-1}), proline (average 6.4 μg L^{-1}), glycine (average 2.3 μg L^{-1}) and alanine (average 4.5 μg L^{-1}) as the highest concentrations of free amino acids present in both waters (Chinn and Barrett, 2000). Earlier research identified the major amino acids found from hydrolysed NOM, isolated from the Apremont Reservoir, France, included glycine, glutamic acid, aspartic acid, and serine (Krasner *et al.*, 1996). These amino acids have also been reported to be the major amino acids in rivers (Thurman, 1985).

A large scale occurrence survey of free and total amino acids was undertaken in United States drinking waters by Dotson and Westerhoff (2009). In this study, 16 full-scale US drinking water treatment plants affected by upstream algal blooms or wastewater discharges were investigated. Measurements of specific organic nitrogen species (free and total amino acids) and the bulk organic matter surrogates (DOC, DON and UV at 254 nm) were undertaken. Free amino acids accounted for < 1.3% DON, while total amino acids accounted for 15% of the DON on average, and varied between 0.17 and 47%. Serine, glycine and alanine were statistically most likely to occur in the five most concentrated amino acid species in any given water. The amino acids found in raw waters varied slightly to those found in treated drinking waters. In treated water, tyrosine was likely to be found as the dominant free amino acid, while threonine was likely to be the most dominant amino acid derived from combined amino acids (Dotson and Westerhoff, 2009).

There have been few studies within Australia of the occurrence of amino acids in Australian natural water systems. In a recent study carried out by researchers in our laboratory at Curtin University, the free amino acid concentrations of three different Western Australian surface waters were measured (How *et al.*, 2014). The three amino acids present in highest concentrations in surface waters A, B, and C were tyrosine, leucine and isoleucine (Table 4.2). These amino acids have not previously been observed as major free amino acids in earlier studies (Dotson and Westerhoff, 2009; Chinn and Barrett, 2000). However, natural variation and/or analytical variation could account for these differences. Other amino acids were identified in the waters, however these were below the level of detection of the method (2 μg L^{-1} as N).

Table 4.2: Concentration (μg L^{-1} as N) of measured free amino acids in surface waters A, B and C. Reproduced from How *et al.* (2014).

Name	Surface water A	Surface water B	Surface water C
Proline	<2	2	2
Isoleucine	3	3	5
Leucine	4	4	8
Tyrosine	4	4	7
Phenylalanine	3	3	4
Tryptophan	1	2	1
Total free amino acids	15	16	26

4.1.2 Reactions of Chlorine with Nitrogen Compounds: The Breakpoint Phenomenon

As the practice of disinfecting drinking water with chlorine grew rapidly in the 1920s and 1930s the disinfection process was the subject of much research, and a number of early investigators recognised that chlorine reacted with ammonia. Chloramines are formed when chlorine reacts with ammonia nitrogen which is present naturally in the water, or added for the purpose of forming chloramines. The chlorination of ammonia in dilute solutions can involve as many as 14 different reactions, but the formation of chloramines can be summarised by the following reaction equations, in sequence (White, 2010):

$$HOCl + NH_3 \leftrightarrows NH_2Cl \text{ (monochloramine)} + H_2O \quad \textbf{Equation 4.1}$$

$$HOCl + NH_2Cl \leftrightarrows NHCl_2 \text{ (dichloramine)} + H_2O \quad \textbf{Equation 4.2}$$

$$HOCl + NHCl_2 \leftrightarrows NCl_3 \text{ (trichloramine)} + H_2O \quad \textbf{Equation 4.3}$$

The early investigators began to study the formation of chloramines, reactions of chlorine with other nitrogenous compounds and taste and odour control (Griffin and Chamberlin, 1941a; Calvert, 1940; Faber and Cox, 1939; Griffin, 1939). However, the analytical methodology in use at this time was unable to distinguish between free and combined chlorine. Free chlorine refers to all chlorine present in the water as Cl_2, HOCl, OCl^- and Cl_3^-. When ammonia is present in water, it can quickly react with free chlorine to form chloramines, as described in Equations 4.1 - 4.3. These species are known as combined chlorine. The sum of free and combined chlorine is known as the total chlorine and, accordingly, the combined chlorine concentration can also be calculated by subtracting the free chlorine concentration from the total chlorine concentration (White, 2010).

During the initial studies, Griffin (1939) investigated the sudden loss of chlorine residual and the simultaneous disappearance of ammonia nitrogen at treatment plants that were experimenting with higher than usual chlorine residuals (2-15 mg L^{-1}) to combat tastes and odours. Griffin found that increasing the chlorine dose in some waters did not increase the residual, but rather reduced it significantly (Griffin and Chamberlin, 1941a; Griffin, 1939). The point of maximum reduction of the residual was termed the “breakpoint” and part of the “breakpoint reaction”. Other researchers

at the time also observed and understood from a chemical point of view the "breakpoint reaction"(Calvert, 1940; Faber and Cox, 1939), and it is these researchers (including Griffin) who are credited with elucidating the breakpoint curve. Calvert (1940) recognised that the breakpoint was directly related to the concentration of ammonia in the water.

The breakpoint curve (the typical curve is shown in Figure 4.2) is a plot of the residual chlorine concentration versus the dose of chlorine added to a water containing ammonia nitrogen. The chlorine dose is typically shown in units of milligrams per litre as Cl_2 or as a Cl_2:N weight or molar ratio (White, 2010).

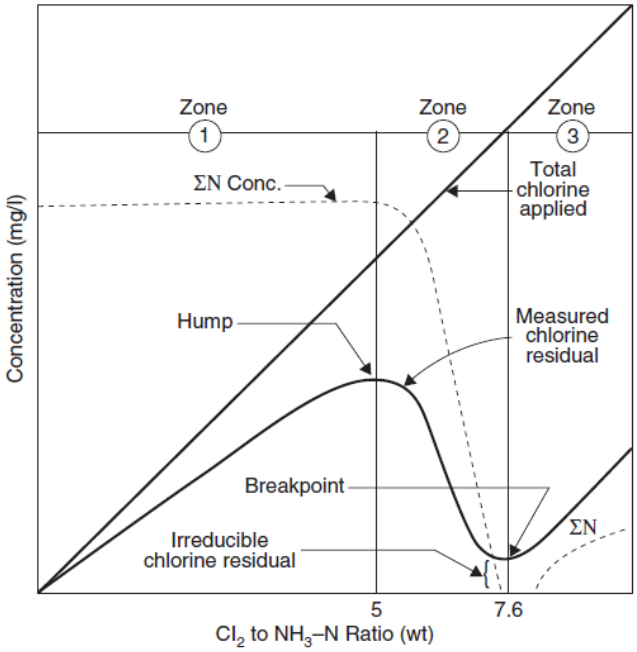


Figure 4.2: The classic breakpoint curve (where ΣN is the sum of nitrogen species, including both NH_3-N and chloramines). Reprinted with permission from White (2010). Copyright John Wiley & Sons, Inc 2010.

There are several characteristics of the theoretical breakpoint curve for the addition of varying amounts of chlorine to waters containing ammonia nitrogen. In zone 1, chlorine reacts with ammonia to form chloramines, primarily monochloramine. At the end of zone 1, at a chlorine:ammonia weight raio of 5:1, the combined chlorine concentration reaches a maximum, commonly referred to as the "hump". In zone 2, chlorine reacts with ammonia and monochloramine to form dichloramine. However, dichloramine undergoes a series of reactions that result in both loss of chlorine and nitrogen. At the breakpoint, ammonia is oxidised to nitrogen gas and chlorine is

reduced to chloride in a series of complex reactions. In practice, an "irreducible minimum", or "nuisance residual", chlorine residual remains, typically a few tenths of a milligram per litre as Cl_2. The composition of this residual is still unknown, even though it has been extensively studied. When measured, it appears to be predominantly dichloramine, with traces of monochloramine and free chlorine, but also may include organic chloramines which present as dichloramine. In zone 3, to the right of the breakpoint, free chlorine increases in direct proportion to the dose of chlorine applied (White, 2010).

The shape of the curve is affected by contact time, temperature, concentration of chlorine and ammonia, and pH. Sometimes at pH values greater than 7 or 9, and if the measurement is taken after a short contact time, the hump or dip may appear as an inflection point in a continually rising curve (White, 2010).

The organic nitrogen component in water includes a variety of functional groups that can form numerous products upon reaction with chlorine. Free amino acids contain available amine groups that can be readily chlorinated. A review by Morris (1975) into the reactions of chlorine with amines, amides, amino acids, proteins, and heterocyclic compounds reported that the reaction between chlorine and these compounds is often rapid, especially for compounds having more basic nitrogen atoms. They also noted that amino acids, after first forming *N*-chloro derivatives, can undergo oxidative deamination to yield NH_2Cl or $NHCl_2$ and keto acids (Morris, 1975). Deamination results in the loss of organic nitrogen. This was consistent with several studies involving high doses of chlorine (Taras, 1953; Taras *et al.*, 1950).

In a study by Taras *et al.* (1950), chlorine (1.5 to 3 mg L^{-1}) was added to a range of amino acids and the chlorine demand was measured after 15 minutes. The chlorine demand, or chlorine consumed, was determined by subtracting the chlorine residual from the chlorine dose applied. They reported that the rate of chlorination of the amino acid was dependent upon the structure of the amino acid, where an increase in structural complexity, such as the introduction of an aromatic or heterocyclic ring, resulted in prolonged chlorine demand, *i.e.,* the chlorine was not consumed as quickly (Taras, 1953).

Studies by Griffin and Chamberlin (1941b) found that waters containing a mixture of ammonia and organic nitrogen do not display the classic dip of the breakpoint reaction, but instead display a plateau effect. Figure 4.3 illustrates this effect for a water containing 0.3 mg L^{-1} of NH_3-N and 0.3 mg L^{-1} of organic nitrogen, with approximately half of the organic nitrogen associated with simple amino acids. There is only a small drop in residual chlorine beyond the hump, which suggests continuing and competing reactions between monochloramine and dichloramine and little loss of nitrogen relative to what would occur in the absence of organic nitrogen, thus the plateau is observed. Beyond this, free chlorine residual begins to appear, but the irreducible minimum, or nuisance residual is considerably higher than when only inorganic nitrogen is present (as illustrated in Figure 4.2).

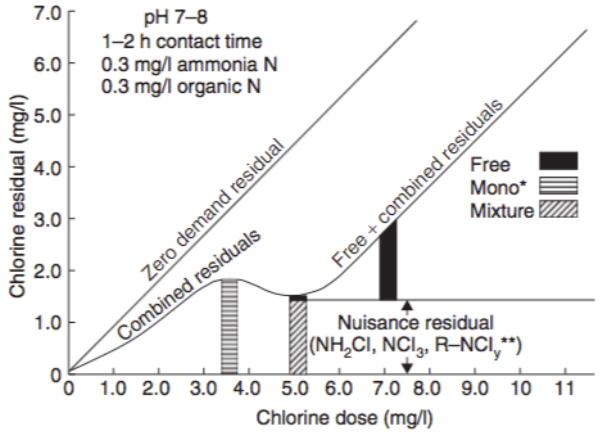


Figure 4.3: Breakthrough curve for water containing a mixture of ammonia and organic nitrogen. *Predominantly monochloramine at first, with dichloramine increasing over time. **R-NCl_y represents organic chloramines. Reprinted with permission from White (2010). Copyright John Wiley & Sons, Inc 2010.

4.1.2.1 Analysis of Chlorine Species in the Presence of Nitrogen Compounds

Like inorganic chloramines, organic chloramines also exist in equilibrium with free chlorine (as shown in Equations 4.1 - 4.3 for inorganic chloramines), but the equilibrium strongly favours organic chloramine formation in most cases (White, 2010). Since they contain active chlorine, organic chloramines can also be titrated as residual free chlorine using various methods of measurement. While they are usually titrated as dichloramine, they can also impact on the free chlorine measurement. However, when amino acids are present, the syringaldazine (SYRING) and diethyl-*p*-phenylene diamine (DPD) methods of measurement seem to be the most specific

for free chlorine (Wajon and Morris, 1980a). A study by Wajon and Morris (1980a) compared a range of available methods to measure free chlorine in the presence of organic nitrogen. They found it was only possible to measure free chlorine reliably when the type of organic nitrogen was known, and that in the presence of amino acids, the DPD or SYRING method should be used as they are most specific for free chlorine (Wajon and Morris, 1980a).

4.1.3 The General Reaction of Chlorine with Amino Acids

An important characteristic of amino acids is the active amine functionality, which can readily react with chlorine to form an assortment of by-products. Each amino acid has different chemical moieties that will be responsible for the production of various reaction products. It was in an effort to determine the health effects associated with disinfection of water and wastewater, that the reactions and by-products of the chlorination of amino acids have received some attention (Hureiki *et al.*, 1994; Trehy *et al.*, 1986), and a number of studies into the chlorination of specific amino acids were carried out in the late 1980s and early 1990s (Conyers and Scully Jnr, 1993; Conyers *et al.*, 1993; McCormick *et al.*, 1993; Nweke and Scully Jnr, 1989). Earlier work had previously investigated the rates of decomposition of intermediate products and established that the main final products identified were aldehydes and nitriles (Pereira *et al.*, 1973).

In fact, initial studies carried out by Langheld (1909) into the action of sodium hypochlorite on amino acids demonstrated the production of an aldehyde, carbon dioxide and ammonia. Langheld postulated that the first stage of the reaction was likely dependent on the formation of a monochloramino acid, which subsequently underwent decomposition, as shown below in Figure 4.4.

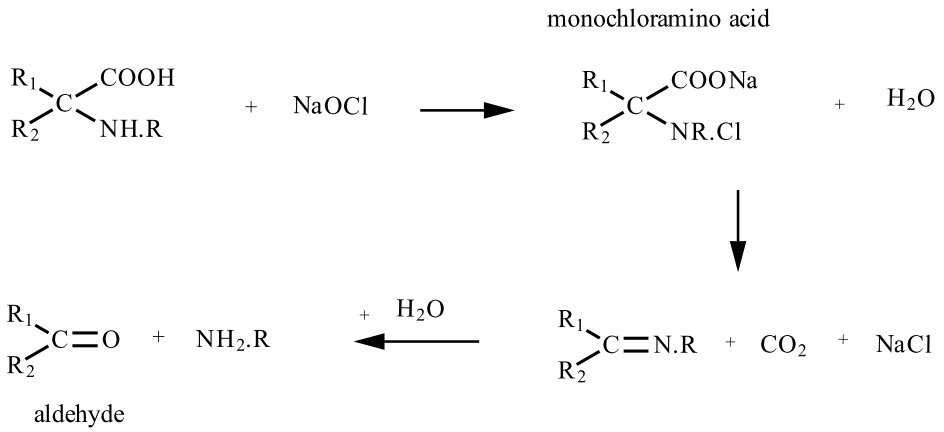


Figure 4.4: The pathway for the reaction of an amino acid with sodium hypochlorite (Langheld 1909).

Langheld isolated the monochloramino acid from the reaction of leucine with sodium hypochlorite as an unstable solid and obtained evidence of the production of a dichloramino acid when excess sodium hypochlorite was used (Dakin, 1916). Subsequent work by Dakin (Dakin *et al.*, 1916; Dakin, 1916) established that nitriles are produced by the decomposition of the dichloramino acids (Figure 4.5).

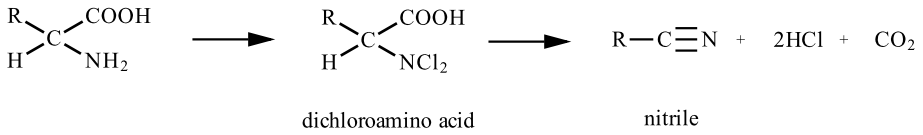


Figure 4.5: The pathway for the formation of a nitrile from the decomposition of a dichloramino acid (Dakin, 1916).

The formation of the aldehyde and nitrile species was therefore found to be dependent upon the ratio of sodium hypochlorite reagent to amino acid, *i.e.,* 1 mole equivalent of sodium hypochlorite converted the amino acids to their corresponding aldehyde, while if two mole equivalents of sodium hypochlorite were used, the corresponding nitrile was formed. Later studies by Pereira *et al.* (1973) investigated the reaction of 4 mmol of sodium hypochlorite with 2 mmol of phenylalanine, *i.e.* a two mole equivalent. Using GC-MS, they identified two products as phenylacetonitrile (95% of the reaction mixture) and phenylacetaldehyde (5% of the reaction mixture), thus confirming that hypochlorous acid, by oxidative decarboxylation, converts amino acids into a mixture of the corresponding nitriles (major products) and aldehydes (minor products) (Pereira *et al.*, 1973).

4.1.4 Chlorination of Specific Amino Acids

The chlorination of amino acids and resulting production of aldehydes was thought to be responsible for several taste and odour episodes (Bruchet *et al.*, 1992; Hrudey *et al.*, 1988), as discussed in Section 2.4.4. This led to a number of studies involving the chlorination of specific amino acids which had either been identified in natural water, or their resulting aldehyde had been identified in water treated with chlorine. This research identified the reactions of aqueous chlorine with amino acids and detailed pathways were proposed. These pathways are similar to those presented in Figures 4.4 and 4.5, however they identified another intermediate species prior to the production of the nitrile, which was thought to possibly be the cause of the off-flavour, as discussed below.

After a taste and odour episode in Paris, researchers treated phenylalanine (chosen for its physical and chemical properties and/or since the aromatic ring made it easy to detect by HPLC-UV with chlorine and monitored the by-products by HPLC over a period of time (Freuze *et al.*, 2004). The monochlorinated amino acid and then the aldehyde were the major products formed for lower chlorine to amino acid ratios. As this molar ratio increased above 1, the nitrile and an *N*-chloroaldimine appeared and increased as the ratio increased. The *N*-chloroaldimine species, as shown in Figure 4.6, had been earlier identified (and is discussed in further detail below) as another intermediate in the chlorination process in a series of three studies, but there had been difficulty in isolating and quantifying its presence (Conyers and Scully Jnr, 1993; McCormick *et al.*, 1993; Nweke and Scully Jnr, 1989). Freuze *et al.* (2004) initially suggested that the monochlorinated amino acid may be responsible for the odour problems, however this product dissociated prior to reaching consumer's taps. On the other hand, the *N*-chloroaldimine (*N*-chlorophenylacetaldimine) appeared to be very odorous, being a water-stable product with a strong swimming pool odour with a floral background (Freuze *et al.*, 2004). Freuze *et al.* (2004) determined its odour threshold to be about 3 μg L^{-1} and found that it could persist for more than one week at 18 °C. They therefore suspected this, and other *N*-chloroaldimine species, as being a source of off-flavour concerns amongst consumers (Freuze *et al.*, 2004).

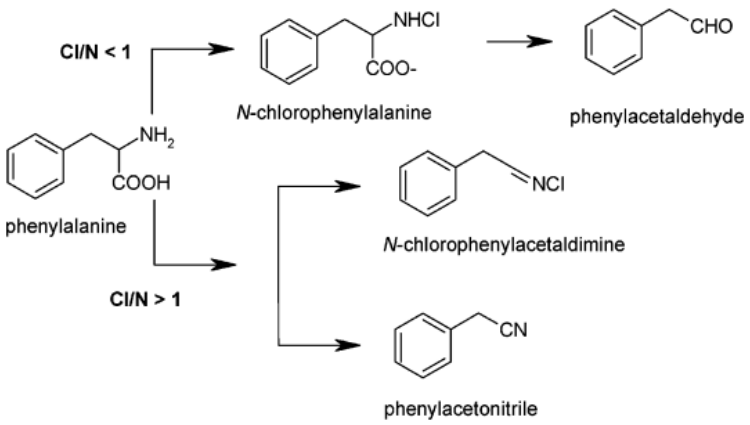


Figure 4.6: Proposed scheme for the chlorination of phenylalanine. Reprinted with permission from Freuze *et al.* (2004). Copyright 2004 American Chemical Society.

A study into the chlorination of isoleucine by Nweke and Scully (1989) observed that, as chlorine was added to isoleucine, *N*-chloroisoleucine (the monochloramino acid species) was formed. 2-Methylbutyraldehyde and 2-methylbutyronitrile, being the corresponding aldehyde and nitrile species, were also identified in the reaction mixture by headspace gas chromatographic techniques. Subsequent analysis of the reaction mixture by UV suggested that *N,N*-dichloroisoleucine formed (the dichloramino acid species), however this rapidly decayed to a *N*-chloroaldimine species. They also found that the aldehyde was the only volatile product detected at chlorine to nitrogen molar ratios of less than 1. The nitrile was detected at higher chlorine to nitrogen molar ratios, but the overall percentage conversion to aldehyde continued to increase at these higher ratios. Based on these observations, they proposed a reaction pathway (depicted in Figure 4.7) where the aldehyde was formed *via* the loss of CO_2 from the chloramine (I) species to produce an unstable imine which rapidly hydrolyzes to form the aldehyde (V). Analysis of the headspace of the chlorination mixture by GC-MS identified that further aldehyde was formed by the dechlorination of the *N*-chloroaldimine (IV) species to form imine (III) which in turn hydrolyses to the aldehyde (V) (Nweke and Scully Jnr, 1989).

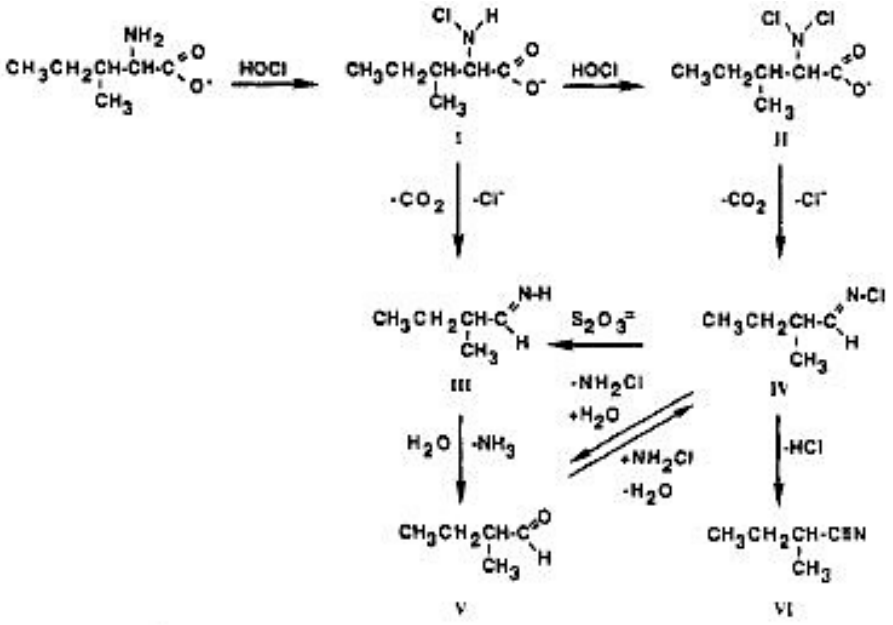


Figure 4.7: Proposed reaction scheme for the production of aldehydes and nitriles from chlorination of isoleucine. Reprinted with permission from Nweke and Scully Jnr (1989). Copyright 1989 American Chemical Society.

The chlorination of valine has also been studied (McCormick *et al.*, 1993). McCormick *et al.* (1993) identified *N*-chlorovaline (monochlorinated amino acid) and isobutyraldehyde as the main products at chlorine to nitrogen molar ratios of < 1, while isobutyronitrile and *N*-chloroisobutyraldimine (the *N*-chloroaldimine species) were the major products at chlorine to nitrogen molar ratios greater than 2. The final study in this series was of the chlorination of phenylalanine by Conyers and Scully Jnr (1993). Aqueous chlorine was found to react with phenylalanine to form *N*-chlorophenylalanine (monochloramino acid) which decomposed to form phenylacetaldehyde, phenylacetonitrile and *N*-chlorophenylacetaldimine, similar to the reaction pathways reported by Freuze *et al.* (2004) (Figure 4.6).

Each of these studies (Conyers and Scully Jnr, 1993; McCormick *et al.*, 1993; Nweke and Scully Jnr, 1989) confirmed the formation of an *N*-chloroaldimine species during the chlorination of amino acids, and essentially characterised a new class of disinfection by-products. *N*-Chloroaldimines form when amino acids react with 2 or more equivalents of chlorine (to amine nitrogen) to form *N,N*-dichloramino acids. These dichloramino acids are unstable and decompose to form nitriles and *N*-chloroaldimines (Conyers *et al.*, 1993). Conyers *et al.* (1993) identified, using gas chromatographic techniques, four *N*-chloroaldimine species in municipal

wastewaters. Since the actual compounds were not available in pure form, it was not possible to quantify them in the wastewaters. Also, due to the proposed thermal instability of some of the *N*-chloroaldimines, it was proposed that quantification might not be possible using the gas chromatographic method of Conyers *et al.* (1993). There was no information presented on the odour properties of the *N*-chloroaldimines in any of these studies.

Improvements in analytical techniques allowed Freuze *et al.* (2004) to successfully quantify the phenylalanine *N*-chloroaldimine species using HPLC and UV detection, after synthesising it in-house as no commercial standards were available. A further analytical method utilising GC-MS (to investigate more amino acids without an aromatic ring) was later developed to demonstrate the presence of such compounds in water at concentrations close to their odour detection threshold (Freuze *et al.*, 2005b). In this latter study, valine and leucine, as well as phenylalanine, were chlorinated. The three *N*-chloroaldimine species were also identified in water samples spiked with amino acids at the μg L^{-1} level, however the method was not able to quantify them.(Freuze *et al.*, 2005b).

To summarise, Figure 4.8 combines the findings of all of the abovementioned studies to give an overall reaction scheme for the chlorination of amino acids. Chlorination of the amino acid results in the formation of mono- and di-chlorinated amino acids (where the chlorine(s) is attached to the amine nitrogen). Decarboxylation, elimination and hydrolysis result in the formation of the final by-products, the aldehyde and nitrile species.

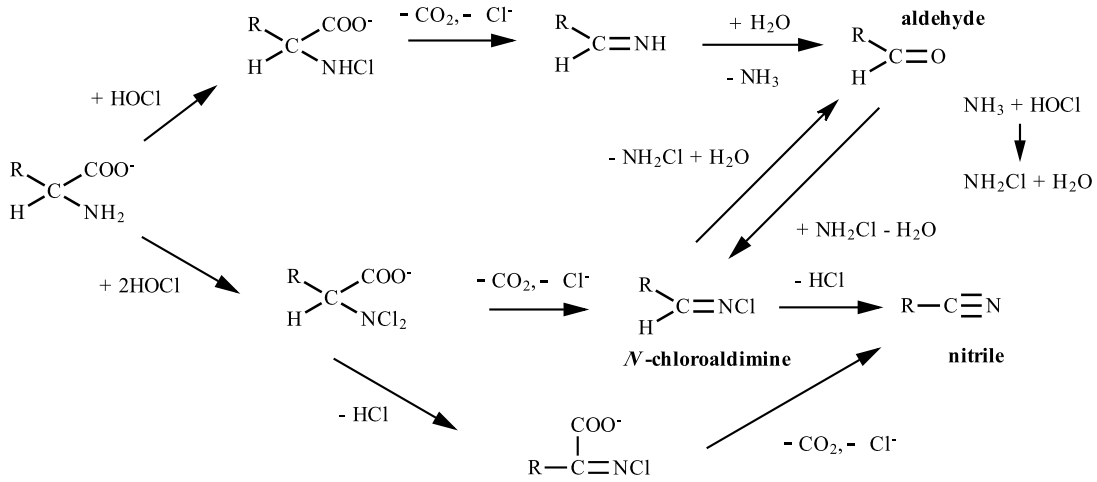


Figure 4.8: The reaction pathways for the chlorination of amino acids based on existing knowledge.

4.1.4.1 Kinetic Studies of the Chlorination of Specific Amino Acids

More extensive research on the chlorination of amino acids has focused on the formation of the corresponding *N*-chloramino acid (monochloramino acid) species (Abia *et al.*, 1994; Armesto *et al.*, 1993; Antelo *et al.*, 1988; Hand *et al.*, 1983; Stanbro and Smith, 1979). These studies all reported the decomposition kinetics of the *N*-chloramino acid species upon chlorination of the specified amino acid. A large range of amino acids have been studied, including, but not limited to alanine, leucine, valine and isoleucine. The kinetics in all studies were followed by UV spectroscopy by monitoring the decrease in the monochloramino acid. The decomposition rate was determined by preparing a mixture of the amino acid and chlorine and observing the decrease in absorbance over time at the wavelength of maximum absorbance of the monochloramino acid.

Stanbro and Smith (1979) followed the kinetics of chlorination of alanine using chlorination at a molar amino acid to chlorine ratio of 6:1. The concentration of the *N*-chloroalanine was monitored by measuring absorbance at 250 nm (previously determined to be the maximum absorbance for this species). By plotting the logarithmic concentration of the *N*-chloroalanine against time, the decomposition kinetics of the *N*-chloroalanine appeared to be first order (Stanbro and Smith, 1979).

Antelo *et al.* (1988) reported the decomposition kinetics of the chlorination of leucine. *N*-Chloroleucine (the monochloramino acid species) was rapidly formed upon chlorination of leucine and monitored by UV at 250 nm. The molar extinction

coefficient (ε) for *N*-chloroleucine was found to be 350. The rate constant for the decomposition of *N*-chloroleucine was reported to be 3.20 x 10^{-4} s^{-1} over a pH range of 5 – 12, with concentrations of 0.002 M chlorine and 0.003 M leucine. In another study by this group, Abia *et al.* (1994) reported the decomposition kinetics of *N*-chlorovaline. *N*-Chlorovaline was formed upon chlorination of valine and its concentration monitored by UV at 255 nm. The molar extinction coefficient (ε) was determined to be 345. The reaction rate was also deemed to follow first order kinetics, where the rate of reaction was equal to the rate constant (*k*) multiplied by the concentration of the monochloramino acid and *k* was reported to be 1.8 x 10^{-4} s^{-1} at 25 °C for a pH range of 4-13 (Abia *et al.*, 1994).

4.1.5 Reaction of Sodium Hypobromite with Amino Acids

Amino acids react similarly with sodium hypobromite (NaOBr), to form aldehydes and nitriles, as they do with chlorine (Wajon and Morris, 1982; Wajon and Morris, 1980b; Friedman and Morgulis, 1936). NaOBr reacts with water to form HOBr which then reacts with ammonia and many nitrogenous compounds to form *N*-bromoamines. The reactions with ammonia are depicted in Equations 4.4 to 4.6 (Wajon and Morris, 1982).

$$HOBr + NH_3 \leftrightarrows NH_2Br + H_2O \qquad \textbf{Equation 4.4}$$

$$HOBr + NH_2Br \leftrightarrows NHBr_2 + H_2O \qquad \textbf{Equation 4.5}$$

$$HOBr + NHBr_2 \leftrightarrows NBr_3 + H_2O \qquad \textbf{Equation 4.6}$$

A comprehensive study of the bromination of amino acids by Friedman and Morgulis (1936) reported that the formation of bromamino acids is very rapid, and that they have half-lives of under one minute.. At higher pH, aldehyde formation was found to be favoured. Friedman and Morgulis (1936) postulated that, in an alkaline solution, there is greater tendency for dehydrohalogenation of the brominated amino group, losing HBr, and forming the imine, hydrolysis of which forms the aldehyde. In less alkaline solutions, there is a greater tendency for the formation of the dibromo substitution product and subsequent nitrile formation. Also, the longer the carbon chain of the amino acid, the greater the amount of nitrile formed (Friedman and Morgulis, 1936). They proposed the following reaction scheme for the oxidation of amino acids by sodium hypobromite (Figure 4.9).

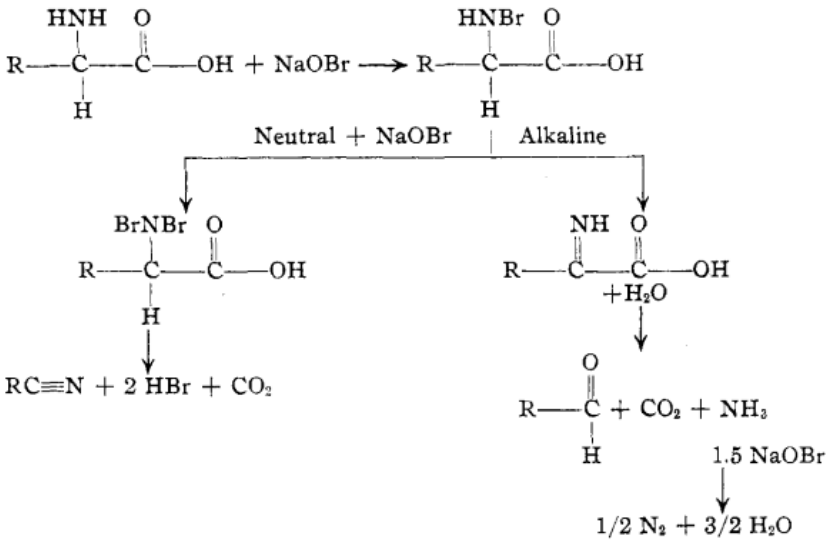


Figure 4.9: Reaction scheme for the bromination of amino acids. Reprinted with permission from Friedman and Morgulis (1936). Copyright 1936 American Chemical Society.

From later studies (Johnson and Overby, 1971; Galal-Gorchev and Morris, 1965) on the conditions of formation of inorganic bromamines, it was reported that the inorganic bromamines form more rapidly than do the inorganic chloramines (Wajon and Morris, 1980b).

4.1.5.1 Kinetic Studies of the Bromination of Specific Amino Acids

Research into the bromination of amino acids has also focused on the formation of the corresponding *N*-bromamino acid species (Wajon and Morris, 1982; Wajon and Morris, 1980b). Kinetic studies followed the reaction of sodium hypobromite with ammonia and a number of amino acids by monitoring the disappearance of OBr^- or the appearance of the *N*-bromo products, spectrophotometrically. Wajon and Morris (1982) measured the rates of formation of bromamines and bromamino acids and found that they were formed within 2-200 milliseconds between pH 7 and 13. They also found that bromamines and bromamino acids decompose rapidly, with half-lives of 0.2 to 20 hours (Wajon and Morris, 1982).

Decomposition kinetics of the monobromamino acids formed upon bromination of several amino acids have also been studied (Antelo *et al.*, 1993; Antelo *et al.*, 1991). The monobromamino acid species were measured spectrophotometrically by monitoring the absorbance at the wavelength of maximum absorbance for the corresponding monobromamino acid species (Antelo *et al.*, 1993; Antelo *et al.*,

1991). Chlorination of the amino acid was also carried out for some of these amino acids, and the monochloramino acid species were also measured. From these measurements, the rate constants for the first order decomposition of the bromo/chloro-amino acids were calculated and are shown in Table 4.3. From the pH range of these experiments, it was proposed that the decomposition reaction would take place entirely through decarboxylation of the *N*-bromamino acid to yield an imine which rapidly converts to an aldehyde (Antelo *et al.*, 1993). The same process was proposed to occur for the *N*-chloramino acids, however it was expected that the *N*-bromamino acids would degrade at a faster reaction rate (due to the dissociation energy of the N-Br bond being 272 kJ mol^{-1}, as opposed to N-Cl being 385 kJ mol^{-1}) (Antelo *et al.*, 1993).

Table 4.3: Rate constants for the first order decomposition of the monobromo/chloro-amino acids.

Monohalo derivatives of Amino Acids	k_{Cl} / s^{-1}	k_{Br} / s^{-1}	**pH**	**Ref**
Alanine	2.67×10^{-4}	1.2×10^{-3} (1)	9-11	Antelo *et al.* (1993)
2-Aminobutyric acid		1.37×10^{-3} (1)	9-11	Antelo *et al.* (1993)
Norvaline		1.28×10^{-3} (1)	9-11	Antelo *et al.* (1993)
Leucine	3.2×10^{-4}	1.27×10^{-3} (1)	9-11	Antelo *et al.* (1993)
Isoleucine		7.5×10^{-4}	9-12	Antelo *et al.* (1991)
Serine	2.97×10^{-4}	1.67×10^{-3} (1 & 2)	9-11	Antelo *et al.* (1993)
Glutamic Acid	4.5×10^{-4}	2.06×10^{-3} (1)		Antelo *et al.* (1993)

Conditions:

[1] [AA] = 0.02M, [BrO^-] 1.8×10^{-3}M, pH 9-11

[2] [AA] = 0.2M, [BrO^-] 1.9×10^{-2}M

4.1.6 Scope of Work in Chapter 4 of this Study

As outlined in Sections 1.7 and 2.6, the first part of this Thesis focused on the investigation of certain chlorination by-products as potential source analytes in chlorinous off-flavour incidents. Previous studies (Bruchet *et al.*, 1992; Hrudey *et al.*, 1988) identified a range of specific compounds, namely aldehydes whose presence was found to have arisen as a result of chlorination. Accordingly, the aim of the study described in this Chapter was to investigate the chlorination reactions of four specific amino acids- valine, isoleucine, leucine, and phenylalanine. The presence and subsequent chlorination by-products of these amino acids have been

previously implicated in taste and odour episodes (Bruchet *et al.*, 1992; Hrudey *et al.*, 1988), and which have also been found in Australian waters (How *et al.*, 2014). Breakpoint chlorination experiments were undertaken and the formation of the corresponding aldehyde and nitrile species (structures shown later in Figure 4.10) was measured. Due to the occurrence of naturally occurring bromide in Australian waters, the effect of the presence of bromide ion on the production of these by-products was also investigated. Previous studies had investigated the action of bromine as the oxidant on the amino acid, rather than chlorine, however they had not looked at naturally occurring concentrations of bromide during the chlorination process. The reaction pathways for the chlorination of the three more simple (in terms of structure) amino acids were explored by determining the rate constants for the decomposition of the monochlorinated amino acid intermediates and comparing these to the rate constants for formation of the corresponding aldehyde species. The analytical method developed in Chapter 3 was used for the analysis of the aldehyde and nitrile by-products. Odour analysis of the respective aldehydes was also undertaken to determine the appropriate odour description of these by-products.

4.2 Experimental

4.2.1 Chemicals and Reagents

Inorganic reagents, organic solvents, and organic compounds were of analytical grade purity (Technical grade ≥ 90% pure) or better, and were used without further purification. The following chemical solutions were prepared:

pH 7 buffer: 3.53g Na_2PO_4 (AR, Univar Ajax) and 3.4 g KH_2PO_4 (AR, BDH) in 500 mL of MilliQ water.

pH 9 buffer: 7.2 g borax (AR, BDH) and 600 µL HCl (10.2 M, AR, Univar) in 500 mL of MilliQ water.

Bromide solution: 0.113 g KBr (AR, ChemSupply) in 250 mL of MilliQ water.

Chlorine solution: 10 mL of NaOCl (12.5%, technical grade, APS Ajax) in 250 mL of MilliQ water.

*Quenching agent***:** 5.6 g sodium thiosulfate in 100 mL of MilliQ water.

4.2.2 Chlorination of Amino Acid Experiments

Individual stock solutions of amino acids were prepared as follows: 0.53 g valine (≥ 99%, Fluka) in 50 mL of MilliQ water; 0.58 g isoleucine (≥ 99%, Fluka) in 50 mL of MilliQ water, 0.74 g of leucine (≥ 99%, Fluka) in 50 mL of MilliQ water, and 0.75 g phenylalanine (98 %, Aldrich) in 50 mL of MilliQ water. 5 to 10 drops of HCl (10.2 M, AR, Univar) was added to each solution.

Chlorination of amino acid experiments were carried out at pH 7 and pH 9 with and without the addition of bromide. For valine, isoleucine, leucine, and phenylalanine, chlorination experiments were carried out at the following Cl_2 to N molar ratios: 0.2, 0.8, 1.6, 2.4 and 4. The concentrated NaOCl solution was prepared and its concentration checked using a Hach Pocket Colorimeter prior to chlorination of the amino acid solutions. The required aliquots to achieve the desired molar ratios were determined at this stage.

An aliquot (200 µL) of the amino acid stock solution was buffered to pH 7 (25 mL buffer solution) or 9 (13.5 mL buffer solution) using the appropriate buffer solution. Bromide (0.3 mg L^{-1}), if required, was then added (500 µL of stock solution). The required volume of NaOCl solution was then added to the reaction solution and the solution was quickly made up to 500 mL with MilliQ water and allowed to stand in the dark at room temperature. After an hour, and then 24 hours, an aliquot of the reaction mixture was collected and the residual oxidant quenched with aqueous sodium thiosulfate solution (750 µL of stock solution) and the solution stored at 4 °C for further analysis. At the time of sample collection, the concentration of free and total chlorine equivalents was measured using a Hach Pocket Colorimeter (DPD Free and Total Chlorine). Some of these reaction solutions also had odour testing carried out on them using an Odour Panel.

4.2.2.1 Kinetic Experiments

Kinetic experiments were conducted on valine, isoleucine, and leucine at pH 7 and at an amino acid to chlorine molar ratio of 5:1. An aliquot (200 µL) of the amino acid stock solution was buffered to pH 7 (25 mL buffer solution). The required volume of NaOCl solution was then quickly added by syringe to the reaction solution and the solution was quickly made up to 500 mL with MilliQ water and allowed to stand in

the dark at room temperature. An aliquot of the reaction solution was taken, and at ten different time intervals over one hour, the UV absorbance of the reaction solution was measured at 256 or 250 nm. Separate aliquots of the reaction mixture were removed and quenched with aqueous sodium thiosulfate solution (500 µL of stock solution) and the quenched solution stored at 4 °C for further analysis.

The UV absorbance of the water samples was determined used a HP 8452A diode array spectrophotometer with a 5 cm quartz cell. Background measurements were done using MilliQ water.

4.2.3 Sensory Analysis

4.2.3.1 Odour Panel

An Odour Panel was established. This panel consisted of staff and students of the Department of Chemistry (Curtin University) and staff from the Water Corporation of Western Australia. Panellists covered a range of ages and genders and were trained to describe odours and their intensities in accordance with the Standard Method for Flavour Profile Analysis (APHA, 2012) described in Section 1.4.1.1. Common odour descriptors used included earthy, musty, chlorinous, hay/sweet, septic and solventy/alcohol.

Once trained, the panellists were assigned to detect the presence or absence of chlorinous and/or other odours in standard aqueous solutions of the aldehydes studied in this Thesis and three reaction solutions. Panellists received a 250 mL wide mouthed conical flask containing 100 mL sample at room temperature. They analysed the samples by holding the bottom of the flask, swirling the contents, removing the glass lid and then smelt the odours produced from the solution. The panellists recorded their findings on a survey sheet, which included the order in which the odour(s) were detected and the intensity of the odour(s). The intensity of the odour was described according to Table 4.4. Panellists smelt up to five samples during any one panel event and were encouraged to smell a piece of blank paper in between each sample.

Table 4.4: Intensity rating scale for odours in drinking water for the odour panel.

Rating	Description
-	Odour free
T	Threshold
2	Very Weak
4	Weak
6	Weak to Moderate
8	Moderate
10	Moderate to Strong
12	Strong

4.2.3.2 Preparation of Odour Free Water for Odour Analysis

Odour free water was prepared by filtering MilliQ water through a bed of activated carbon and was used as a 'blank' for odour analyses by the panel. This water was stored in a glass holding tank fitted with a tap.

4.2.3.3 Preparation of Standard Solutions of Aldehydes for Odour Analysis

Individual stock solutions of aldehydes were prepared by adding the aldehydes (0.0516 g of 2-methylbutyraldehyde (>90%); 0.0506 g of isobutyraldehyde (99%); 0.0487 g of 3-methylbutyraldehyde (97%); and 0.0489g of phenylacetaldehyde (>90%); Sigma Aldrich) to 10 mL of HPLC grade methanol (Mallinckrodt Baker). These stock solutions were then further diluted to prepare individual working standards where an aliquot of each aldehyde stock solution (50 µL) was made up to 100 mL with MilliQ water to give a concentration of 25 µg L^{-1} for each aldehyde.

Odour free water (1 L) was added to glass bottles (prepared according to Section 4.2.3.2) with ground glass stoppers. An aliquot (250 µL) of the aldehyde working standard was quickly added and the bottle was capped and swirled and left at room temperature until the time of odour analysis. For each panellist, approximately 100 mL of the solution was then quickly poured into a wide mouthed conical flask (250 mL), and a glass lid placed on top to prevent loss of volatile compounds, for immediate analysis.

4.2.3.4 Preparation of Chlorination of Amino Acid Mixtures for Odour Analysis

Odour analysis was performed on separate solutions of leucine, isoleucine and valine at pH 7 at a Cl_2 to N molar ratio of 1:2. An aliquot (200 µL) of the amino acid stock solution (described in Section 4.2.2) was buffered to pH 7 (25 mL). An aliquot

(580 μL) of the standard chlorine solution was then added to the reaction solution and the solution was quickly made up to 500 mL with MilliQ water and allowed to stand in the dark at room temperature. The solution was prepared in a glass bottle with a ground glass stopper. After approximately one hour, for each panellist, 100 mL of the solution was then quickly poured into a wide mouthed conical flask (250 mL), and a glass lid placed on top to prevent loss of volatile compounds, for immediate analysis. The concentration of free and total chlorine equivalents was also measured after one hour reaction time, using a Hach Pocket Colorimeter (DPD Free and Total Chlorine).

4.2.4 Analysis of Aldehyde and Nitriles

Aldehydes and nitriles were extracted from the reaction mixture samples *via* automated HS SPME using a 75 μm CAR-PDMS fibre, followed by GC-MS. Details of the analytical method and instrumentation are given in Section 3.2.4. All samples were analysed within 14 days of collection.

The standard solutions, solvents and chemical solutions used for the analysis of aldehyde and nitriles are the same as those described in Section 3.2.4.

4.3 Results and Discussion

The formation of odorous aldehydes and nitriles upon chlorination of amino acids was evaluated. Chlorination experiments at varying chlorine concentrations were carried out on four specific amino acids (valine, isoleucine, leucine, and phenylalanine) to construct breakpoint curves. The concentrations of respective aldehydes and nitriles formed after chlorination were measured for each experiment. The effect of bromide ion on breakpoint and by-product formation at a concentration relevant to natural waters was studied. The structures of the amino acids and their corresponding aldehyde and nitrile species are shown in Figure 4.10.

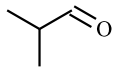
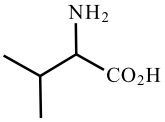
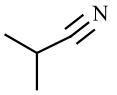
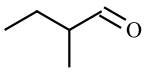
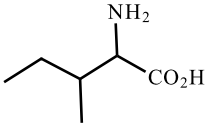
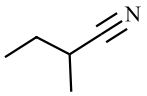
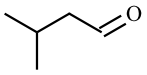
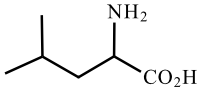
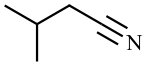
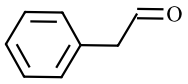
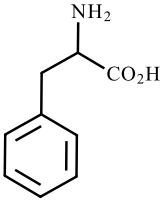
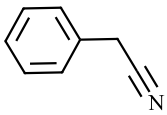
Aldehyde	Amino Acid	Nitrile
 Isobutyraldehyde (IBA)	 Valine	 Isobutyronitrile (IBN)
 2-Methylbutyraldehyde (2-MBA)	 Isoleucine	 2-Methylbutyronitrile (2-MBN)
 3-Methylbutyraldehyde (3-MBA)	 Leucine	 3-Methylbutyronitrile (3-MBN)
 Phenylacetaldehyde (PA)	 Phenylalanine	 Phenylacetonitrile (PN)

Figure 4.10: Amino acids, and their corresponding aldehyde and nitrile species, used in this study.

4.3.1 Chlorination of Amino Acids with and without Bromide

Valine, isoleucine, leucine, and phenylalanine, at concentrations of 0.5 mg L^{-1} as nitrogen, were treated with chlorine (initial concentrations ranging from 0.5 to 10 mg L^{-1}) to achieve chlorine to amino acid molar concentration ratios of 0.2, 0.8, 1.6, 2.4 and 4. The experiments were carried out at pH 7 and 9 and also in the presence and absence of 0.3 mg L^{-1} bromide ion.

4.3.1.1 Free and Total Chlorine Equivalent Residuals

After one hour reaction time, free and total chlorine equivalent measurements, measured as chlorine, were made for each experiment (Figures 4.11 and 4.12, respectively). Each of the reaction mixtures had a strong chlorinous odour. The formation of *N*-chloramino acid species was identified by the fact that concentrations of the total chlorine equivalent were greater than the free chlorine equivalent for each experiment. Total chlorine refers to chlorine that is combined with ammonia or organic nitrogen to form chloramines, which are to be expected based on the reaction scheme in Figure 4.8. In this study, each amino acid displayed similar breakpoint

curves (Figures 4.11 and 4.12), at each pH. However, pH influenced the reaction of the amino acids noticeably, with different free and total chlorine equivalent concentrations being recorded for the same initial dose concentrations at each pH. The breakpoint curves for each amino acid, showing both free and total chlorine equivalents, are also plotted in Figures 4.16 to 4.19.

At pH 9 (blue and red lines in Figures 4.11 and 4.12), a greater concentration of both free and total chlorine equivalents can be observed for all amino acids, in comparison to pH 7 (orange and green lines in Figures 4.11 and 4.12). As the ratio of chlorine to amino acid was increased, there was also a steep increase in the concentration of both free and total chlorine equivalents. In terms of total chlorine, this represents a greater amount of chloramino acid being produced at pH 9 in comparison to pH 7, or that the destruction of the chloramino acid happens more readily at the lower pH, resulting in its lowered concentration. This is likely to be as a result of the presence of a greater proportion of the effective chlorinating agent, HOCl, at pH 7 (pKa of 7.54), compared to pH 9, where the weaker OCl^- predominates (Rook *et al.*, 1978).

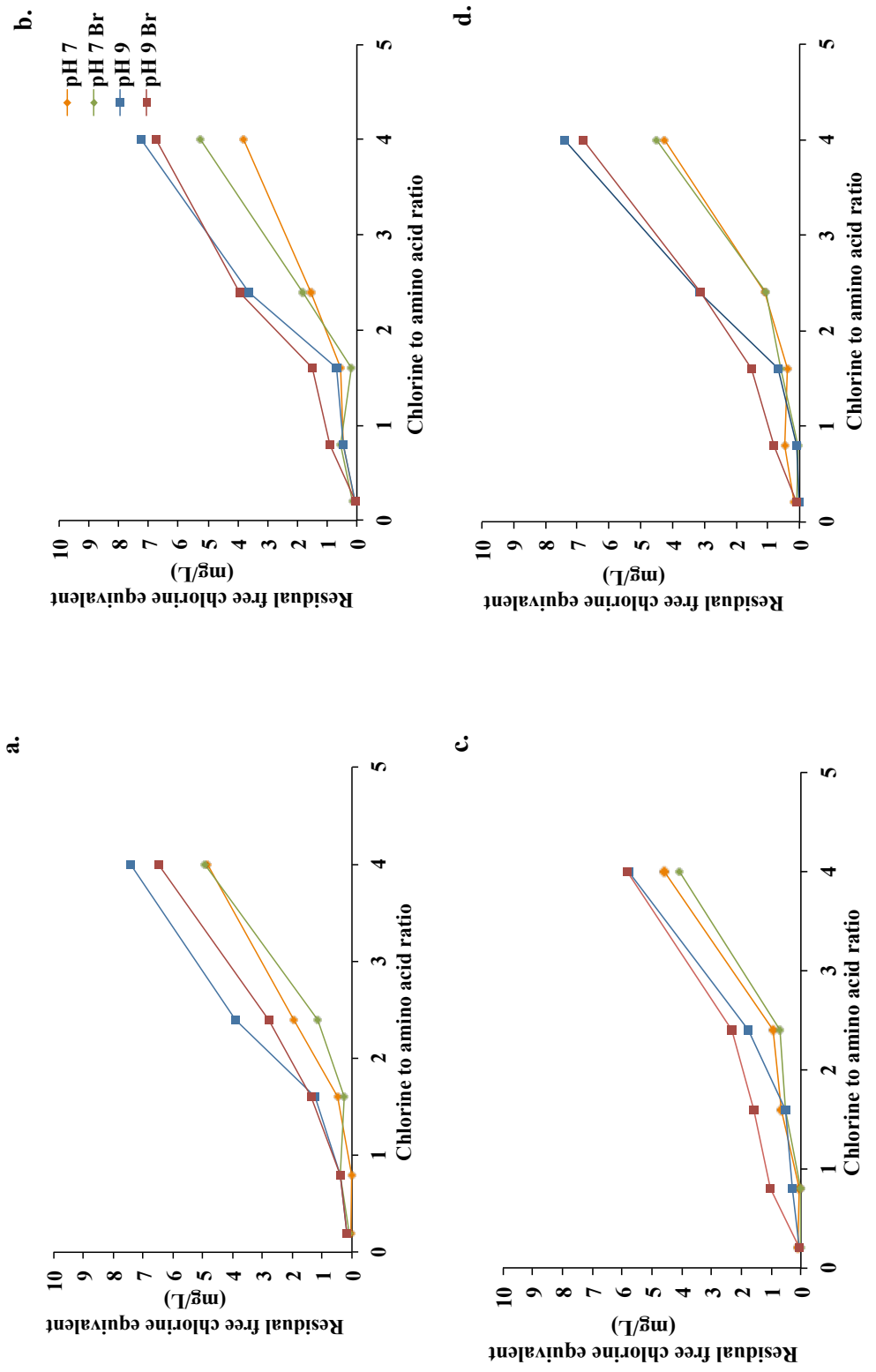


Figure 4.11: Residual free chlorine equivalent concentrations following chlorination of amino acids a) valine b) isoleucine c) leucine and d) phenylalanine; at pH 7, pH 7 + Br, pH 9 and pH 9 + Br, after one hour reaction time.

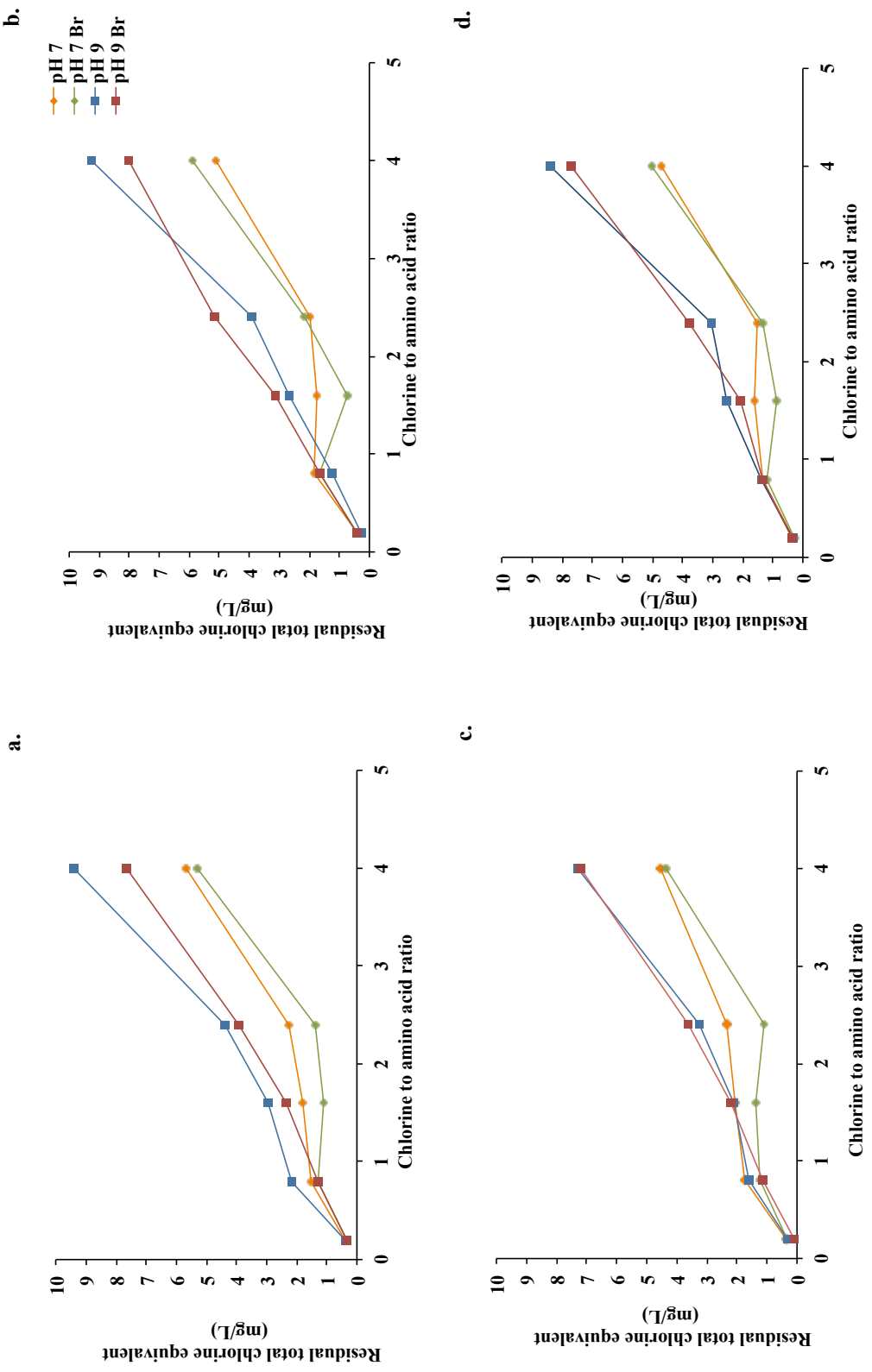


Figure 4.12: Residual total chlorine equivalent concentrations following chlorination of amino acids a) valine b) isoleucine c) leucine and d) phenylalanine; at pH 7, pH 7 + Br, pH 9 and pH 9 + Br, after one hour reaction time.

At the higher pH, a greater amount of the nitrogen group of the amino acid will be protonated, based on the pK_b values for each amino acid being within the range of pH 9-10, making it more susceptible to chlorination (Table 4.5).

Table 4.5: Dissociation constants of amino acids at 25 °C at zero ionic strength (Sharma, 2012).

Amino Acid	pK_a (COOH)	pK_b (NH_2)
Isoleucine	2.32	9.76
Leucine	2.58	9.93
Valine	2.28	9.54
Phenylalanine	2.28	9.34

At pH 9, bromide ion appeared to have little impact on the free and total chlorine equivalent concentrations for each of the amino acids, with similar residuals regardless of its presence. Low concentrations of bromine may have been present in the reaction mixtures due to the addition of the bromide ion. However, it is unlikely to have had an impact on the breakpoint phenomenon due to its low concentration, relative to chlorine.

In the experiments at pH 7 (orange and green lines in Figure 4.12), the total chlorine equivalent concentration plateaued between chlorine to amino acid ratios of 0 to 2.5, followed by a sharp increase after 2.5, which also correlates to an increase in the free chlorine equivalent concentration. This trend can be explained by the breakpoint phenomenon. Breakpoint is based upon the three reactions detailed earlier (Equations 4.1 to 4.3). These reactions compete with one another, and are dependent upon a number of factors. The latter two reactions (Equations 4.2 and 4.3) relate to the breakpoint phenomenon.

While the plots/curves of chlorine concentration versus chlorine to amino acid N ratio do not show the typical breakpoint hump for the chlorination of inorganic nitrogen (Figure 4.2), they follow the trend expected for organic nitrogen chlorination (Figure 4.3). An increase in both free and total chlorine equivalent residual can be observed, corresponding with the amount of excess chlorine not consumed during the formation of the aldehydes and nitrile species, once all of the mono- and di-chloramino species have been converted to the corresponding aldehyde or nitrile species. This sharper increase in free and total chlorine equivalent, correlating to the breakpoint for each of the amino acids, tended to occur at a

chlorine to nitrogen molar ratio of between 1.5 to 2.5 for all amino acids, regardless of the presence or absence of bromide. This is consistent with the requirement of a molar ratio of greater than one for the formation of the nitrile species, as well as the aldehyde, which upon its formation would release more chlorine back into the reaction mixture (with the reaction scheme depicted in Figure 4.8).

The free chlorine demand for each amino acid was also calculated, based on the moles of initial free chlorine equivalent dose minus the moles of final free chlorine equivalent, divided by the moles of amino acid, and the results are shown in Table 4.6. This is the first report of such a range of different molar ratios and pH values for these amino acids.

Similar chlorine demands were exhibited for valine, isoleucine and phenylalanine at each chlorine to amino acid ratio and pH. The effect of pH was noticeable, with each amino acid consuming approximately twice as much chlorine at pH 7 in comparison to pH 9, at Cl:N > 2. In the case of leucine, there was only a slightly lower consumption at pH 9. The large differences between pH 7 and pH 9 chlorine consumption are likely to be due to the relative oxidisability of the chloramines, diimides, nitriles and aldehydes at these pHs, as large differences were only found at Cl:N ratios > 2 when most of the reactants had been converted to /were aldehydes and nitriles, and nearly all of the extra added HOCl was being used for oxidation, not N-chlorination.

Table 4.6: Free chlorine demand of valine, leucine, isoleucine and phenylalanine following chlorination at pH 7 and 9 and in the presence and absence of bromide ion (0.3 mg L^{-1}) at chlorine to amino acid ratios of 0.2, 0.8, 1.6, 2.4 and 4 after one hour reaction time.

Amino Acid	pH	Chlorine to amino acid ratio	Chlorine demand	Chlorine demand in the presence of bromide
Valine	7	0.2	0.18	0.16
		1	0.79	0.63
		1.6	1.40	1.48
		2.4	1.60	1.92
		4	2.04	2.01
Valine	9	0.2	0.13	0.13
		1	0.65	0.63
		1.6	1.10	1.05
		2.4	0.84	1.28
		4	1.03	1.39
Isoleucine	7	0.2	0.17	0.14
		1	0.61	0.57
		1.6	1.36	1.51
		2.4	1.76	1.66
		4	2.46	1.88
Isoleucine	9	0.2	0.17	0.18
		1	0.61	0.42
		1.6	1.31	1.00
		2.4	0.94	0.81
		4	1.09	1.28
Leucine	7	0.2	0.15	0.20
		1	0.77	0.78
		1.6	1.33	1.39
Leucine	7	2.4	2.00	2.10
		4	2.14	2.36
Leucine	9	0.2	0.18	0.18
		1	0.67	0.38
		1.6	1.39	0.96
		2.4	1.68	1.46
		4	1.66	1.64
Phenylalanine	7	0.2	0.13	0.16
		1	0.60	0.78
		1.6	1.43	1.35
		2.4	1.94	1.96
		4	2.29	2.18
Phenylalanine	9	0.2	0.19	0.16
		1	0.76	0.47
		1.6	1.31	0.99
		2.4	1.13	1.15
		4	1.03	1.27

These results are consistent with a study carried out on the same amino acids, but instead only a single chlorine to amino acid molar ratio of 8 and a single pH (8) was

used (Hureiki *et al.*, 1994). In this instance, chlorine demands ranging from 2.6 to 2.8 where observed for each amino acid. The chlorine demands observed in this study, at the conditions most similar (pH 7, chlorine to amino acid ratio of 4:1) to the study carried out by Hureiki *et al.* (1994), ranged from 2.04 to 2.46. These slightly lower values are expected due to the lower chlorine dose. Hureiki *et al.* (1994) reports that chlorine demand is related to the structure of the amino acid, and that since phenylalanine has a non-activated aromatic ring, it behaves similarly to the neutral, non-aromatic amino acids like valine, leucine and isoleucine.

In the case of leucine, where there is little difference in chlorine demand at pH 9 in comparison to pH 7, this could be due to the difference in structure of leucine in comparison to valine and isoleucine. Leucine does not have a methyl group attached to the carbon chain at C-3.

4.3.1.2 The Formation of Aldehydes and Nitriles upon Chlorination of the Amino Acids

Each amino acid was allowed to react with chlorine in different molar ratios (as described in Section 4.3.1), where after the one hour contact time and measurement of free and total chlorine equivalent concentrations, the oxidant residual was quenched and the sample analysed for the corresponding aldehyde and nitrile species for each amino acid. Figures 4.13 a-d and 4.14 a-d display the concentrations of the respective aldehydes and nitriles formed from chlorination of the amino acids at pH 7 and pH 9, and in the presence of bromide, at various chlorine to amino acid ratios.

Differences in concentrations of aldehydes and nitriles formed from the different amino acids, pH values, and also the presence or absence of bromide ion can be observed. The concentration of nitrile species formed in all cases was less than the aldehydes; also, the nitrile species were not formed unless a chlorine to amino acid ratio of 1 was used. This result supports the original reaction mechanism proposed by Dakin (1916), where the formation of the nitrile is dependent upon the production of the dichloramine species, which will not occur until the ratio of chlorine to nitrogen is equal to or greater than 2, or in the case of the chlorine to amino acid ratio, the production will not occur until a molar ratio of 1 is achieved (Bruchet *et al.*, 1992).

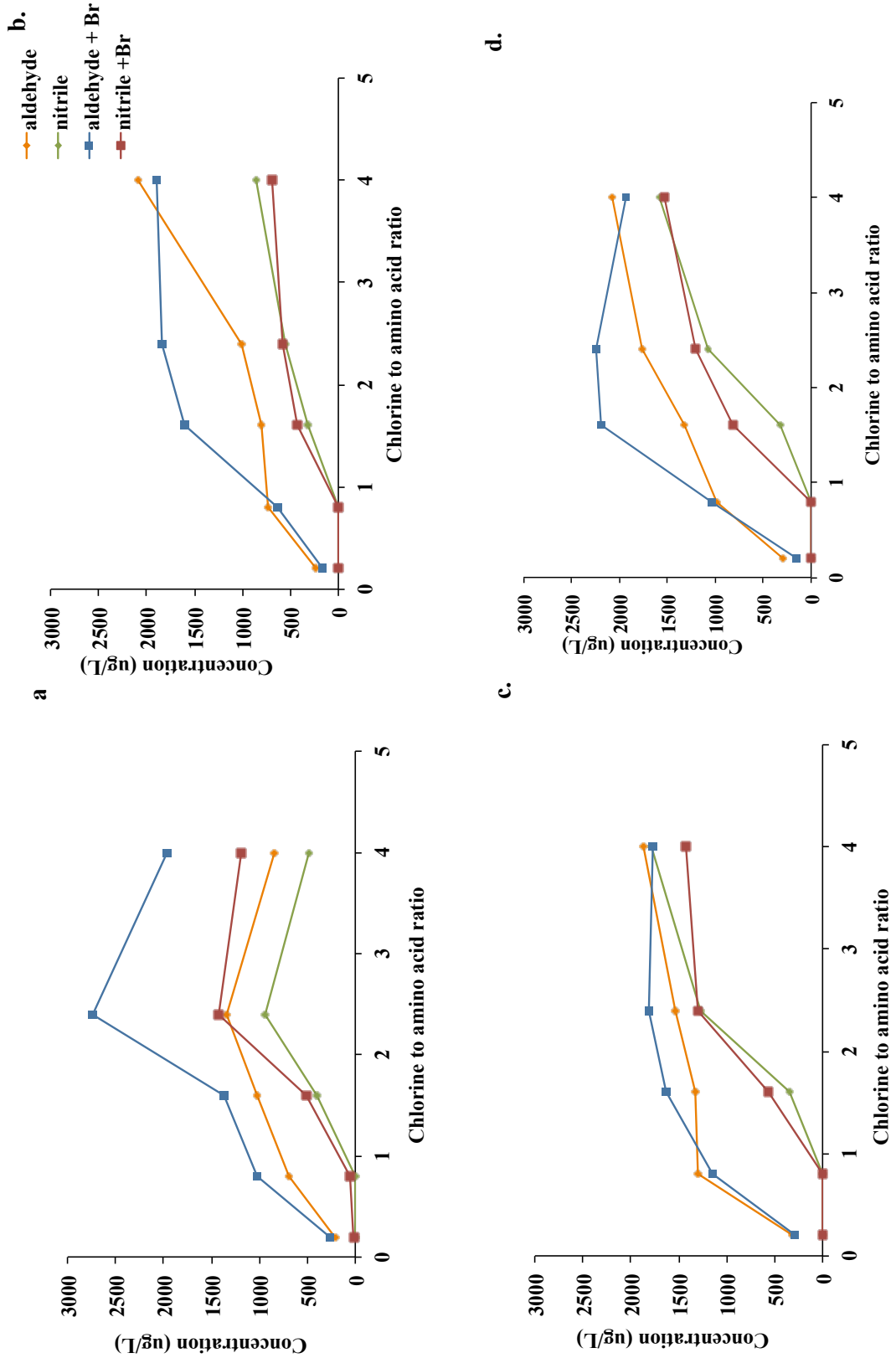


Figure 4.13: Aldehyde and nitrile concentrations following chlorination of amino acids a) valine b) isoleucine c) leucine and d) phenylalanine; at pH 7 and pH 7 + Br, after one hour reaction time.

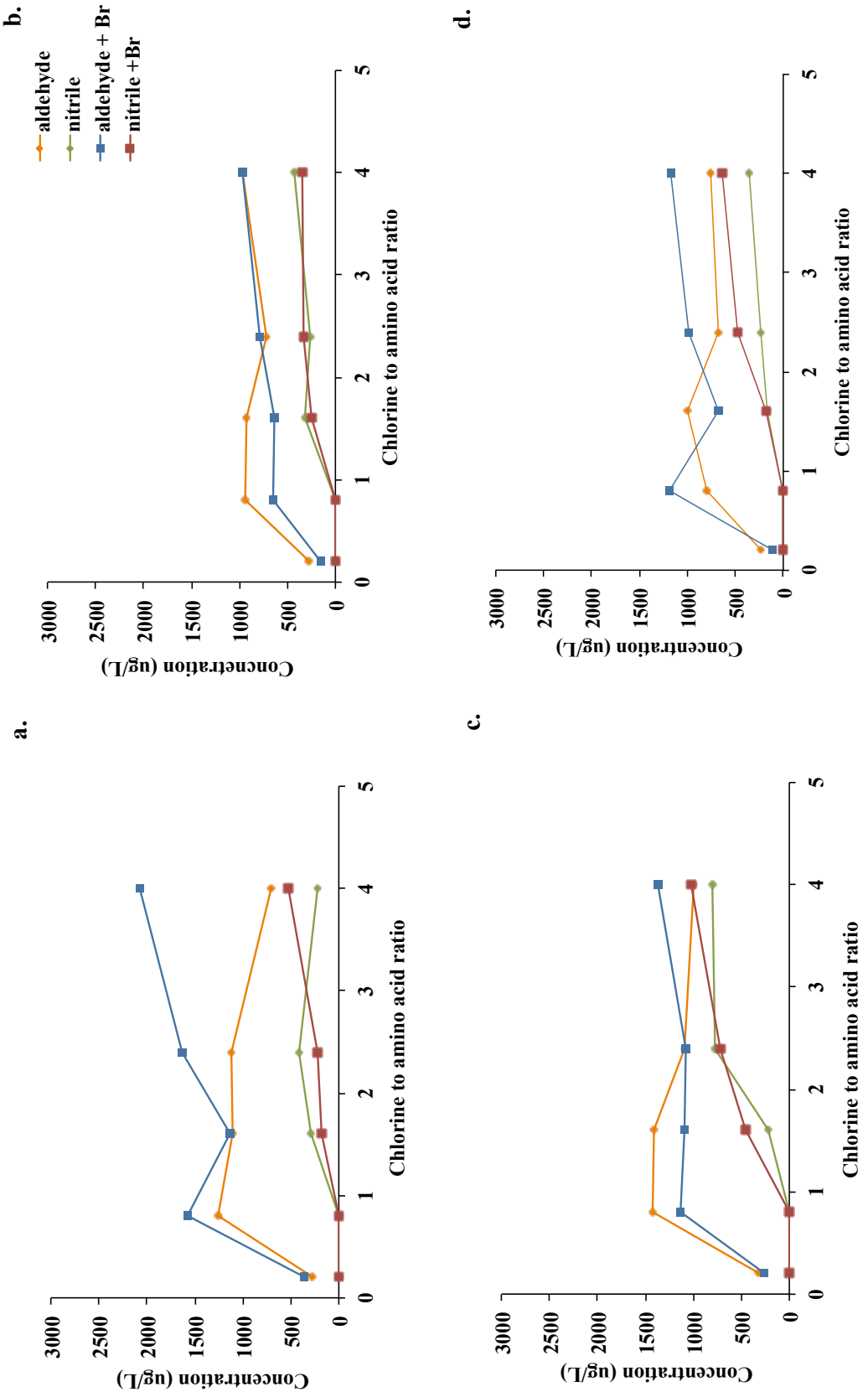


Figure 4.14: Aldehyde and nitrile concentrations following chlorination of amino acids a) valine b) isoleucine c) leucine and d) phenylalanine; at pH 9 and pH 9 + Br, after one hour reaction time.

An example of the reaction pathway for formation of the aldehyde and nitrile from isoleucine is shown in Figure 4.15, where upon addition of excess chlorine (chlorine to amino acid ratio >1), the dichloramino acid forms, as well as the monochloramino acid species, allowing the formation of both the nitrile and aldehyde species. The nitrile species can also form from the reaction of the aldehyde species with additional chlorine *via* the formation of the *N*-chloroaldimine species as shown in Figure 4.8.

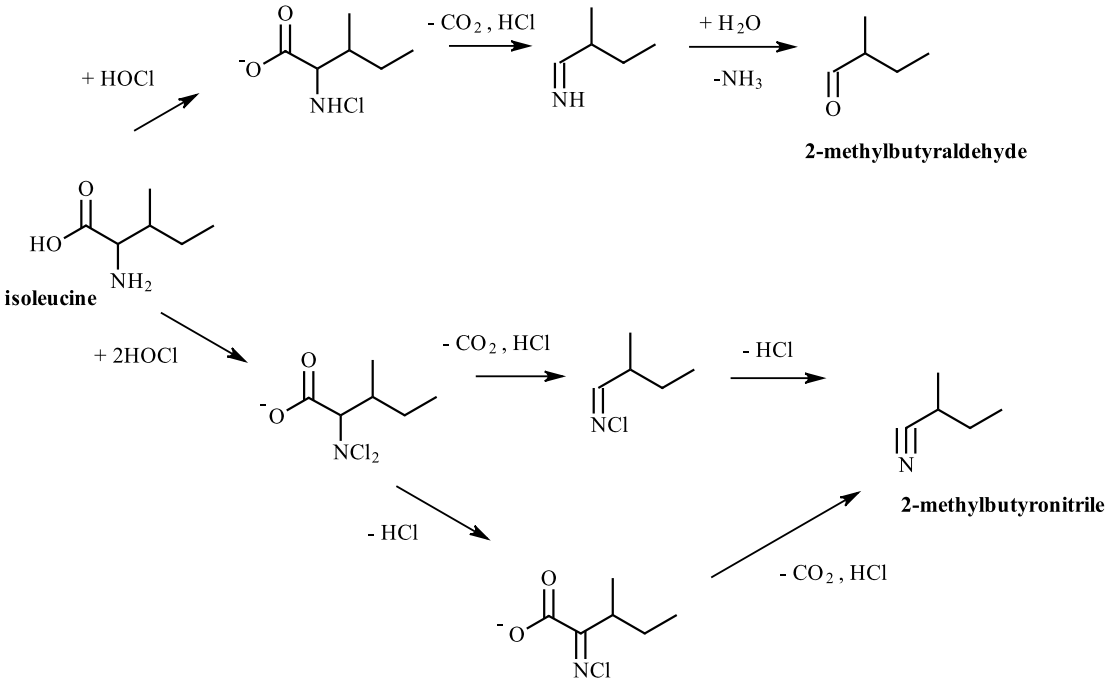


Figure 4.15: Reaction pathway for the formation of 2-methylbutyraldehyde and 2-methylbutyronitrile upon chlorination of isoleucine.

The concentration of each aldehyde and nitrile species produced from each amino acid was converted into a molar percentage conversion of the parent amino acid to each respective by-product (Tables 4.7 - 4.10, Figures 4.16 - 4.19). In Figures 4.16-4.19, the corresponding free and total chlorine equivalent residuals are also shown, which present the breakpoint curves as previously described in Section 4.3.1.1. As observed above, nitriles were not produced until the chlorine to amino acid ratio was above 0.8, at 1.6 for these experiments.

Valine showed a slightly greater conversion of amino acid to by-products at pH 9, in comparison to pH 7 (Table 4.7, Figure 4.16), and was the only amino acid in this

study to demonstrate this. It is also notable that for valine, a percentage conversion of amino acid to by-products of greater than 100% occurred at chlorine to nitrogen ratios of 2.4 and 4 in the presence of bromide ion at pH 7, indicating that this data should be treated with caution, and that there were issues with the analysis.

From all amino acids in this study, at all chlorine to nitrogen ratios, more aldehyde was produced than nitrile, suggesting a higher chlorine to amino acid ratio was required for the production of the nitrile species. It is also likely that not much of the dichloramino acid species was produced (a species which is required for conversion to the nitrile). Higher formation of by-products also occurred in the presence of bromide. This may have occurred due to the presence of bromine, resulting from the addition of the bromide ion. It is possible that the formation of *N*-bromamino acids occurred, compounds which react similarly to *N*-chloramino acids and produce aldehydes and nitriles. By having bromide present, additional by-products have the potential to form, and this appears to have occurred.

Chlorination of isoleucine (Table 4.8, Figure 4.17) resulted in higher production of aldehydes and nitriles at pH 7 compared to pH 9, which is in contrast to the reaction of valine. The presence of bromide appeared to increase the production of aldehydes and then nitriles (if the chlorine to amino acid ratio was greater than 0.8) at pH 7, while at pH 9, it seemed to have little effect.

Chlorination of leucine (Table 4.9, Figure 4.18) resulted in similar conversion of amino acid to aldehyde and then nitrile at molar ratios of 0.2 to 2.4 at pH 7 and pH 9. A greater conversion of amino acid to aldehyde and then nitrile was observed at a molar ratio of 4 at pH 7, in comparison to pH 9. This result is consistent with the chlorine demand results presented in Section 4.3.1.1, Table 4.6, where a similar demand was exhibited at each pH. Bromide appeared to have a slight effect, in that there were slightly more by-products in the presence of bromide. In the case of all of the amino acids investigated in this study, the greatest conversion of amino acid to nitrile was observed for leucine. This is likely to be due to the structure of leucine and its subsequent aldehyde, 3-MBA. Leucine does not have a methyl group attached to the carbon chain at C-3, and as a result, the α carbon of the resultant aldehyde is secondary rather than tertiary. This reduced steric hindrance could cause an increased

reactivity of the monochloramino acid species, thus formation of the aldehyde. This is further explored in Section 4.3.2.

Chlorination of phenylalanine (Table 4.10, Figure 4.19) also resulted in higher conversion of amino acid to aldehyde and then nitrile at pH 7 compared to pH 9. Similarly to leucine, there were slightly more by-products formed in the presence of bromide across both pH ranges.

These results are consistent with those found by Bruchet *et al.* (1992), who carried out a similar study, in terms of addition of chlorine to a range of amino acids (including valine and phenylalanine) and monitoring the chlorine residuals and concentration of resultant aldehydes. The study by Bruchet *et al.* (1992), however, was only carried out at pH 7 and did not examine the influence of pH. Similarly shaped free and total chlorine residual plots were achieved by Bruchet *et al.* (1992).

Table 4.7: Molar percentage conversion of valine to isobutyraldehyde and isobutyronitrile following chlorination at pH 7, pH 7 + Br, pH 9 and pH 9 + Br after one hour reaction time. Data marked with an * should be carefully considered.

	pH 7		**pH 7 + Br**		**pH 9**		**pH 9 + Br**	
Cl/AA ratio	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**
0.2	8	0	10	0	10	0	14	0
0.8	27	0	39	0	48	0	61	0
1.6	39	16	53	20	42	12	43	7
2.4	52	38	105*	57*	43	17	63	9
4	32	19	76*	48*	27	9	80	21

Table 4.8: Molar percentage conversion of isoleucine to 2-methylbutyraldehyde and 2-methylbutyronitrile following chlorination at pH 7, pH 7 + Br, pH 9 and pH 9 + Br after one hour reaction time.

	pH 7		pH 7 + Br		pH 9		pH 9 + Br	
Cl/AA ratio	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**
0.2	6	0	4	0	7	0	4	0
0.8	19	0	16	0	24	0	17	0
1.6	20	9	41	11	24	8	16	7
2.4	26	15	47	16	18	7	20	9
4	53	23	49	19	25	11	25	9

Table 4.9: Molar percentage conversion of leucine to 3-methylbutyraldehyde and 3-methylbutyronitrile following chlorination at pH 7, pH 7 + Br, pH 9 and pH 9 + Br after one hour reaction time.

	pH 7		pH 7 + Br		pH 9		pH 9 + Br	
Cl/AA ratio	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**
0.2	10	0	9	0	10	0	8	0
0.8	42	0	37	0	46	0	36	0
1.6	43	12	53	19	45	7	35	15
2.4	49	42	58	43	35	26	35	24
4	60	59	57	48	32	27	44	34

Table 4.10: Molar percentage conversion of phenylalanine to phenylacetaldehyde and phenylacetonitrile following chlorination at pH 7, pH 7 + Br, pH 9 and pH 9 + Br after one hour reaction time.

	pH 7		pH 7 + Br		pH 9		pH 9 + Br	
Cl/AA ratio	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**	**% aldehyde**	**% nitrile**
0.2	7	0	3	0	5	0	3	0
0.8	23	0	24	0	18	0	27	0
1.6	31	9	51	22	23	5	16	5
2.4	41	29	52	32	16	6	23	13
4	48	43	45	41	17	10	27	17

4.3.1.2.1 Summary of the Effect of pH Upon Chlorination of Amino Acids

Overall, lower concentrations of aldehydes and nitriles were produced at pH 9 (Figure 4.14 a-d) compared to pH 7 (Figure 4.13 a-d). This is consistent with the observation of larger total chlorine equivalent residuals at the same chlorine to amino acid ratios, which suggest more of the combined chlorine species were present (resulting in a greater total chlorine equivalent concentrations), and had not been converted into aldehydes or nitriles. At pH 9, the formation of other by-products or intermediates may have been more favourable. The higher concentrations of aldehydes and nitriles produced at pH 7 is likely to be as a result of the more effective chlorinating agent, HOCl, being present at pH 7 compared to pH 9, where the weaker OCl^- predominates.

It can also be observed that high concentrations of aldehydes and nitriles are present when there are high concentrations of free chlorine present. This demonstrates that these compounds are stable and resistant to oxidation by chlorine, and that they do not contribute significantly to chlorine demand.

4.3.1.2.2 Summary of the Effect of Bromide Upon Chlorination of Amino Acids

The presence of bromide allows for the formation of bromine, as well as chlorine, to react with the amino acid. In the case of valine, more aldehyde and nitrile were formed in the presence of bromide. It is likely that the presence of bromine allowed the formation of more by-products than experiments carried out without bromide present.

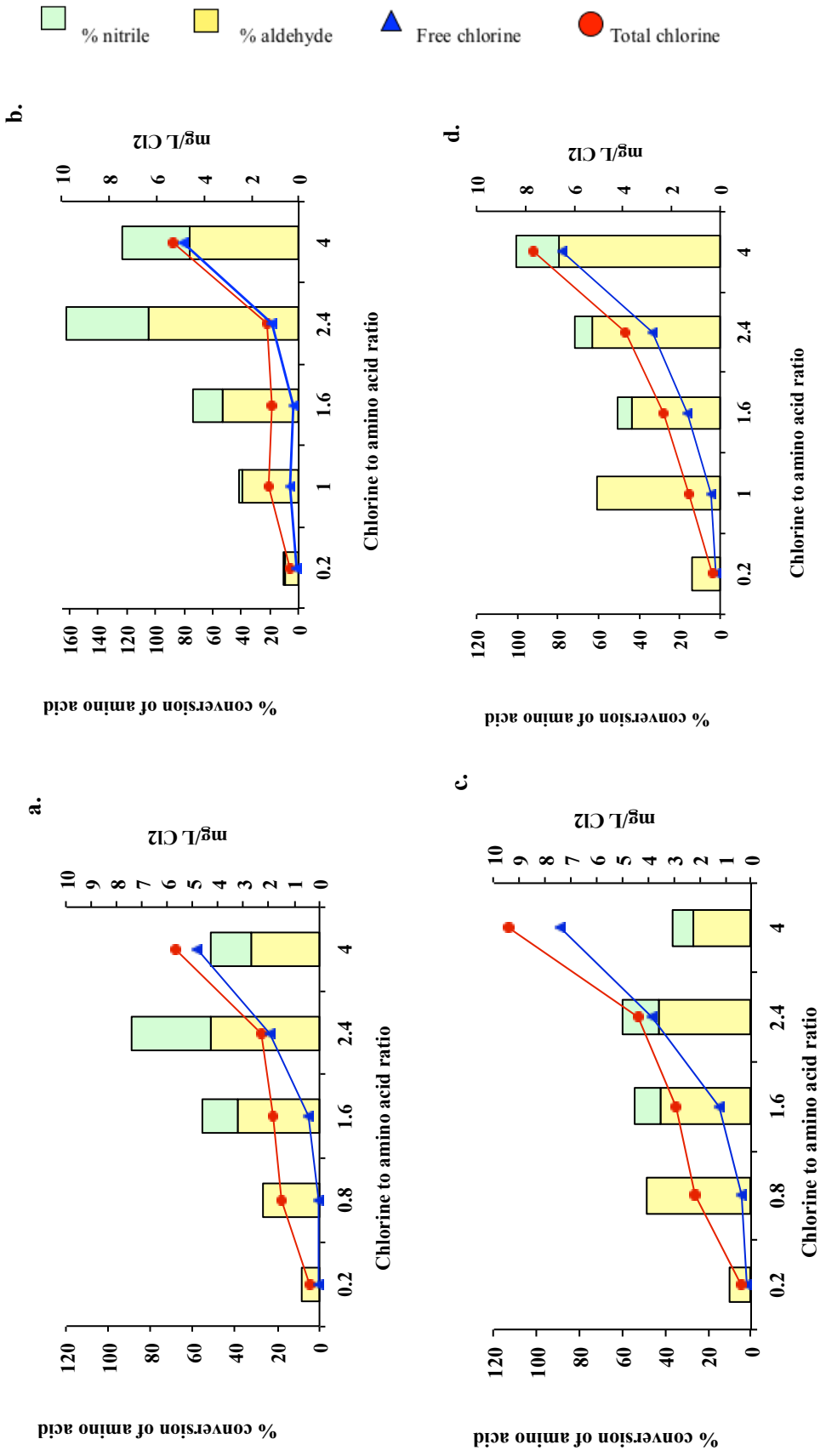


Figure 4.16: Molar percentage conversion of valine to isobutyraldehyde and isobutyronitrile following chlorination at a) pH 7, b) pH 7 + Br, c) pH 9 and d) pH 9 + Br after one hour reaction time.

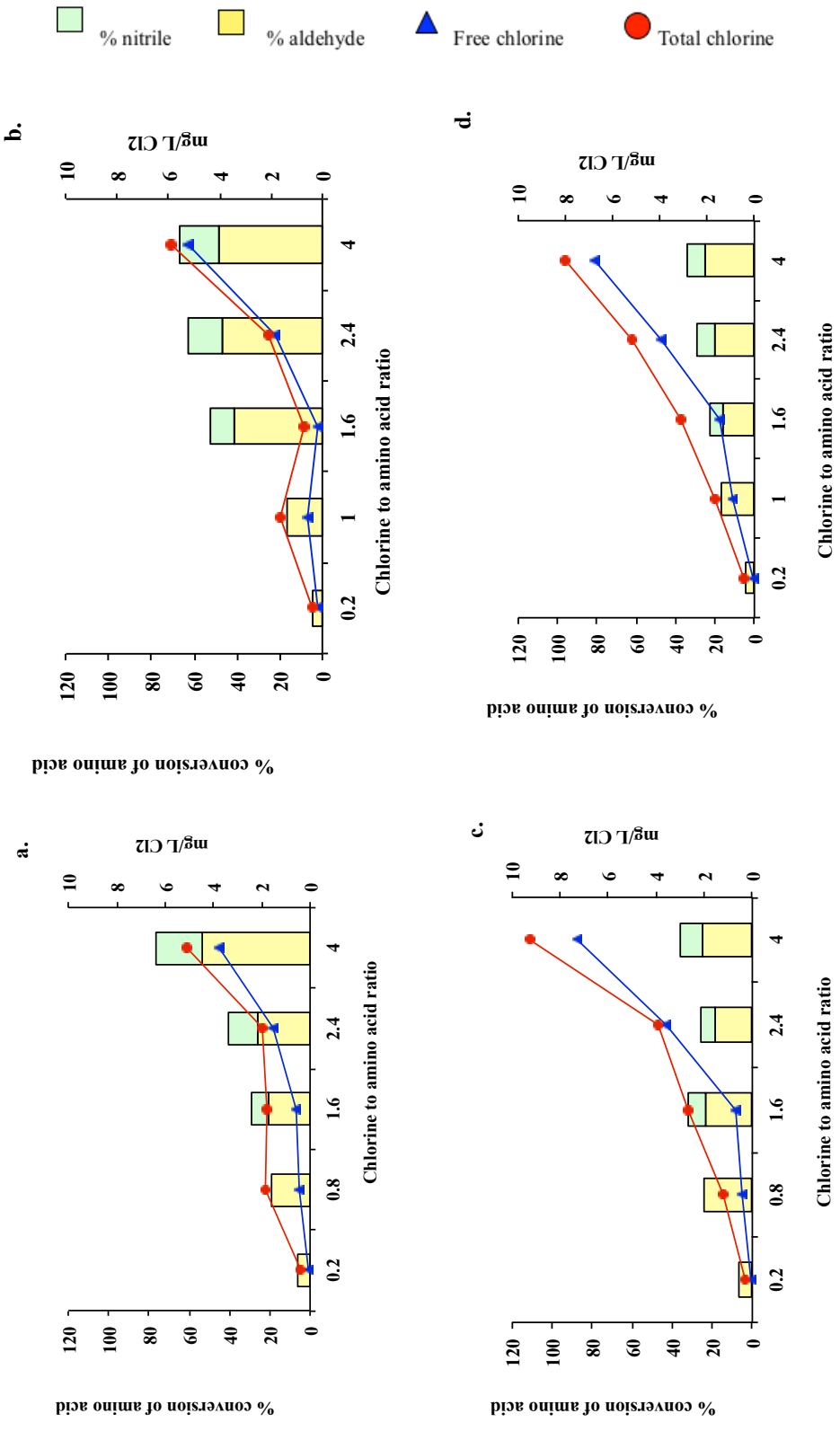


Figure 4.17: Molar percentage conversion of isoleucine to 2-methylbutyraldehyde and 2-methylbutyronitrile following chlorination at a) pH 7, b) pH 7 + Br, c) pH 9 and d) pH 9 + Br after one hour reaction time

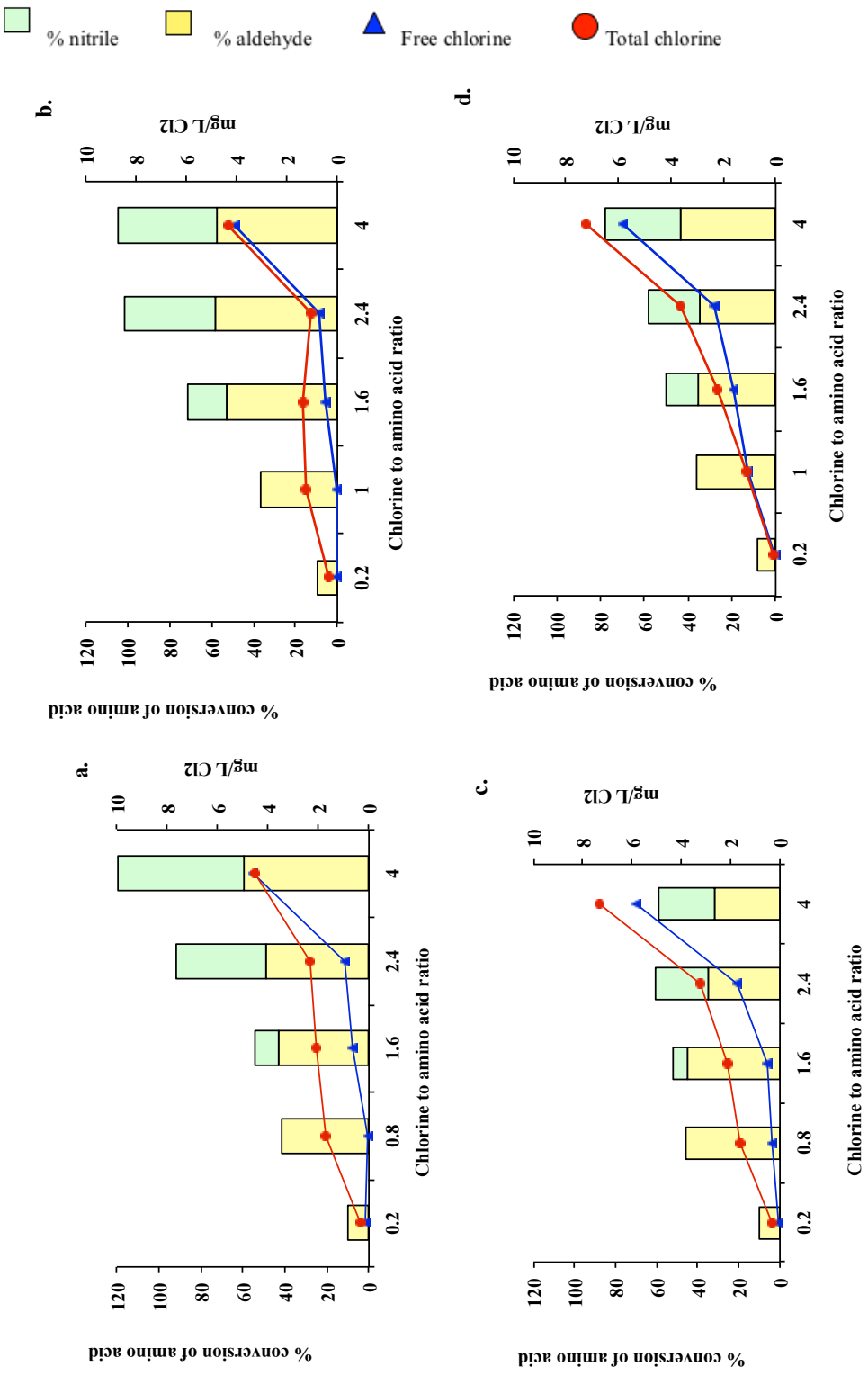


Figure 4.18: Molar percentage conversion of leucine to 3-methylbutyraldehyde and 3-methylbutyronitrile following chlorination at a) pH 7, b) pH 7 + Br, c) pH 9 and d) pH 9 + Br after one hour reaction time.

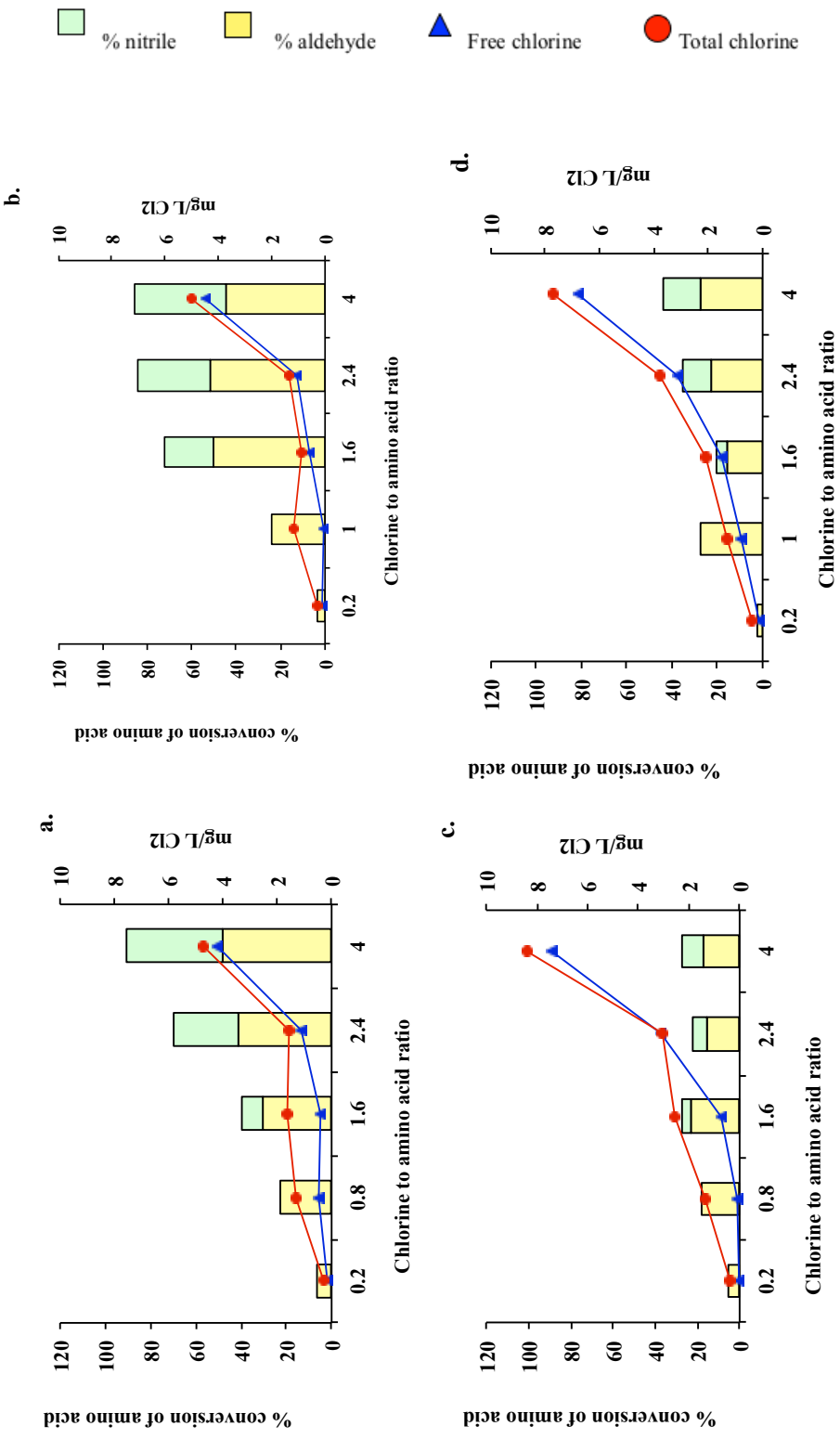


Figure 4.19: Molar percentage conversion of phenylalanine to phenylacetaldehyde and phenylacetonitrile following chlorination at a) pH 7, b) pH 7 + Br, c) pH 9 and d) pH 9 + Br after one hour reaction time.

4.3.2 Kinetics of the Chlorination of Amino Acids

A small further study on the kinetics of the chlorination of amino acids was carried out on the three similarly-structured amino acids, valine, leucine, and isoleucine at pH 7. The ratio of amino acid to chlorine used was 5:1. The rates of the chlorination of these amino acids, and subsequent production of by-products, were investigated by addition of chlorine to the amino acids and measurements of the monochloramino acid degradation *via* UV spectroscopy and aldehyde formation *via* GC-MS. All experiments were carried out at room temperature. The ratio of chlorine to amino acid was not high enough for any nitrile to be formed.

The rate of a reaction (ν) is proportional to the concentrations of the reactants (A and B) raised to a power *i.e.*

$$\nu = k\,[\mathrm{A}][\mathrm{B}] \qquad \textbf{Equation 4.7}$$

where k is the rate constant which is independent of the concentrations of the reactants but dependent on temperature. The rate law can be determined experimentally by the isolation method in which all but one of the reactants is in large excess: so if B is in large excess, the concentration of B is assumed to be constant throughout, and while the true raw law might be $\nu = k\,[\mathrm{A}]\,[\mathrm{B}]$, [B] is approximated by $[\mathrm{B}]_0$, and

$$\nu = k'[\mathrm{A}] \quad \text{where} \quad k' = k[\mathrm{B}]_0$$

which has the form of a first-order rate law.

Rate laws are differential equations, therefore they must be integrated to find concentrations as a function of time. The first-order rate law for the consumption of reactant A is:

$$\frac{\mathrm{d}\,[\mathrm{A}]}{\mathrm{d}\,t} = -k\,[\mathrm{A}] \qquad \textbf{Equation 4.8}$$

which has the solution

$$\ln\left(\frac{[\mathrm{A}]}{[\mathrm{A}]_0}\right) = -kt \qquad \textbf{Equation 4.9}$$

so by plotting $\ln\left(\frac{[A]}{[A]_0}\right)$ against time, a first-order reaction will give a straight line with a slope of $-k$.

In the case of first-order reactions which are close to equilibrium, the composition of a reaction with respect to time can be explored considering the forward and reverse reactions:

$$[A] \rightarrow [B] \qquad \textbf{Equation 4.10}$$

$$[B] \rightarrow [A] \qquad \textbf{Equation 4.11}$$

If the initial concentration of A is $[A]_0$ and no B is present initially, then at all times $[A]_0 = [A] + [B]$, therefore $[A] = [A]_0 - [B]$, using the rate law presented above for the consumption of reactant A;

$$\ln\left(\frac{[A]_0 - [B]}{[A]_0}\right) = -kt \qquad \textbf{Equation 4.12}$$

(Atkins, 1998)

In the case of amino acid chlorination, [A] is equal to the amount of monochloramino acid present in solution, while $[A]_0$ is equal to the concentration of chlorine (assuming all the chlorine is converted to the monochloramino acid instantly upon addition to the amino acid) and [B] is equal to the concentration of aldehyde by-product formed.

4.3.2.1 Measurement of the Monochloramino Acid Species

Valine, leucine, and isoleucine (0.003 M) were treated with chlorine (0.0006 M) (ratio amino acid to chlorine 5:1), at pH 7. As the amino acid was present in excess, it was assumed that all of the chlorine was converted to the monochloramino acid (structures shown in Figure 4.20). The absorbance of each mixture was measured *via* UV spectroscopy from 190 to 350 nm to determine the wavelength of maximum absorbance for the reaction mixture products for further experiments (Figure 4.21). This wavelength was determined to be 256, 256 and 258 nm for each of the amino acids, respectively. Based on the reaction pathway discussed previously and shown

in Figure 4.15, the larger broad hump in the UV spectra was assumed to be the monochloramino acid species.

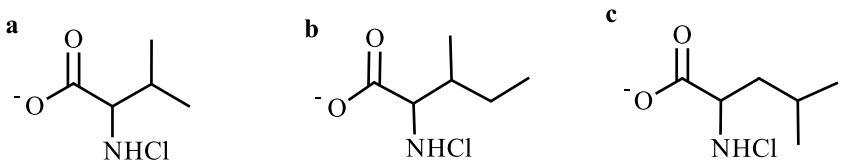


Figure 4.20: Monochloramino acid structures formed following the monochlorination of a) valine, b) isoleucine and c) leucine.

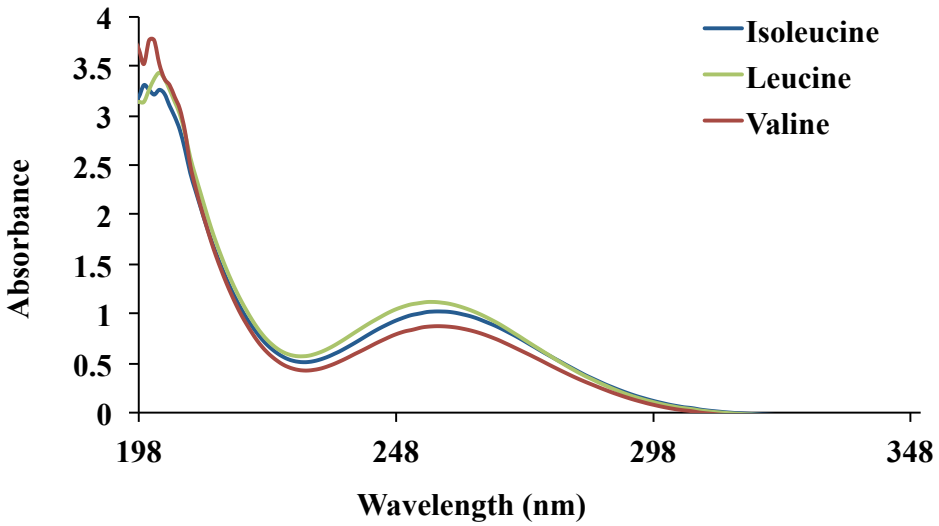


Figure 4.21: The UV absorbance of chlorinated isoleucine, leucine and valine respectively. The spectra were recorded after 1 minute reaction time.

Each amino acid was then treated with chlorine at six different concentrations, while maintaining an excess amino acid to chlorine ratio (to ensure all of the chlorine was converted to the monochloramino acid species), and the absorbance of each mixture was then measured *via* UV spectroscopy at 256 or 258 nm. These values were plotted against concentration and, using the Beer-Lambert law, the molar extinction coefficient (ε), the slope of the concentration plotted *versus* absorbance, was determined for each monochloramino acid (Figure 4.22: valine, Figure 4.23: isoleucine and Figure 4.24: leucine). These values of ε were required for later experiments to convert the absorbance of monochloramino acid into the corresponding concentration. The ε values are presented in Table 4.11 and are reasonably similar to those determined by other researchers.

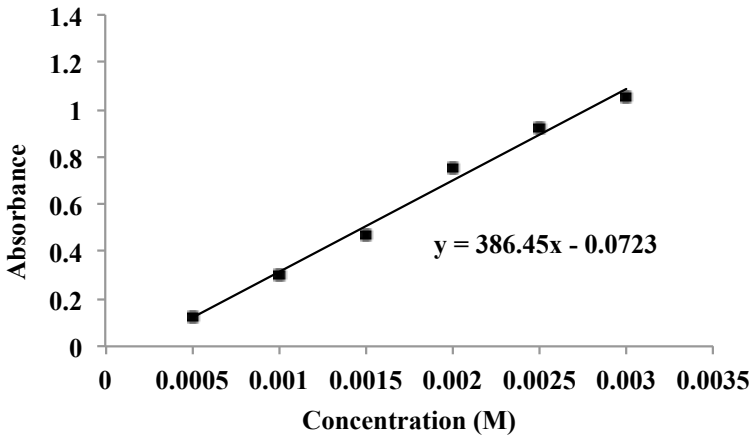


Figure 4.22: UV absorbance at 256 nm *versus* concentration plot for monochlorinated valine at pH 7. Derived value of ε = 386.

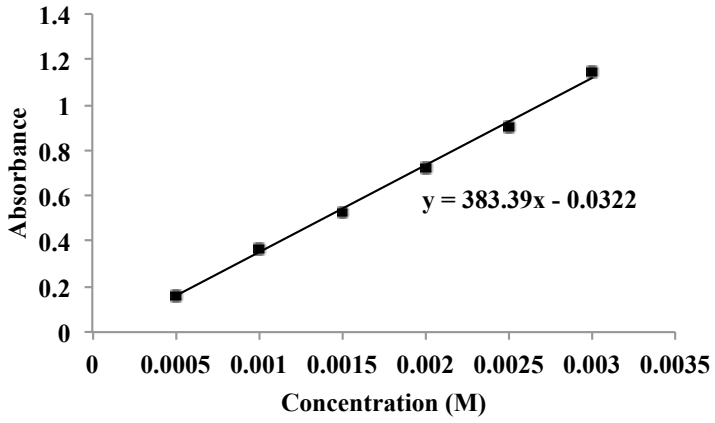


Figure 4.23: UV absorbance at 256 nm *versus* concentration plot for monochlorinated isoleucine at pH 7. Derived value of ε = 383.

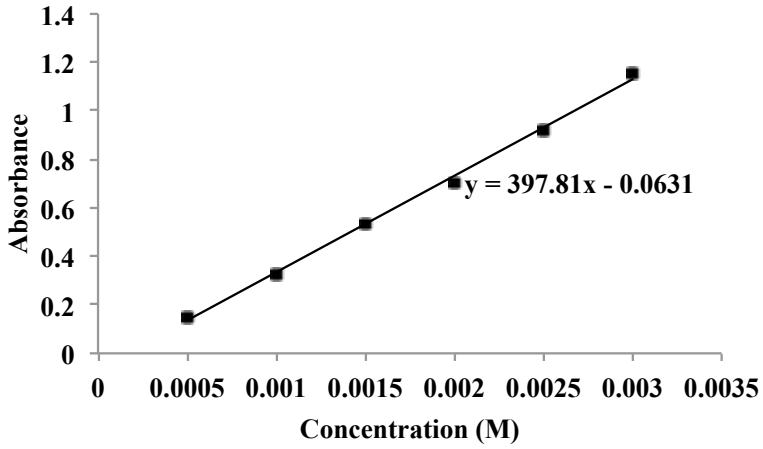


Figure 4.24: UV absorbance at 258 nm *versus* concentration plot for monochlorinated leucine at pH 7. Derived value of ε = 398.

Table 4.11: The molar extinction coefficient values calculated for each amino acid. Each amino acid adhered to the Beer-Lambert Law.

Amino Acid	Molar coefficient extinction (ε)	Literature values
Valine	386	345[a]
Isoleucine	383	
Leucine	398	350[b]

a- Abia *et al.* (1994), b- Antelo *et al.* (1988)

4.3.2.2 Degradation of Monochloramino Acid Species

Valine, leucine and isoleucine (0.003 M) were treated with chlorine (0.0006 M) (ratio amino acid to chlorine 5:1), at pH 7. The rate constant for the degradation of the monochloramino acid formed was determined by measuring the absorbance of the reaction mixture *via* UV spectroscopy at 256-258 nm over one hour, converting this to a concentration of monochloramino acid (where c = ε x absorbance), and plotting $\ln\left(\frac{[A]}{[A]_0}\right)$ *vs.* time (Figure 4.26). According to Equation 4.6, the degradation rate constant was the slope of the line of best fit (Figure 4.25).

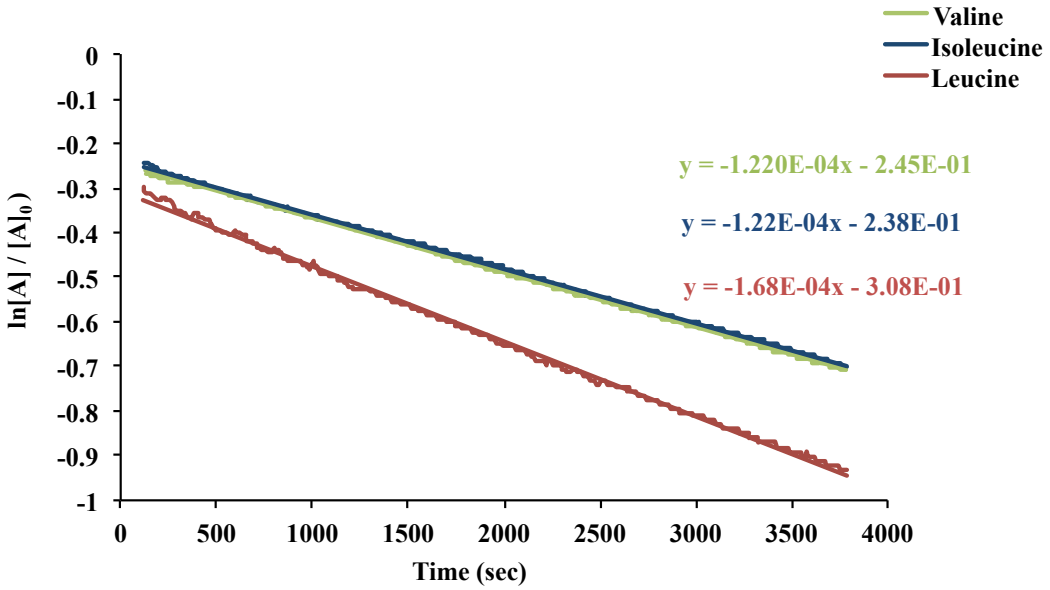


Figure 4.25: The rate constant for the degradation of the monochloramino acid species for each chlorinated amino acid at pH 7, amino acid to chlorine ratio 5:1, where [A] is [monochloramino acid] at any time and $[A]_0$ is [chlorine].

4.3.2.3 Formation of Aldehyde Species

The rate of formation of the aldehydes from the reaction mixture described in Section 4.4.2.2 was monitored by taking aliquots of the reaction mixture at ten increasing time intervals (over one hour), and quenching the oxidant residual with sodium thiosulfate solution, measuring concentration of aldehydes *via* GC-MS and

plotting $\ln\left(\frac{[A]_0-[B]}{[A]_0}\right)$ *vs.* time (Figure 4.27). According to Equation 4.12, the formation rate constant was the slope of the line of best fit (Figure 4.26).

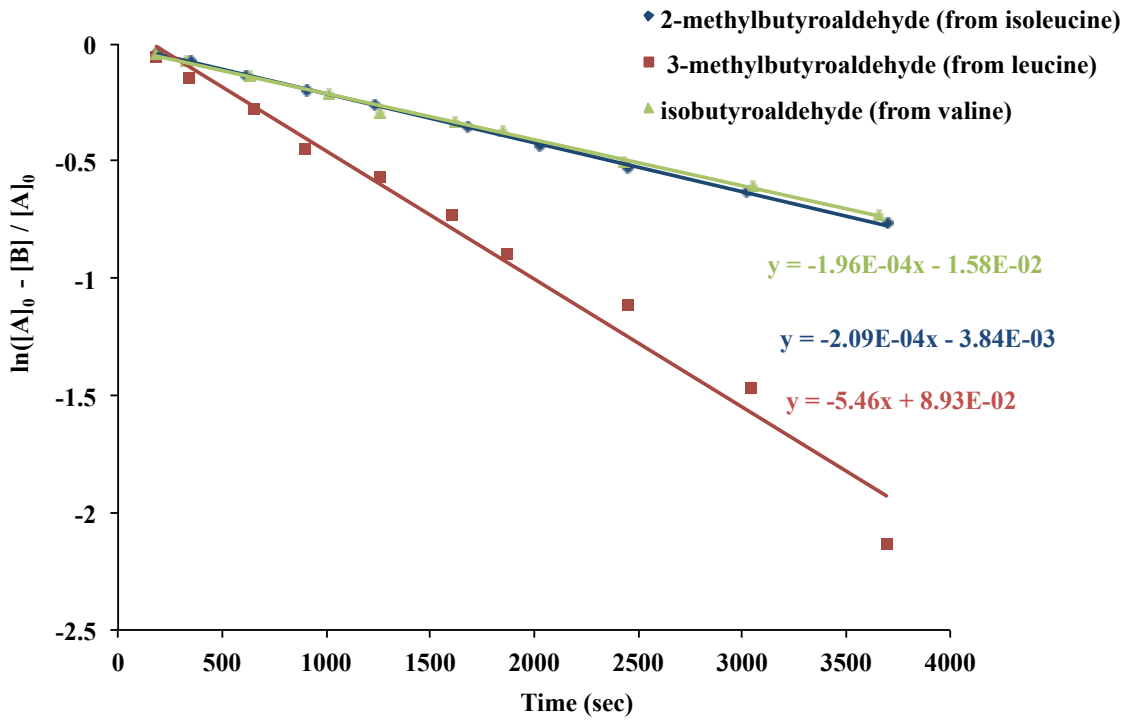


Figure 4.26: The rate of formation of the aldehyde species for each chlorinated amino acid at pH 7, amino acid to chlorine ratio 5:1, where $[A]_0$ is [chlorine], [B] is [aldehyde].

4.3.2.4 Summary of the Kinetics of the Chlorination of Amino Acids in this Study

A summary of the rate constants determined for decomposition of the monochloramino acid species and formation of the aldehyde species is shown in Table 4.12.

Table 4.12: The rate constants for monochloramino acid degradation and aldehyde formation for each amino acid treated with chlorine at a ratio of 5:1 chlorine:nitrogen, at pH 7.

	Valine	**Isoleucine**	**Leucine**
Monochloramino acid degradation	1.22×10^{-4} s^{-1}	1.22×10^{-4} s^{-1}	1.68×10^{-4} s^{-1}
Aldehyde formation	1.96×10^{-4} s^{-1}	2.08×10^{-4} s^{-1}	5.46×10^{-4} s^{-1}

Valine and isoleucine exhibited similar rates of decomposition of the monochloramino acid and formation of the respective aldehyde, while the monochloramino acid for leucine degraded faster and the aldehyde formed faster in comparison. The rate constant observed for the decomposition of valine is similar to that observed by Abia *et al.* (1994), who, at a range of pH values of 5 to 12, found $k = 1.8 \times 10^{-4}$ s^{-1}. They determined a larger rate constant in the case of the decomposition of leucine, however, finding $k = 3.2 \times 10^{-4}$ s^{-1} (Antelo *et al.*, 1988). In their study, however, the ratio of amino acid to chlorine was only 1.5:1, so the smaller excess of amino acid may have impacted the formation of the monochloramino acid species and its subsequent degradation. Another study of a range of differently structured amino acids found that the rate constants were highly dependent upon the amino acid structure and among the compounds with the same number of substituents, the larger substituents increased reactivity (Hand *et al.*, 1983). As discussed in Section 4.3.1.2, the structure of leucine could allow for the production of a monochloramino acid with less steric hindrance than valine or isoleucine, resulting in a larger rate constant and a faster degradation to the aldehyde species.

This is the first report of the rate constants of the formation of these aldehydes. While each of the previous studies on monochloramino acid degradation acknowledged the formation of the aldehyde products, they did not look at the rate of their formation.

Based on the rate constants determined in this study (Table 4.12), the rates of aldehyde formation were faster than the degradation of the monochloramino acid. It could be possible that the dichloramino acid had also been formed, and in Figure 4.21, may be the first peak in the spectrum at around 190 nm. The dichloramino acid

would provide another pathway for aldehyde formation (the dichloramino acid forms a chlorinated imine which produces an aldehyde upon quenching with sodium thiosulfate solution, as shown in Figure 4.8) which may account for the faster aldehyde formation observed.

4.3.3 Odour Analysis of Solutions of Chlorinated Amino Acids

A brief investigation into the odours produced by aldehydes and solutions of chlorinated amino acids was undertaken to determine the likelihood that aldehydes could be responsible for chlorinous related off-flavour complaints.

4.3.3.1 Odour Analysis of Aldehyde Standards

Odour analysis of the four aldehydes (IBA, 2-MBA, 3-MBA and PA) used in this study was undertaken by an Odour Panel. A panel of 13 were presented with individual solutions of each aldehyde at a concentration of 25 µg L^{-1}, which was in excess of their reported odour threshold concentrations (Table 4.13), in order to determine whether the aldehydes themselves were odorous and how the odour would be described.

Table 4.13: Odour threshold concentrations of the aldehydes.

Compound	OTC (µg L^{-1})
IBA	0.90[a]
2-MBA	12.5[b]
3-MBA	0.20 [a]
PA	4

a: (Guadagni *et al.*, 1972; Guadagni *et al.*, 1963), b: (Buttery *et al.*, 1971)

Of these solutions, 92% of panellists detected an odour for IBA, 70% detected an odour from 2-MBA, 100% detected an odour for 3-MBA, while 70% detected an odour for PA. None of the panellists described any of the odours as chlorinous. A range of different descriptors were recorded for each of the aldehydes and these are summarised in Table 4.14. While none of the aldehyde solutions were described as chlorinous, the description of chemical and medicinal have previously been related to chlorinous odours (Mackey *et al.*, 2004). It is also important to consider that the aldehydes were smelled individually and without anything else present in the

solution. In the case of a real water sample, it is likely that other compounds will be present and these can impact on the overall odour of the water.

Table 4.14: Odour descriptions received from panellists analysing aldehyde solutions (25 µg L^{-1}).

Compound	Descriptor
IBA	earthy, dusty, organic, chemical, ants
2-MBA	sweet, ants, earthy, dusty
3-MBA	dirty, septic, ants, chemical
PA	floral, sweet, wood, medicinal

4.3.3.2 Odour Analysis of Chlorinated Amino Acids

Valine, leucine and isoleucine were treated with chlorine at pH 7, at a molar ratio of 1:2 chlorine to amino acid. After one hour, free and total chlorine equivalent measurements were made, and odour analysis was undertaken by a panel of ten people. A molar ratio of 0.5 was chosen to ensure that only aldehydes were present in the reaction mixture (and not nitriles).

Free and total chlorine equivalent measurements for each of the chlorine dosed amino acid solutions are presented in Table 4.15. Valine was the only amino acid to still have a reasonable free chlorine equivalent concentration present in solution. The remaining amino acid mixtures likely consisted of a variety of chlorinated amino acid species, such as the monochloramino acids due to the lack of free chlorine equivalents. Therefore, a variety of compounds, including the aldehyde and monochloramino acid, could be expected to contribute to the odour of the solutions.

Table 4.15: Free and total chlorine equivalent measurements of chlorine dosed amino acid solutions after one hour reaction time.

Amino acid solution	Free chlorine equivalent concentration (mg L^{-1})	Total chlorine equivalent concentration (mg L^{-1})
Valine	0.29	0.58
Leucine	0.00	0.33
Isoleucine	0.07	0.56

Upon odour analysis of the solutions, each panellist detected an odour in each of the solutions. Only one panellist described one of the mixtures as chlorinous, and this was the valine solution. This is consistent with the presence of a free chlorine equivalent concentration for this sample. A range of descriptors were recorded for

each solution and these descriptors are presented in Table 4.16. In a similar case to the aldehydes, where no chlorinous descriptors were received, each solution was odorous and descriptors like chemical and solvent featured.

Table 4.16: Odour descriptions received from panellists analysing chlorine dosed amino acid solutions.

Amino acid solution	Descriptor
Valine	ants, musty, sweet, chlorine
Leucine	musty, unpleasant, ants, chemical
Isoleucine	ants, chemical, solvent, grass

4.4 Conclusions

Chlorination of the amino acids, valine, isoleucine, leucine and phenylalanine, resulted in the formation of aldehydes and nitriles. A variety of increasing chlorine to amino acid ratio experiments were carried out. Free and total chlorine equivalent concentrations were measured at each ratio after a reaction time of one hour and chlorine demand was calculated and presented for the first time for the range of conditions examined.

At pH 9, greater free and total chlorine equivalent concentrations were measured, coinciding with lower chlorine demand. The presence of chlorine and combined chlorine suggests that other chlorinated by-products, such as *N*-chloroaldimine species or dichloramino acids, may have been present and not been converted to the aldehyde or nitrile species at pH 9. This result is consistent with the lower by-product formation at pH 9.

Investigating the rate of monochloramino acid degradation and formation of aldehyde species of three of the amino acids at pH 7 indicated that there was the likelihood that another reaction pathway, other than the decomposition of the monochloramino acid species to produce the aldehyde, which is consistent with reaction schemes presented in Figure 4.8. Other kinetic studies indicated that rate constants were constant across a range of pH values for the degradation of the monochloramino acid species, however looking at the rate of aldehyde formation at a higher pH may be advantageous. Measurement of other intermediates, such as an *N*-chloraldimine or dichloramino acid species may offer more insight into the

reaction pathway, and likely species expected to be present in waters containing amino acids which have been treated with chlorine.

Odour analysis of solutions of the aldehydes themselves indicated that they are odorous. A range of odour descriptors were presented, including medicinal and chemical which have previously been associated with chlorinous off-flavours. Odour analysis of the reaction mixture of a chlorine dosed amino acid solution also indicated that the resultant solution was odorous. This confirms that waters containing amino acids which are treated with chlorine are likely to produce odorous by-products. These by-products include aldehydes and nitriles, which are also odorous. Other by-products or intermediates, such as monochloramino acid species or others, may also be present. These compounds could be responsible for chlorinous off-flavour incidents, and the presence of bromide can increase their formation. The pH of the water is also important, where in a more alkaline water, the production of aldehydes and nitriles may not occur, or occur too rapidly to measure. However, the presence of monochloramino acids and other chlorinated intermediates could occur in a more acidic water.

Chapter 5

Odours from Wastewater Systems: A Review

5.1 Introduction

The second part of this Thesis (Chapters 5, 6 and 7) focuses on wastewater systems and the identification and analysis of odorous compounds arising from them by SPME/GC-MS. This Chapter provides an overview of the different compounds associated with wastewater systems and techniques for their analysis.

Wastewater systems operate to protect public health and the environment. The introduction of reticulated wastewater collection systems is considered to be one of the major advances in public health over recent centuries (Water Corporation, 2006). In Perth, Western Australia, the reticulated wastewater pipe system removes wastewater from households, commercial businesses and industry for treatment prior to either reuse or discharge back into the environment.

Modern municipal wastewater treatment plants utilize a combination of physical separation and biological processes to remove contaminants from water, producing stabilized sludge and water suitable for discharge to the environment. In a typical flow path (Figure 5.1) (UNEP, 2002), coarse solids are removed during initial grit removal and screening, followed by primary wastewater treatment which includes gravity sedimentation of the screened wastewater to remove settlable solids. Secondary wastewater treatment involves the use of multiple biological processes (aerobic, anoxic and anaerobic) to remove biodegradable material. Microorganisms consume the dissolved and suspended organic material and produce carbon dioxide, methane and other by-products. As the microrganisms feed, their density increases and they settle at the bottom of the processing tanks, separating from the clarified water a material referred to as secondary sludge, biological sludge, waste activated sludge or trickling filter humus (UNEP, 2002). This sludge then undergoes a stabilisation process to accelerate the biodegradation of the organic materials and reduces the microbial population to render the material safe for land application. Different varieties of stabilisation include- biological, chemical, thermal drying and pasteurisation. Finally, the sludge is dewatered to reduce volume and improve the handling techniques. The major processes used are a belt filter press, centrifuge or a recessed chamber filter press (Novak, 2001).

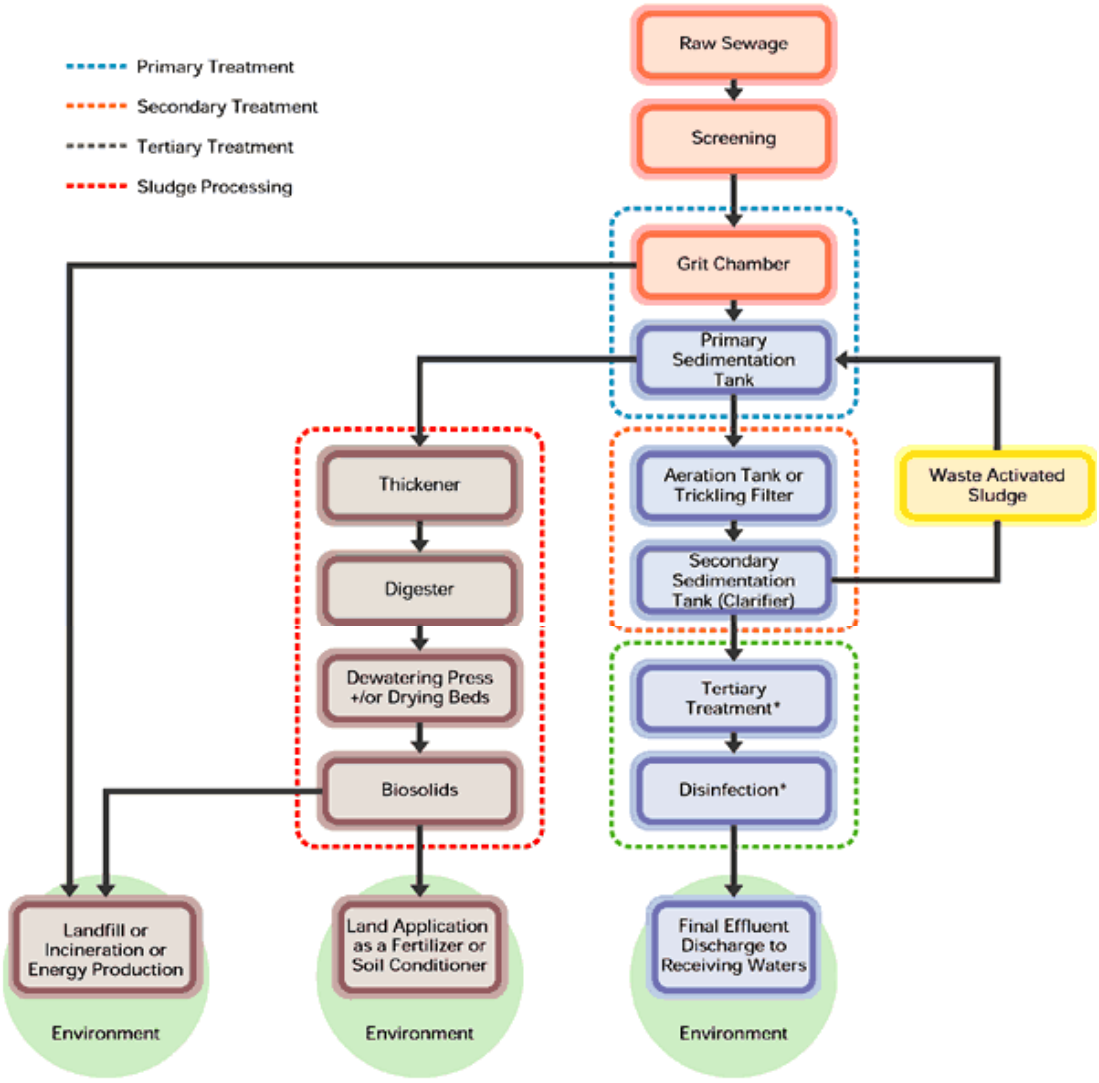


Figure 5.1: Typical wastewater treatment process (adapted from UNEP (2002)).

The main odours that arise from wastewater treatment works are due to decomposition of organic matter. These are particularly associated with anaerobic microbial activity which results in the release of volatile organic compounds, including alcohols, aldehydes, volatile fatty acids, ammonia and other nitrogen compounds, and various sulfur compounds (Suffet *et al.*, 2004a).

5.2 Compounds that Cause Wastewater Odours

Many compounds have been identified in wastewater treatment works. Typically, these compounds are reduced sulfur or nitrogen compounds, organic acids, aldehydes or ketones.

The odorants released from WWTPs vary, depending upon the raw wastewater entering the system, and the operation of the treatment processes. An odorous emission is composed of hundreds of chemical compounds, most of which are typically present at very low concentrations (*i.e.*, in the ppbv range; see Table 5.1). An understanding of the odorous chemicals present in wastewater was previously discussed in Section 1.3.

The development of the Wastewater Odour Wheel (Figure 1.3) by Burlingame *et al.* (2004) provided a summary of the different odour types and the chemicals responsible for these odours. This wheel provides sensory and chemical links between odour descriptors. A summary of this wheel is shown in Table 5.1.

Table 5.1: Main odour descriptors and the chemical responsible for the odour. Reproduced from Suffet *et al.* (2004a) Copyright IWA Publishing, 2004.

Sulfur compounds	Odour descriptor	Air odour threshold (ppmv)
Ethyl mercaptan	Rotten cabbage	0.00001a
Hydrogen sulfide*	Rotten eggs	0.0005a,e
		0.0085–1c,d
Carbon disulfide	Disagreeable, sweet	0.0077–00.96c,d
	Vegetable sulfide, Aromatic	
Dimethyl sulfide*	Rotten cabbage	0.001a
	Decayed vegetables	0.0006–0.04c,d, 0.001e
	Decayed cabbage	
Dimethyl disulfide	Rotten cabbage,	0.000026a
	Putrefaction	0.0001–0.0036c,d
Dimethyl trisulfide	Rotten cabbage	0.0012a
Diphenyl sulfide	Unpleasant	0.0001e
Methyl mercaptan*	Rotten cabbage	0.00002a
		0.0005e
Ethyl mercaptan*	Decayed cabbage	0.0003e
Allyl mercaptan*	Garlic, coffee	0.0001e
	Disagreeable, garlic	0.0001a
Propyl mercaptan*	Unpleasant	0.0001a
		0.0005e
Amyl mercaptan*	Putrid	0.00002a
	Unpleasant, putrid	0.0003e
Phenyl mercaptan*	Putrid, garlic	0.0003e
Benzyl mercaptan*	Unpleasant	0.0003a
	Unpleasant, strong	0.0002e

Sulfur compounds	Odour descriptor	Air odour threshold (ppmv)
Thiocresol*	Skunky, irritating	0.0001e
Sulfur dioxide*	Irritating	0.449a
	Pungent, irritating	2.7
Nitrogen compounds	**Character**	**Air odor threshold (ppmv)**
Ammonia*	Pungent	0.038a
	Pungent, irritating	17e
Methyl amine*	Fishy	3.2b
	Putrid fishy	4.7e
Ethyl amine*	Ammonia like	0.27e
Triethyl amine	Fishy	0.48b
Dimethyl amine*	Putrid, fishy	0.34e
Trimethyl amine*	Fishy	0.00044b, 0.0004e
	Pungent fishy	
n-Butyl amine*	Sour, ammonia	0.08e
Dibutyl amine*	Fishy	0.016e
Diisopropyl amine*	Fishy	0.13e
Indole*	Fecal, nauseating	0.0001e
Skatole*	Fecal, nauseating	0.001e
Pyridine*	Pungent, irritating	0.66e
Volatile fatty acids		
Formic acid	Biting	0.024a
Acetic acid	Vinegar	1.019a
Propionic acid	Rancid, pungent	0.028a
Isobutyric and butyric	Rancid	0.0003a
Isovaleric acid	Unpleasant	0.0006a
Valeric acid	Unpleasant	0.0006a
Formaldahyde	Unpleasant	1.199a
Ketones and aldehydes		
Acetaldehyde*	Green sweet	0.0001a
Acetone	Pungent, fruity	0.067e
	Sweet, minty	20.6a
Acreolin	Burnt, sweet	0.0228a
Propionaldehyde	Sweet, ester	0.011a
Crotonaldehyde	Pungent, suffocating	0.037a
Methyl ethyl ketone	Sweet, minty	0.25a
Butanaldehyde	Sweet	9.5a
Valeraldehyde	Pungent	0.028a

a, Ruth (1986) (lowest OTC); b, Amoore and Hautala (1983); c, de Zwart and Kuenen (1997); d, M. Devos *et al.* (1990); e, Sullivan (1969); * Identified in waste

A review of the literature regarding odorous sources, composition and typical concentrations measured in wastewater treatment plants was carried out by Adams *et al.* (2003) and is summarised in Table 5.2.

Table 5.2: Odour source characterisation from wastewater treatment processes (Adams *et al.* (2003) and references therein).

Source	**Compound**	**Concentration**
Pump station	H_2S	40-140 ppmv
Pump station	H_2S Mercaptans DMDS	100 ppmv 240 ppbv 195 ppbv
Lift station	H_2S Mercaptans DMDS	5-150 ppmv 1140 ppbv 290 ppbv
Headworks- bar screen	H_2S	9 ppmv (avg) 3-20 ppmv (range)
Headworks- inlet structure	H_2S	32 ppmv (avg) 6-94 ppmv (range)
Headworks	H_2S DMS	20-3000 ppbv 2-15 ppmv
Grit channels	H_2S	13 ppmv (avg) 6-20 ppmv (range)
Headworks/primary clarifiers	H_2S	250-300 ppmv
Headworks/primary clarifiers	H_2S Mercaptans Carbonyl sulfide Carbonyl disulfide DMDS	50-290 ppbv 3-9 ppbv 5-8 ppbv 4 ppbv 3 ppbv
Headworks	H_2S	20-70 ppmv
Headworks	H_2S	0.007-70 ppmv
Aerated grit tanks	H_2S	0.01-3.9 ppmv
Primary clarifier launder	H_2S	15 ppmv (avg) 4-35 ppmv (range)
Primary sedimentation tanks	H_2S	2.6 ppmv (avg) 6 ppmv (peak)
Primary sedimentation tanks	H_2S	0.009-12 ppmv
Primary clarifiers	H_2S	5.6 ppmv
Primary clarifier feed wells/launders	H_2S	77ppmv
Aeration tanks	H_2S	7.4 ppmv (avg) 10 ppmv (peak)
Trickling filter	NH_3 Mercaptans	0.25 ppmv 10-45 ppbv
Digester vents	H_2S	0.91-19.5 ppmv
Digester gas	H_2S Carbonyl sulfide	58-80 ppmv 7-15 ppbv
Biosolids holding tank	H_2S	100ppmv
Biosolids gravity thickener tank	H_2S	50-200 ppmv
Biosolids dewatering and storage	H_2S NH_3	0.24 ppmv (avg) 1.5 ppmv (peak) 0.33 ppmv
Biosolids dewatering	H_2S Mercaptans Carbonyl sulfide	3 ppmv 5 ppbv 30-35 ppbv

Source	Compound	Concentration
Biosolids dewatering	H_2S Mercaptans Methyl sulfide DMDS	34-42 ppmv <0.3 ppmv 2-7 ppbv 5.5 ppbv
Biosolids holding tank	H_2S	11 ppmv (avg) 20-300 ppmv (range)

ammonia (NH_3); dimethyl sulfide (DMS); dimethyl disulfide (DMDS)

This data highlights that the majority of compound specific data which has been collected has focused on H_2S. While other odorous compounds, such as methanethiol and dimethyl sulfides, can be emitted, monitoring for these compounds is not routinely performed. Instead, subjective measurements of odour through an odour panel are completed to provide information on the total odour strength. Odour, which is expressed in units of dilution-to-threshold (D/T), is determined relative to the detection threshold which is defined as the level at which half of the individuals in a trained odour panel can detect the odour. The D/T value corresponds to the number of dilutions required for one half of the panel members to record detection of odour (Adams *et al.*, 2003).

From June 2011 to May 2012, Wang *et al.* (2015) characterised the volatile sulfur compounds of different sewer systems in Sydney and Melbourne, Australia. Gas samples were collected from 18 sewer sites, which included a range of sewage types, sewer structures and chemical dosing treatments, to be representative of conditions present in Australian sewers. Six sulfur species were detected in the samples, which appeared in over 50% of the sampling events. Hydrogen sulfide (H_2S), methanethiol (MeSH), ethanethiol (EtSH), DMS, carbon disulfide (CS_2), 1-butanethiol, DMDS and dimethyl trisulfide (DMTS) were indentified in varying concentrations at different sewer locations over winter and summer (Wang *et al.*, 2014). A similar monitoring program, carried out between January and June 2011 in Sydney, Australia, surveyed 12 different sewer sites, where gas samples were collected and analysed (Sivret *et al.*, 2013). Several reduced sulfur compounds were consistently identified from the samples collected: CS_2, MeSH, DMS, DMDS and DMTS in addition to H_2S. The sewer air was also found to contain a range of odorous VOCs, including limonene, eucalyptol, *a*-pinene, toluene, ethylbenzene and benzaldehyde (Sivret *et al.*, 2013).

Sun *et al.* (2014) found MeSH, DMS and DMDS in wastewater samples from a full-scale sewer pipe system. Lebrero *et al.* (2013) identified MeSH, toluene, limonene, benzene, DMTS, acetic acid, benzaldehyde and propionic acid in a sludge sample from a Spanish WWTP. Godayol *et al.* (2013a) detected DMDS, limonene and phenol in air samples collected from around a Spanish WWTP. Another study by Godayol *et al.* (2013b), identified DMDS, toluene, ethylbenzene, *m,p*-xylene, *o*-xylene, phenol, limonene, 1,4-diethylbezene, *m*-cresol, and nonanal in an air sample taken at the inlet to the biological treatment processes of a Spanish WWTP. Urase and Sasaki (2013) measured MIB, geosmin and 2,4,6-trichloroanisole in treated wastewater from a Tokyo WTTP. Agus *et al.* (2012) identified butanethiol, hexanal, *n*-decanoic acid, gamma-heptalactone, 2-pyrollidine, 1-methylnaphthalene, 2-6-dichlorophenol, 2,3-dichloroaniline, vanillin and 5-hydroxyvanillin in a secondary wastewater effluent. Gruchlik *et al.* (2012) found DMS, DMDS and DMTS present in a fresh biosolid sample from a Western Australian WWTP. They further identified indole and skatole from a biosolid sample that had been stored for a number of months.

Further discussion of the analytical techniques used in these methods is provided in Section 5.3.

5.2.1 Analytes Responsible for Sulfurous Odours in Wastewater Systems

Both anoxic and anaerobic conditions at WWTPs can produce sulfur-type odours. H_2S is commonly found under anaerobic conditions. The formation of H_2S arises from two sources: the reduction of sulfate and the desulfurisation of organic compounds containing sulfur in a reduced form. As presented in Table 5.2, H_2S is widely detected in WWTPs and, accordingly, it is commonly used as the surrogate for all sulfur compounds (Suffet *et al.*, 2004a). It is however, questionable as to whether this is the major odorant at WWTPs, as its odour threshold concentration is not as low as that of many other sulfur compounds found in wastewaters. For example, the odour threshold concentration of DMDS is approximately 50 times lower than that of H_2S (0.000026 ppmv *versus* 0.0005 ppmv, respectively (Table 5.2). Since odorous compounds may act additively or synergistically, attributing the odour of a complex material such as wastewater to a single compound is clearly simplistic. In addition, at elevated pH (*e.g.,* as for biosolids which can have a pH

around 8.5), H_2S deprotonates to sulfide (S^{2-}) and H_2S is readily oxidised under aerobic conditions (Suffet *et al.*, 2004a). Bacteria and fungi can also remove sulfide by promoting methylation, producing thiols and various methyl sulfides, the latter of which are oxidised to dimethyl sulfides. A summary of the main sulfur compounds found in wastewaters with their odour threshold concentrations is presented in Table 5.3.

Table 5.3: Main malodorous sulfur compounds present in wastewater systems.

Compound Name	**OTC (ppmv)**
Hydrogen sulfide (H_2S)	0.0005
Dimethyl sulfide (DMS)	0.001
Dimethyl disulfide (DMDS)	0.000026
Dimethyl trisulfide (DMTS)	0.0012
Methanethiol (MeSH)	0.00002

Anaerobic conditions also promote the fermentation of fats, polysaccharides and proteins. During fermentation, these compounds are hydrolysed first to fatty acids, shorter chain saccharides, amino acids, and peptides, and then to shorter chain compounds. The hydrolysis of proteinaceous material (which contains the sulfur-amino acids, cysteine and methionine) and organic sulfur compounds leads to the production of hydrogen sulfide and organic sulfides and disulfides (Vincent, 2001). Domestic wastewater normally contains 3 to 6 mg L^{-1} of organic sulfur in the form of proteinaceous material, and additional organic sulfur in the form of sulfonates (around 4 mg L^{-1}), derived from household detergents (Boon, 1992).

The bacteria responsible for hydrolysis resulting in the production of sulfides are anaerobic or facultative anaerobic species, such as *Proteus* spp,. *Bacteroides* spp. and some *Clostridium* spp (Crowther and Harkness, 1975). The products of fermentation, which are volatile and odorous with low odour thresholds, include the following: organic sulfides, such as EtSH, DMS, DMDS and H_2S (Vincent, 2001). These compounds have been found in numerous studies as discussed in Section 5.2.

5.2.2 Non-Sulfurous Compounds that Cause Odours in Wastewater Systems

In addition to sulfur-containing compounds, nitrogenous compounds, aldehydes and ketones and volatile acids have also been identified as malodorous compounds in wastewater processes (Table 5.1). Biochemical transformations in the growth and metabolism of microrganisms feeding on wastewater components can lead to the formation of such malodorous products (Mosier *et al.*, 1977). This is summarised in Table 5.4.

Table 5.4: Relation of odour constituent to microbial metabolic activity. Reproduced from Mosier *et al.* (1977) Copyright Soil Science Society of America, 1977.

Odour constituent	Microbial metabolic activity
Volatile fatty acids; ketones	Anaerobic carbohydrate fermentation and amino acid deamination
Alcohols	Anaerobic carbohydrate fermentation
Amines	Amino acid decarboxylation; phosphatidylethanolamine-methionine interaction
Mercaptans	Decarboxylation of cysteine, methionine
Hydrogen sulfide	Aerobic and anaerobic catabolism of cysteine and methionine
Ammonia	Aerobic and anaerobic amino acid deamination; denitrification; urea and uric acid decomposition

Ammonia, methyl amine, ethyl amine, dimethyl amine, trimethyl amine, n-butyl amine, dibutyl amine, diisopropyl amine, indole, skatole and pyridine have all been previously identified in waste (Burlingame *et al.*, 2004). Ammonia and trimethyl amine comprise a high concentration of the odorous nitrogen emissions from wastewater. The major biological forms of nitrogen include amino acids and nucleic acids (Suffet *et al.*, 2004a). Decomposition of organic matter containing nitrogen could be responsible for the presence of these compounds, however the relationship between many nitrogen type compounds and other chemicals present and their relative concentrations has not been clearly defined (Suffet *et al.*, 2004b).

Aerobic secondary treatment processes produce oxygenated compounds, such as aldehydes, ketones, alcohols, and volatile fatty acids. Aldehydes and ketones form during biosolids productions due to incomplete organic matter decomposition (Mosier *et al.*, 1977). Ketones and aldehydes can form *via* decomposition of cellulose, starch, hemicellulose, and pectins. Volatile fatty acids also form *via* breakdown of these materials, that are broken down by acid forming bacteria into short-chain fatty acids.

5.3 Analysis of Odorous Compounds in Wastewater Systems

Appropriate analytical techniques are required to measure volatile sulfur compounds, nitrogenous compounds and other volatile compounds in odorous emissions from WW and WW systems. Reported techniques range from onsite measurements with handheld equipment, to more sophisticated instrumentation, usually used offsite. The latter is required for measurement of compounds at ultra-trace levels, for separation of multiple compounds in complex mixtures or for identification of unknown analytes and usually utilises gas chromatography-based methods. Off-site measurements are complicated by the need for gas sampling methods which must retain the integrity of the sample and withstand the rigors of transportation and storage.

Analytical measurement for WWTPs fall into two classes, either quantitiative measurement of a single odorant or qualitative-quantitative measurements for a range of odorants (Gostelow *et al.*, 2001). Often, a particular odorant can be dominant and can give an overall indication of the odour concentrations. H_2S and ammonia are often present in concentrations much higher than any other odorant, and accordingly can be measured directly, without any pre-concentration steps.

H_2S measurement is very common at WWTPs and offers a number of advantages: it is the dominant odorant associated with sewer odours; even when it is not the major odorant it is generally present and acts as a marker compound; the gas phase concentrations can be related to liquid phase concentrations and it is easily measured in low parts per billion concentrations with handheld equipment. Some common handheld analysers use a gold film sensor to adsorb H_2S. The amount of adsorbed H_2S is measured as a proportional change in electrical resistance (Adams *et al.*, 2003). Some instruments also have a response curve for organic sulfur compounds, however, there is no specific method for determining the individual organic sulfur compound being measured.

A field direct measurement for organic sulfur compounds involves the use of sorption tubes. A fixed volume of sample air is drawn through the tube where a

chemical reaction takes place and the sorbent changes colour. These can be used to measure H_2S, DMS, thiols, ammonia and many other compounds. While the accuracy of these devices can vary from source to source, the tube provides a quick indication of the presence of odorants in the air. They have relatively high detection limits, and other compounds can interfere and give false positive results. Their main purpose, is therefore as screening tools (Adams *et al.*, 2003).

Ammonia is another compound determined by specific methods such as colorimetry and titimetry. Ion-selective electrodes are also used for this analysis (Kaelin *et al.*, 2008).

However, due to the complex nature of most odours, comprising a number of different odorants, usually in trace concentrations, it is often difficult to identify the individual compounds without the use of a separation technique. GC is frequently used for this purpose, often followed by FID or MS detection. This analysis is sometimes done in parallel with olfactometry analysis (Urase and Sasaki, 2013; Agus *et al.*, 2012). In most cases however, these techniques are not sensitive enough and it is necessary to pre-concentrate samples prior to analysis, as discussed in Section 1.5.2. Recent analysis of samples from wastewater treatment processes have utilised a variety of different pre-concentration and separation techniques that have identified a broad range of analytes as listed in Table 5.1. The following sections provide further discussion of the methods used for the most recent studies (in the last five years) of wastewater systems.

5.3.1 Analysis of Air Samples from Wastewater Systems

A variety of different methods exist for the analysis of air samples and, while most of these use a pre-concentration step, direct analysis of the air by dilution may also occur (USEPA, 2014a; USEPA, 2014b). USEPA Methods 15 and 16 (USEPA, 2014a; USEPA, 2014b) describe standard methods to sample and measure sulfur in air emissions from air samples. These methods utilise a GC equipped with a FID. Method 15 is for light weight sulfur compounds, such as H_2S, carbonyl sulfide and carbonyl disulfide, while Method 16 is for heavier compounds, such as MeSH, DMS and DMDS (Adams *et al.*, 2003). A sulfur chemiluminescence detector is utilised in another standard method for determining sulfur compounds in gaseous fuels.

Solid sorbent capture is also used as a reference method for the sampling of VOCs and volatile sulfur compounds in air samples. USEPA Method TO17 (USEPA, 1999) describes the analysis of volatile compounds after trapping onto a sorbent followed by thermal desorption and GC-MS. A large variety of sorbents are commercially available, with the choice of sorbent depending upon the target compounds of interest. TO17 details the range of sorbents, with recommendations for their usage. Sorption as a method of pre-concentration is still widely used, however SPME is an alternative pre-concentration technique also being used for volatile sulfur compounds (VSCs) and VOCs (this will be discussed in detail in Chapter 6).

Wang *et al.* (2015) developed a method for the analysis of volatile organic sulfur compounds utilising thermal desorption and GC, coupled to sulfur chemiluminescence detection for a broad range of compounds; H_2S, MeSH, EtSH, DMS, carbon disulfide, ethyl methyl sulfide, 1-butanethiol, DMDS, diethyl disulfide (DEDS) and DMTS. The method detection limits were in the range of 0.1-5.26 ug m^{-3}, with thermal desorption recoveries higher than 66%. Active collection of the samples on a cold trap enabled small volumes of gas to be sampled without breakthrough. The method was then utilised to analyse sewer air samples (Wang *et al.*, 2015).

Godayol *et al.* (2013a) developed an analysis method, using SPME followed by GC coupled to MS to monitor a group of odour-causing compounds previously found in wastewater samples, including DMDS, phenol, indole, skatole, octanal, nonanal, benzothioaole and some terpenes. Sampling was performed in a glass bulb, which had been flushed with sample. The SPME fibre was inserted and exposed to the analytes for 10 minutes. The method was validated with method detection limits ranging from 0.1 – 20 ug m^{-3}. These method detection limits were below the respective odour threshold concentrations for each of the target compounds. The method was then applied to air samples collected from a WWTP, which identified only DMDS, phenol and limonene. The identification of 26 compounds was however possible using this developed method (Godayol *et al.*, 2013a).

A further study by Godayol *et al.* (2013b) utilised thermal desorption, followed by GC-MS for the determination of 16 volatile organic compounds in air samples, including: 1,4-dioxane, DMDS, toluene, ethylbenzene, *m*-xylene, *p*-xylene, *o*-xylene, phenol, 1,2,3-trimethylbenzene, limonene, 1,4-diethylbenzene, *m*-cresol, nonanal, benzothiazole, carvone, indole and skatole. For calibration purposes, liquid standards were loaded on to Tenax TA/Carbograph 1TD tubes, followed by helium. The tube was then immediately desorbed and analysed. This study looked at a range of compounds belonging to different chemical families thus having different volatilities and chromatographic behavior. The method was optimised to ensure good separation of target compounds within a reasonable analysis time. The limits of detection for the method ranged from 0.2 to 2 ug m^{-3}, except for nonanal which had an LOD of 20 ug m^{-3}. A stability study on the storage of tubes loaded with all analytes found that there was no significant loss of analytes after three and seven days of storage (Godayol *et al.*, 2013b). The method was then used to analyse air samples which were collected onto tubes from the inlet and sludge pre-treatment areas of a WWTP.

5.3.2 Analysis of Aqueous Samples from Wastewater Systems

The presence of odour compounds can be investigated directly in wastewater samples. Conventionally, purge and trap and closed-loop stripping methods have been applied as pre-concentration techniques. These methods use a gas stream to liberate compounds from the aqueous phase, after which they are trapped on a solid sorbent. In the case of closed loop stripping, the analytes are desorbed from the sorbent using a liquid solvent prior to introduction to the GC inlet. For purge and trap methods they are generally thermally desorbed directly onto the GC column (Ginzburg *et al.*, 1999; Mayer *et al.*, 1995) (James and Stack, 1997). SPME is now also being used for the pre-concentration of odorous compounds for the analysis of wastewater samples.

Sun *et al.* (2014) developed a static headspace method utilising GC coupled with a sulfur chemiluminescence detector for the analysis of VSCs in wastewater samples. The method was developed for MeSH, DMS and DMDS. No pre-concentration technique was required. The method achieved detection limits ranging 0.12 to 0.21 ug L^{-1}. The method was then utilised for studies of wastewater samples from a full scale sewer pipe.

Urase and Sasaki (2013) examined the concentration of the earthy musty compounds contained in biologically treated wastewater using HS SPME coupled with GC-MS. The compounds of interest were 2,4,6-trichloroanisole, geosmin and MIB. They used analysis methods by Godayol *et al.* (2011), with modifications for the 2,4,6-trichloranisole. The fibre was introduced into the headspace of the sample for extraction, after being desorbed in the GC-MS. They achieved quantification limits ranging between 0.1 and 0.5 ng L^{-1}. The method was used to identify all of the target analytes in treatment wastewater.

Godayol *et al.* (2011) also used HS SPME as an extraction technique coupled with GC-MS to characterise a list of compounds belonging to different chemical families in wastewater matrices. These compounds included DMDS, phenol, octanal, limonene, *m*-cresol, nonanal, carvone, indole and skatole. A variety of fibre phases were investigated, as well as time and extraction temperature. The optimised method was validated with detections limits ranging between 0.03 and 0.6 ug L^{-1}. Each target compound was then identified in analysis of a wastewater sample collected from a WWTP (Godayol *et al.*, 2011).

As evident by some of the more recent wastewater studies, SPME is increasingly being used as a pre-concentration technique for the detection of odorous compounds. The use of SPME as a pre-concentration technique for the analysis of compounds associated with wastewater odours is the focus of Chapters 6 and 7 and will be further discussed in these chapters.

5.4 Formation of Odours in Wastewater Systems

Odour sources in WWT systems can be broadly divided into two classes (Gostelow *et al.*, 2001):

1. "those which promote mass transfer for odorants which have already formed (for example in the sewage system), and
2. those in which new odorants are formed" (Gostelow *et al.*, 2001).

Accordingly, the main odour sources at a WWTP tend to be at the inlet, if the incoming sewage is septic, or associated with the sludge forming process when anaerobic conditions result in formation of new odorants (Gostelow *et al.*, 2001).

Lebrero *et al.* (2011) carried out an extensive review of odours within WWTPs and concluded that primary treatments and sludge handling activities constituted the two major contributors to odour impact in most WWTPs with a significant contribution from receiving influent. This was based on findings from a variety of studies. Vincent (2001), based on the evaluation of 26 sewerage and WWTPs, reported that primary settlers, sludge digestion tanks and sludge thickening and dewatering facilities were the main causes of odour problems.

Frechen (2004) reported similar results, measuring the OEC from several stages within a German WWTP, with the greatest OEC being produced from raw sludge dewatering. Dewatering of other material, such as excess sludge and digested sludge also produced high OECs. Domestic wastewater without odour problems should be below 5,000 $OU_E\ m^{-3}{}_{liquid}$; yet Frechen reported values up to 10,000,000 $OU_E\ m^{-3}{}_{liquid}$ for the raw sludge dewatering process and 1,000,000 $OU_E\ m^{-3}{}_{liquid}$ for a sewer (Frechen, 2004).

Adams *et al.* (2003) carried out an extensive review of the sources of odours in wastewater treatment processes as shown in Table 5.2. Most of the unit processes within a treatment plant were shown to have an odour problem at some stage, with varying concentrations of odorants and odour intensities.

5.5 Management of Odours

Odour management of emissions from sewer systems and WWTPs has traditionally been maintained by a number of different processes, including the use of buffer zones to separate odorous sources from population centers, the dispersion of emissions through ventilation stacks and the use of chemical dosing to prevent anaerobic conditions and control odour production (Sivret and Stuetz, 2012). However, due to increasing numbers of complaints and the pressure on the land surrounding sewer ventilation points, pumping stations and WWTPs, the installation

of odour abatement processes (including activated carbon, biofilters, biotrickling filters and chemical scrubbers) has become necessary in order to manage the production of odours to a limit which is acceptable.

5.5.1 Prevention of Odours from Wastewater Systems

Odour prevention in WWTPs requires efficient design and operation practices. The prevention of odour formation in sewers is crucial, however this is often difficult due to existing design parameters. The design of gravity sewers is one of the most common approaches (Lebrero *et al.*, 2011). In gravity sewers, fermentative and sulfur-metabolising microorganisms establish in larger numbers in the deposit, however, natural aeration provides a supply of oxygen to the wastewater which is often enough to maintain aerobic conditions. By promoting aerobic metabolism, the anaerobic metabolism required for the production of sulfide and odour formation is prevented (Yang and Hobson, 2001).

5.5.2 Treatment of Odorous Emissions from Wastewater Systems

If the prevention or minimisation of odorant emissions is not possible, odour abatement and treatment technologies are applied. A range of technologies is available to treat odorous air emitted from wastewater treatment plants, sludge handling facilities, and industrial processes. According to Burgess *et al.* (2001) there are three methods of odour treatment:

1. Biochemical *i.e.,* biofilters, bioscrubbers, activated sludge
2. Chemical *i.e.,* chemical scrubbers, thermal oxidation, catalytic oxidation, ozonation; and,
3. Physical *i,e.,* condensation, adsorption (activated carbon), absorption (clean water scrubbers).

5.5.2.1 Biochemical Treatment

Biofilters are the most commonly employed biotechnology in WWTPs odour treatment. In 2005, more than 300 biofilters were under operation in the United States WWTPs (Iranpour *et al.*, 2005). Air in biofilters is forced through a fixed bed hosting the microbial community capable for odorants mineralization (Figure 5.2 a). Odorants first diffuse from the gas phase into the biofilm before degradation can occur.

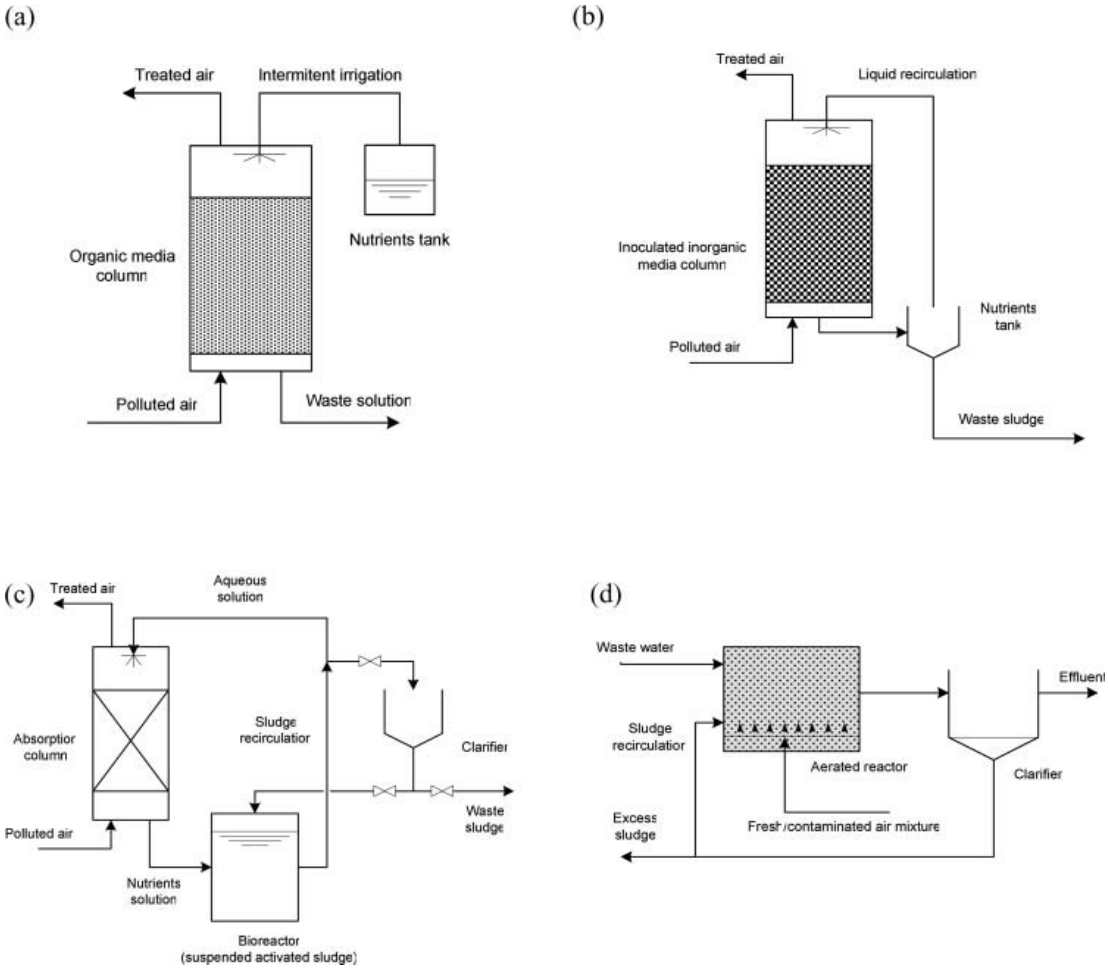


Figure 5.2: Biological treatment technologies for odour treatment: (a) biofilter, (b) biotrickling filter, (c) bioscrubber, and (d) activated sludge diffusion system. Reprinted with permission from (Lebrero *et al.*, 2011). Copyright Taylor & Francis, 2011.

In biotrickling filters, odorous emissions are passed through a packed bed of microrganisms immobilised onto a structured material, generally inorganic packing and continuously irrigated by nutrients in aqueous solution (Figure 5.2 b). Iranpour *et al.* (2005) carried out a survey that indicated both biofilters and biotrickling filters were capable of combining a high H_2S and odour removal efficiency with VOC removal. The removal of VOCs holds potential for reducing the overall toxicity and carcinogenicity of the waste air (Iranpour *et al.*, 2005). Further discussion of biotrickling filters is provided in Chapter 7.

Bioscrubbers and activated sludge diffusion systems are also employed to treat odorous emissions (Figure 5.2 c and d).

5.5.2.2 Chemical and Physical Treatment

Chemical scrubbers are among the most commonly employed abatement techniques in WWTPs due to the extensive experience in their design and operation (Lebrero *et al.*, 2011). Odorants are transferred from the air into an aqueous solution (often sulfuric acid or sodium hydroxide) and then can be destroyed by chemical oxidation. Oxidants used in this process may include sodium hypochlorite, sodium hydroxide, potassium permanganate, or hydrogen peroxide (Lebrero *et al.*, 2011).

Activated carbon, silica gel or zeolite based adsorption systems in WWTPs have also been widely employed. For this process, odorants are adsorbed onto a fixed bed of adsorbent by intermolecular forces. This process is convenient when hydrocarbons, thiols or other oxygenated organic compounds are the main odorants in WWTP emissions (Lebrero *et al.*, 2011).

5.6 Scope of Work in Chapters 6 and 7 of this Study

Chapters 6 and 7 in this Thesis describe studies of odorous compounds associated with wastewater systems, and their identification and measurement using SPME/GC-MS. Chapter 6 focuses on the development of an analytical method for detection of sulfurous compounds and some other volatile compounds which have been associated with nuisance odours from wastewater systems. Chapter 7 focuses on application of the analytical method developed in Chapter 6 to investigate the compounds that are present during the collection and treatment of wastewater and associated by-products and their subsequent reduction and/or removal.

Chapter 6

Development of an Analytical Method using Solid-Phase Microextraction for the Identification of Compounds Associated with Wastewater System Odours

6.1 Introduction

Volatile sulfur compounds (VSCs) are one of the main classes of compounds responsible for odours in wastewater systems. They are well known for their characteristic odour, even when present at very low concentration, due to their low sensory threshold value. As discussed in Section 5.3, there are a number of different analysis techniques for the identification and quantification of VSCs. Quite often samples require pre-concentration due to low concentrations present in the sample and volatility of the analyte of interest. The most commonly used pre-concentration techniques for the analysis of VSCs include trapping the analytes onto a solid state sorbent (sample tube) and SPME. Traditionally, purge and trap and closed-loop stripping techniques were employed. The focus of this study is the use of SPME and this will be further discussed in the following sections.

6.1.1 Solid-Phase Microextraction for Analysis of Sulfur Compounds

The analysis of VSCs by SPME has been applied to many different sample types, namely wine and beer, various food products, water and wastewater samples (termed aqueous) and also air samples. A summary of the use of SPME to detect the presence and measure the concentrations of certain VSCs in all of these sample matrices is provided in Table 6.1 and the analysis of sample types relevant to this study (aqueous and air samples) is further discussed in the following Section, focusing on more recent studies.

Table 6.1: Studies utlising SPME as a pre-concentration technique for the analysis of volatile sulfur compounds across various sample types.

Sample Type	References
Wine	López *et al.* (2007); Fang and Qian (2005); Mestres *et al.* (2000); Mestres *et al.* (1997)
Beer	Li *et al.* (2008); Sulak *et al.* (2008); Pinho *et al.* (2006); Hill and Smith (2000)
Food products	Carpino *et al.* (2010); Burbank and Qian (2008); Burbank and Qian (2005); Garcia-Esteban *et al.* (2004); Fan *et al.* (2002)
Aqueous samples	Kristiana *et al.* (2010b); Abalos *et al.* (2002); Godayol *et al.* (2011); Ding *et al.* (2014); Lu *et al.* (2012); Sekyiamah *et al.* (2006); Popp *et al.* (1999)
Air samples	Haberhauer-Troyer *et al.* (1999); Lestremau *et al.* (2003); Lestremau *et al.* (2004); Murray (2001); Nielsen and Jonsson (2002a, 2002b); Ras *et al.* (2008b); Sekyiamah *et al.* (2006)

6.1.1.1 Analysis of Aqueous Samples for Sulfurous Compounds using Solid-Phase Microextraction

A range of different aqueous samples have been analysed for the presence of VSCs utilising SPME (Table 6.1), including beer and wine and also water and wastewater samples, referred to here as aqueous samples. The following discussion focuses on the analysis of these aqueous samples.

Popp *et al.* (1999) developed a range of methods for the determination of volatile and semi-volatile sulfur compounds. The volatile sulfur compounds (DMS, CS_2, ethyl methyl sulfide (EMS), thiophene and DMDS) were analysed using a CAR-PDMS fibre *via* HS SPME followed by GC-MS. Method detection limits were 1 ng L^{-1} for each analyte. For the semi-volatile sulfur compounds (which included various thiophene analogues) a PA fibre was found to be more suitable, utilising direct immersion of the fibre into the sample, followed by GC-MS. Detection limits of 0.4 – 1.5 ng L^{-1} were achieved. These methods were then applied to the analysis of a number of environmental samples, including a contaminated groundwater sample from a landfill and a wastewater sample. DMS and DMDS were identified using the CAR-PDMS fibre, while the semi-volatile compounds were identified using the PA fibre. Each of the volatile compounds were identified in all of the samples analysed. This study highlighted that use of multiple fibres may be required for optimum analysis of a range of sulfur compounds with a wide range of boiling points (Popp *et al.*, 1999).

Abalos *et al.* (2002) developed a quantitative SPME method for the determination of several alkyl sulfide analytes (DMS, EMS and DMDS) from wastewater samples. The linear dynamic range was close to three orders of magnitude for all compounds studied. Low detection limits (ranging from 0.7 to 4 ng L^{-1}) were also achieved. The method was then applied to several wastewater samples.

Sekyiamah *et al.* (2006) identified and quantified a range of significant VSCs associated with activated sludge treatment at a large wastewater treatment plant in the United States using HS SPME coupled to GC-MS. DMS and DMDS were identified in both water and air samples collected from the treatment plant. Air samples were collected in Tedlar® bags, in which the fibre was introduced after

sample collection. For water samples, the fibre was introduced into the headspace of 20 mL of sample. The fibre used in this study was an 85 μm CAR-PDMS fibre. The method had been optimised for the analysis of CS_2, MeSH, EtSH, propanethiol, butanethiol, DMS and DMDS, with method detection limits ranging from 1 ppbv to 13 ppbv (Sekyiamah *et al.*, 2006).

Kristiana *et al.* (2010b) were also successful in the development of an SPME method to be used for the analysis of polysulfide compounds present in drinking water distribution systems. A quantitative analysis method for DMS, DMDS and DMTS utilising deuterated analogues for internal standards was validated and then applied. Detection limits ranging from 50 to 240 ng L^{-1} were achieved. In both studies the different parameters that affected optimisation of the SPME analytical method were discussed.

Godayol *et al.* (2011) developed an HS SPME method coupled with GC-MS to characterise a range of compounds found in wastewater samples. These included DMDS (as well as a range of other volatile non-sulfur compounds). Detection limits ranged from 300 – 600 ng L^{-1} (Godayol *et al.*, 2011).

A simple and efficient method based on HS SPME followed by GC-FID was developed by Lu *et al.* (2012) for the determination of five VSCs (H_2S, MeSH, DMS, DMDS and DMTS) in freshwater lakes where odour compounds were associated with excessive algal growth. The CAR-PDMS fibre was chosen, and method quantification limits ranged from 10 to 500 ng L^{-1}. High reproducibility and recovery was achieved by this method, and it was successfully applied to the analysis of odorous freshwater lakes, in which all five target compounds were identified and quantified (Lu *et al.*, 2012).

Ding *et al.* (2014) developed an SPME method for the analysis of five odorants present in environmental waters which included DMTS. GC-MS detection was used. The limits of detection achieved were all below 1.3 ng L^{-1}. The fibre phase chosen for this study was a DVB-CAR-PDMS fibre (due to the range of other compounds of interest, including geosmin, MIB and cyclocitral) (Ding *et al.*, 2014).

6.1.1.2 Analysis of Air Samples for Sulfurous Compounds using Solid-Phase Microextraction

As detailed in Table 6.1, a number of studies have investigated the quantitative analysis of VSCs with SPME followed by GC analysis. The success of these studies was varied, and some of the difficulties and limitations that were encountered are detailed below. Some of the common difficulties encountered include (but are not limited to) loss of analytes from the fibre during storage, contamination of fibres within the storage container and loss of analytes due to competition for active sites on the fibre phase. The susceptibility of dimethylpolysulfides to disproportionation and thermal degradation was only encountered in a few of the studies..

Haberhauer-Troyer *et al.* (1999) generated gaseous standard mixtures of four volatile sulfur compounds and then spiked them into air samples and sampled with the SPME fibre. Three different fibre coatings were examined. They found that several factors limited the usefulness of the method for quantitative on-site analysis. These included the presence of artefacts during analysis, low storage stability of the samples pre-concentrated on the SPME fibre, the effect on extraction of relative humidity and differences in sensitivities between fibres.

A study carried out by Murray (2001) on volatile organic sulfur compounds produced by brassica plants highlighted the limitations in the use of CAR-PDMS phase SPME fibres. In this study, a comparison of the analytical data derived from SPME and direct gas sampling of standard mixtures found that higher molecular weight compounds displaced the lower molecular weight ones, as a consequence of competition for active sites on the fibre. This resulted in the proportions of components being absorbed onto the fibre depending on their ratio in the sample and in erratic calibration curves. For calibration purposes it is necessary for all components present in the sample to be present in the standard and to also be at the same relative concentrations. The study suggested that this practicality may limit the use of this fibre for quantitative analysis of standard mixtures. It should be noted that the concentration range examined within this study was in the mg L^{-1} range which is well above the odour threshold concentrations of these compounds and often well above the concentrations of these compounds in environmental air samples.

Lestremau *et al.* (2003) examined the CAR-PDMS coated SPME fibre for the analysis of several sulfur compounds. Due to competitive adsorption from compounds on this fibre phase, external calibration was carried out at a predetermined concentration range, based on Fick's diffusion law. The method was tested on a real gaseous sample and it was found that for the heavier compounds (EtSH, DMS and DMDS) the external calibration method was suitable. However for the lighter compounds (H_2S and MeSH) a standard addition method was required for accurate quantification.

In a subsequent study, Lestremau *et al.* (2004) went on to examine the formation of artefacts during the analysis of volatile sulfur compounds using SPME with a CAR-PDMS coated fibre. This fibre has been found to be the most sensitive (lowest detection limits) for sulfur compounds. Among the large range of compounds studied, thiols were found to react to form the corresponding dimers, for example, MeSH formed DMDS during analysis using a CAR-PDMS coated fibre. Further investigation into the likely cause of the dimerisation led researchers to postulate that the artefact formation was due to the presence of metal in the carboxen of the fibre phase, and to be independent of sample matrix. In the presence of several oxygenated and amine compounds, which are common industrial effluent components, no further artefact formation or reaction was noticed, therefore the metal phase of the fibre was believed to cause the dimerisation. Thermal oxidation was also noticed during the desorption step in the GC injection port. Some possible suggestions were to use a PDMS fibre, which required a lower desorption temperature, however the sensitivity of methods using this phase was not sufficient to achieve detection limits at or near the odour threshold concentrations of the analytes.

Two studies carried out by Nielsen and Jonsson (2002a, 2002b) described successful quantification of nine volatile sulfur compounds found in complex gaseous samples by extraction using SPME with a CAR-PDMS fibre. In the initial study Nielsen and Jonsson (2002b) optimised the conditions using experimental design, and detection limits ranged from 1 ppt to 350 ppt, while the linear range of calibration was found to be up to 20 ppb for all of the compounds. They reported issues in quantifying methanethiol due to its oxidation to dimethyl disulfide and stated that this must be taken into consideration if using the method to quantify these compounds. They then

applied this method to the analysis of samples collected at a biogas production plant (Nielsen and Jonsson, 2002a).

Ras *et al.* (2008b) utilised SPME for the determination of seven sulfur compounds (ethanethiol, DMS, carbon disulfide, propanethiol, butanethiol, DMDS and 1-pentanethiol), optimising temperature and time extraction conditions and then validating the method. It was then applied to characterisation of odour in air samples taken from a sewage treatment plant. Good method detection limits (0.01 and 0.08 $\mu g\ m^{-3}$) and linearity (0.1 to 1000 $\mu g\ m^{-3}$) were achieved and the method was shown to be suitable for the analysis of complex samples.

6.1.1.3 Analysis of Solid Samples for Sulfurous Compounds using Solid-Phase Microextraction

A variety of solid samples associated with wastewater treatment have been analysed by SPME followed by GC. A number of biosolids projects have utilised SPME to analyse sludges or biosolids. For example, Kim *et al.* (2005; 2002a; 2002b) have used SPME followed by GC-MS for the analysis of carbon disulfide, DMS, methyl and butyl thiols and DMDS in thickened and dewatered sludge and biosolids samples. They also analysed trimethyl amine and propionic and butyric acids with the same method. Visan and Parker (2004) used SPME followed by GC-MS for the analysis of DMS, DMDS and MeSH in stored biosolids, while Turkmen *et al.* (2004) used SPME/GC-MS for analysis of DMS, DMDS, MeSH, hydrogen sulfide and carbon disulfide in anaerobically digested wastewater sludge. Ramos *et al.* (2002) utilised SPME followed by GC-MS to identify a range of volatile compounds from composting biosolids, including CS_2 and DMDS. The method also identified a range of other compounds including benzene, chloroform, toluene and ethylbenzene.

6.1.2 Analysis of Nitrogen Compounds using Solid-Phase Microextraction

Other classes of odour compounds also associated with wastewater systems include nitrogen-containing compounds. A range of volatile nitrogen compounds have been identified in wastewater systems and SPME has been used as the extraction techinque in other areas.

SPME analysis of odorous nitrogen compounds has focused largely on samples from the livestock industry, mainly from cattle and pig farms. Most work was carried out on gas samples (Cai *et al.*, 2006a; Cai *et al.*, 2006b), however some studies have been carried out on slurry samples (Larreta *et al.*, 2006) and varying methods of analysis were investigated for their ability to provide reliable quantitative results. Analyses were also routinely carried out coupled with olfactometry measurements (Cai *et al.*, 2006a; Cai *et al.*, 2006b).

Cai *et al.* (2006a; 2006b) carried out studies to characterise the odours from swine barns using SPME and GC-MS-olfactometry. A large number of analytes were investigated, including a suite of VOCs, and the nitrogen-containing compounds, indole and skatole. The method was developed trialling several fibre phases, with the final choice being CAR-PDMS fibre. The method was successful in identifying analytes, but was not used for quantitative analysis.

Larreta *et al.* (2006) employed an experimental design to optimise the analysis of volatile compounds in cow slurry by HS SPME/GC-MS. A Plackett-Burman design was sufficient in order to discriminate which variables affected the HS SPME process. The variables investigated were type of fibre, pH of the slurry, addition of sodium chloride, sample volume, exposure time and the temperature of the sample when it was exposed to the fibre. A large set of analytes was selected for simultaneous determination of a range of volatile fatty acids, phenols and indoles. The different physicochemical properties of the analytes resulted in differing interactions with the various fibre phases used: the DVB-CAR-PDMS fibre was optimal for the indoles and phenols, while the CAR-PDMS was more suited to the acids. The method was validated by constructing calibration curves, exploring repeatability and also matrix effects. It was found to be suitable for most of the analytes, however it was suggested that derivatisation might improve detection of the acids due to improvement in chromatographic behaviour.

Tong *et al.* (2010) compared SPME and SPE as a pre-concentration technique for the analysis of odorous substances, including the nitrogenous indole, skatole and also two methylphenols from piggery wastewater samples. SPME gave better limits of quantification (0.09 - 0.96 $\mu g\ L^{-1}$) and linearity but SPE was more suited to

recovering a wider analyte range, allowing for more volatile compounds to be identified and quantified.

6.1.3 Analytical Methods Using Solid-Phase Microextraction for Multiple Groups of Analytes

A variety of analytical methods using SPME have been developed for multiple groups of analytes, to allow for the simultaneous detection of a variety of compounds causing odours. The more common multiple analyte methods include analysis for VOCs, nitrogen compounds and volatile fatty acids. The detection of sulfur compounds generally requires a separate analytical method due to their higher reactivity. Compounds analysed using SPME are summarised in Table 6.2, while SPME methods for multiple analytes are summarised in Table 6.3. Some of these methods have already been described in Sections 6.1.1.3 and 6.1.2.

Table 6.2: Summary of published data on sewage odorants, their odour properties and analytical methods using SPME.

Compound	Odour Character	Air odour threshold (ppmv)	Water odour threshold (ppm)	Method of Analysis	*LOD* and/or LOQ	Reference
Sulfur Compounds						
Ethyl mercaptan	Rotten cabbage	0.00001	0.0000075	SPME-GC/MS	*0.026* & 0.25 µg m^{-3}	Ras *et al.* (2008b)
Hydrogen sulfide	Rotten eggs	0.0005	0.000029	SPME-GC/MS	Not stated	Turkmen *et al.* (2004)
Carbon disulfide	Disagreable, sweet	0.0077	0.00039	SPME-GC/MS	*0.003* & 0.10 µg m^{-3}	Ras *et al.* (2008b)
				SPME-GC/MS	Not stated	Turkmen *et al.* (2004)
				SPME-GC/MS	*0.150* ppbv	Kim *et al.* (2002a)
Dimethyl sulfide	Rotten cabbage	0.001		SPME-GC/MS	*0.017* & 0.15 µg m^{-3}	Ras *et al.* (2008b)
				SPME-GC/AED	*0.003-0.004* ppb	Haberhauer-Troyer *et al.* (1999)
				SPME-GC/MS	Not stated	Turkmen *et al.* (2004)
				SPME-GC/MS	*0.074* ppbv	Kim *et al.* (2002a)
				SPME-GC/MS (Aqueous media)	*0.23* & 0.39 µg L^{-1}	Kristiana *et al.* (2010b)
				SPME-GC/MS (Aqueous media)	*0.004* µg L^{-1}	Huang *et al.* (2004)

Compound	Odour Character	Air odour threshold (ppmv)	Water odour threshold (ppm)	Method of Analysis	*LOD* and/or LOQ	Reference
Volatile Fatty Acids						
Acetic acid	Vinegar	1.019	97	SPME-GC/MS NCI mode (Aqueous media)	*150* µg L^{-1}	Huang *et al.* (2004)
				SPME-GC/MS (also with derivatision)	*1358* µg L^{-1} *707* µg L^{-1}	Larreta *et al.* (2007); Larreta *et al.* (2006)
Propionic acid	Rancid, pungent	0.028		SPME-GC/FID	*1.85* ppbv	Kim *et al.* (2002a)
				SPME-GC/MS (also with derivatision)	*1441* µg L^{-1} *250* µg L^{-1}	Larreta *et al.* (2007); Larreta *et al.* (2006)
Isobutyric and butyric acid	Rancid	0.0003		SPME-GC/FID	*1.32* ppbv	Kim *et al.* (2002a)
				SPME-GC/MS (also with derivatision)	*0.11 (I) &* *0.7* µg L^{-1} *341 (I) &* *53* µg L^{-1}	Larreta *et al.* (2007); Larreta *et al.* (2006)
Isovaleric acid	Unpleasant	0.0006		SPME-GC/MS NCI mode (Aqueous media)	*2* µg L^{-1}	Huang *et al.* (2004)
				SPME-GC/MS (also with derivatision)	*1.8* µg L^{-1} *0.05* µg L^{-1}	Larreta *et al.* (2007); Larreta *et al.* (2006)
Valeric acid	Unpleasant	0.0006		SPME-GC/MS NCI mode (Aqueous media)	*2* µg L^{-1}	Huang *et al.* (2004)
				SPME-GC/MS (also with derivatision)	*0.056* µg/L *0.11* µg/L	Larreta *et al.* (2007); Larreta *et al.* (2006)
Odorous Volatile Aromatic Compounds						
Indole	Faecal nauseating	0.13-1.5 ppbv		SPME-GC/MS (also with derivatision)	*1* µg/L *0.017* µg/L	Larreta *et al.* (2007); Larreta *et al.* (2006)
Skatole	Faecal nauseating	0.065-0.15 ppbv		SPME-GC/MS (also with derivatision)	*0.05*1 µg/L *0.004* µg/L	Larreta *et al.* (2007); Larreta *et al.* (2006)
p-cresol	Medicine	0.011-5.4 ppbv		SPME-GC/MS (also with derivatision)	*0.14* µg/L *0.010* µg/L	Larreta *et al.* (2007); Larreta *et al.* (2006)
Toluene	Sweet, pungent	2.9				Numerous
Ethylbenzene	Gasoline	2.3				Numerous
Styrene	Sweet	320 ppbv		SPME-GC/MS	Not stated	Vilaplana *et al.* (2010)

Table 6.3: Summary of SPME-GC analytical methods for multiple odorants

Analytes	Fibre	Reference
Carbon disulfide , dimethyl sulfide, dimethyl disulfide, ethyl mercaptan, propyl mercaptan, butyl mercaptan and 1-pentanethiol	CAR-PDMS 75 µm coating	Ras *et al.* (2008b)
Methyl mercaptan, dimethyl sulfide, isopropane mercaptan, isobutane mercaptan	CAR-PDMS 75 µm coating	Haberhauer-Troyer *et al.* (1999)
Dimethyl sulfide, methyl mercaptan, dimethyl disulfide, H_2S, carbon disulfide	CAR-PDMS 75 µm coating	Turkmen *et al.* (2004)
Trimethyl amine, carbon disulfide, dimethyl sulfide, dimethyl disulfide Propionic acid, butyric acid	CAR-PDMS 75 µm coating PA 85µm coating	Kim *et al.* (2002a)
Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide	PDMS fibre 100 µm coating	Kristiana *et al.* (2010b)
Methyl amine, dimethyl amine, trimethyl amine, ethyl amine, propyl amine, isopropyl amine, *sec*-butyl amine, *tert*-butyl amine	PDMS fibre 100 µm coating	Huang *et al.* (2004)
Carbonyl sulfide, H_2S, methanethiol, carbon disulfide , SO_2, diethyl sulfide, ethanethiol, 1-propanethiol, 2-propanethiol, 1-butanethiol, 1-pentanethiol, 2-methyl-2-propanethiol, furfuryl mercaptan, dimethyl sulfide, n-butyl sulfide, n-butyl disulfide, thiophene, 2-methylthiophene, 2,5-dimethylthiophene, 2-ethylthiophene, 1,2-ethanedithiol, benzyl mercaptan, *p*-thiocresol, ethyl methyl sulfide, diethyl disulfide and 3-methylthiophene, *sec*-butyl sulfide , dimethyl disulfide	CAR-PDMS 75 µm coating	Li and Shooter (2004)
Acetic acid, phenol, propanoic acid, isobutyric acid, 4-methylphenol, butyric acid, isopentanoic acid, 4-ethylphenol, pentanoic acid, hexanoic acid, indole, heptanoic acid, 3-methylindole (skatole)	DVB-CAR-PDMS 50/30 µm coating, with derivatisation	Larreta *et al.* (2007); Larreta *et al.* (2006)
Styrene	CAR-PDMS	Vilaplana *et al.* (2010)

6.1.4 Scope of Work in Chapter 6 of this Study

As outlined in Section 1.7, the second part of this Thesis focused on the establishment of an analytical method for the analysis of several VSCs associated with wastewater systems: ethanethiol, dimethyl sulfide, ethyl methyl sulfide, dimethyl disulfide, diethyl disulfide and dimethyl trisulfide. These analytes were chosen as they had been previously identified in conjunction and/or associated with wastewater systems. Accordingly, the aim of the research presented in this Chapter was to develop a simple method for the analysis of these VSCs in aqueous samples and adapt the method to air samples. The process of development and validation of the aqueous method will be described in the following sections of this Chapter.

Utilisation of this method to identify several other odorous compounds associated with wastewater systems is detailed. The difficulties associated with adaptation of the method for air analysis resulting in only a qualitative method are also described. SPME in conjunction with GC-MS for the analysis of VSC and other odorous compounds was chosen as the analytical method by considering the advantages and disadvantages of existing analytical methods as well as the availability of instruments and materials in the laboratory.

6.2 Experimental

6.2.1 Chemicals and Reagents

Inorganic reagents, organic solvents, and organic compounds were of analytical grade purity (AR) or better, and were used without further purification.

6.2.2 Analysis of Aqueous Samples

The samples were analysed *via* automated HS SPME using a 75 μm CAR-PDMS fibre, followed by GC-MS. Automated HS SPME was performed on a Gerstel Multipurpose Sampler MPS2, which was interfaced to an Agilent Technologies Series II GC 6890N with an Agilent Technologies 5973N Mass Selective Detector. The sample volume used for aqueous SPME was 10 mL in a 20 mL glass vial. Sodium sulfate (3.00 g) and an aliquot of the internal standard (5 μL of a 50 ng/μL working solution of dimethyl-d_6 disulfide) were added to each sample. The autosampling device transferred the vial into the agitator, where it was agitated at 500 rpm at 40 °C for 10 minutes. The SPME fibre was then introduced into the headspace of the sample for 10 minutes and then transferred to the GC injector for thermal desorption of analytes.

The SPME fibre was desorbed at 230 °C for 4 minutes in the injector port of the GC. The sample was introduced *via* a splitless injection (100 mL min^{-1}. with a 1 min. purge time) onto a fused silica 5% phenyl – 95% dimethylpolysiloxane capillary column (30 m x 0.25 mm id, column phase thickness of 1 μm (ZB-5; Phenomonex)). Helium was used as the carrier gas operating at a flow of 1 mL min^{-1}. The oven temperature was initially set at 0 °C for 2 minutes prior to being increased at a rate of 5 °C min^{-1}. until 35 °C, then 15 °C min^{-1} to a temperature of 315 °C with a final hold

time of 10 minutes. The samples were analysed by mass spectrometry in the SIM mode with the following ions monitored: m/z 45, 47, 50, 61, 62, 66, 68, 76, 79, 94, 122 and 126. A dwell time of 50 ms was used for all ions.The characteristic m/z for each compound is as follows: ET 47, 62; DMS 45, 47, 62; EMS 61, 76; DMDS 79, 94; DMTS 79 126 and DMDS-d_6 122.

Calibration of sulfur compounds was achieved by analysis of a series of external standards (diethyl disulfide (99 %), dimethyl disulfide (≥ 98 %), dimethyl sulfide (≥ 99 %),), dimethyl trisulfide (> 98 %), ethyl methyl sulfide (96 %); Sigma Aldrich). Individual stock solutions for each analyte and dimethyl-d_6 sulfide (99 atom % D, Sigma Aldrich) were prepared in HPLC grade methanol (Mallinckrodt Baker) at 5000 mg L^{-1} and stored at 4 °C. A working standard solution containing each analyte was prepared from the stock solutions using appropriate dilution with MilliQ water and further standards were prepared by appropriate dilution of this solution as required. Dimethyl-d_6 sulfide was used as an internal standard for aqueous samples, and was added at a concentration of 250 ng L^{-1} to each sample.

6.2.3 Validation Procedure

The aqueous method was validated under optimised conditions as described in Section 6.2.2, by determination of linearity, detection and quantification limits, and repeatability and reproducibility. The effect of sample matrix on analysis of VSCs was investigated. A sample from a wastewater treatment plant was collected and analysed by the optimised method, and then spiked with known concentrations of VSCs and their recovery was calculated.

6.2.4 Analysis of Gaseous Samples

A mixed standard of VSCs at concentrations of 50, 150, 250, 500 and 1000 ng L^{-1} in methanol was added to a 20 mL vial, heated to 100 °C for 30 seconds and then left at room temperature until extraction by SPME. Each sample was agitated at 30 °C for 10 minutes, after which the SPME fibre was placed into the gas sample for 10 minutes before being desorbed in the heated GC injector. The sample was then analysed by GC-MS, as described in Section 6.2.2. This will be referred to as Method A.

Another method of standard preparation was investigated, and this was to add a mixed standard of VSCs at concentrations of 50, 150, 250, 500 and 1000 ng L^{-1} in methanol to a 20 mL vial, transfer it to the sample tray, after which the autosampling device transferred the vial into the agitator, which was set at 100 °C. The fibre was then introduced into the sample for 30 minutes and then transferred to the GC injector for thermal desorption. The sample was then analysed by GC-MS, as described in Section 6.2.2. This will be referred to as Method B.

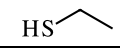
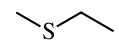
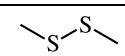
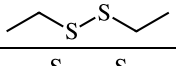
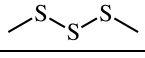
6.2.5 Analysis of a Biosolid Sample

Plant dewatered biosolids cakes (1 kg, dry solid content 16.9 %) were obtained from a Perth WWTP, which uses activated sludge and sequencing batch reactors, egg shaped mesophilic anaerobic digestion and centrifuge dewatering. The sample was kept cool (4 °C) until analysis. The biosolid sample (0.5 g) was added to a 20 mL glass vial and sealed. The sample was then analysed *via* HS SPME/GC-MS as described in Section 6.2.2.

6.3 Results and Discussion

The method for analysis of the VSCs listed in Table 6.4, using HS SPME/GC-MS, was initially developed and optimised for aqueous samples. It was then adapted for qualitative analysis of gaseous samples.

Table 6.4: The VSCs used in this study and their structures.

Compound	Acronym	Structure
Ethanethiol	EtSH	
Dimethyl sulfide	DMS	
Ethyl methyl sulfide	EMS	
Dimethyl disulfide	DMDS	
Diethyl disulfide	DEDS	
Dimethyl trisulfide	DMTS	

6.3.1 Analysis of Volatile Sulfur Compounds in Aqueous Samples

6.3.1.1 Optimisation of the Method

Optimisation of the GC-MS conditions included investigation of the initial oven temperature, hold time and heating rate. An initial oven temperatures of 0 ºC was chosen, due to the volatility of some of the lower molecular weight analytes (namely EtSH, DMS and EtSH). A hold time of 2 minutes was chosen. A heating rate of 5 ºC per minute provided baseline separation of the analytes and this was increased to 15 ºC per minute after the elution of the lower molecule weight analytes. These combined conditions afforded Gaussian shaped peaks for each analyte.

Fibre selection is dependant upon the chemical nature of the analytes. Three fibre phases were investigated for analysis of VSCs, *i.e*. PDMS, CAR-PDMS and DVB-CAR-PDMS. The CAR-PDMS phase is the most widely chosen phase for analysis of this suite of compounds (Table 6.2 and references there-in). The grey DVB-CAR-PDMS fibre was chosen for this study, based on its efficiency on achieving the greatest peak area response and also because it gave the lowest number of interferences.

Due to the volatile nature of certain VSCs, three different desorption temperatures (200 °C, 220 °C and 250 ° C) were trialled while optimising the SPME conditions.

The sample is generally agitated prior to the introduction of the fibre into the headspace to promote volatilisation of analytes. The optimal agitation time was determined by trialing agitation times ranging from 10 to 30 minutes (Figure 6.1 a, b and c). There appeared to be little difference in response after 15 minutes agitation time across all of the analytes and desorption temperatures, with the exception of DMDS at 20 minutes at 220 °C, which appears to be an outlier. There was no increase or decrease as time increased, so 15 minutes was chosen as the optimal agitation time. The effect of desorption temperature was minimal, with the maximum response observed at 220 °C across all the analytes and conditions.

Extraction time profiles constructed for each analyte (Figure 6.2 a, b and c), showed that extraction efficiency reached a maximum after 5 to 10 minutes across each

desorption temperature, with little effect on response observed over a longer time period. An extraction time of 10 minutes was chosen.

Generally the maximum response was observed at a desorption temperature of 250 °C. However, previous studies have shown that, at higher temperatures, DMTS undergoes thermal degradation (Kristiana, 2010). The higher dimethyl(polysulfides), such as DMTS, are thermally unstable and are susceptible to disproportionation, with thermally induced disproportionation resulting in the formation of lower dimethyl(poly)sulfide homologues and elemental sulfur (Wajon *et al.*, 1985a).

At 200 °C, the higher molecular weight compounds, such as DMDS and DMTS, were not as readily desorbed from the fibre. Therefore it was decided to use 220 °C, which is still within the recommended temperature range for the fibre chosen and below the temperature at which Kristiana *et al.* (2010) found evidence of thermal degradation of DMTS. In order to examine the potential for thermal degradation of these compounds, the presence of elemental sulfur was investigated. Elemental sulfur can appear as S_6 or S_8 in the chromatogram and is not usually well resolved, appearing as an un-resolved non-Gaussian peak (Heitz, 2002). The TIC for desorption at 220 °C was investigated and a characteristic broad peak in the chromatogram was not observed, thus indicating elemental sulfur was not present, and therefore that disproportionation did not occur under these conditions.

The response for EtSH was negligible across most of the optimisation testing. Due to its highly volatile nature, it is thought to have either volatilised or reacted with some of the other analytes present in the sample. This is further discussed in Section 6.3.3. It was therefore excluded from further method validation trials.

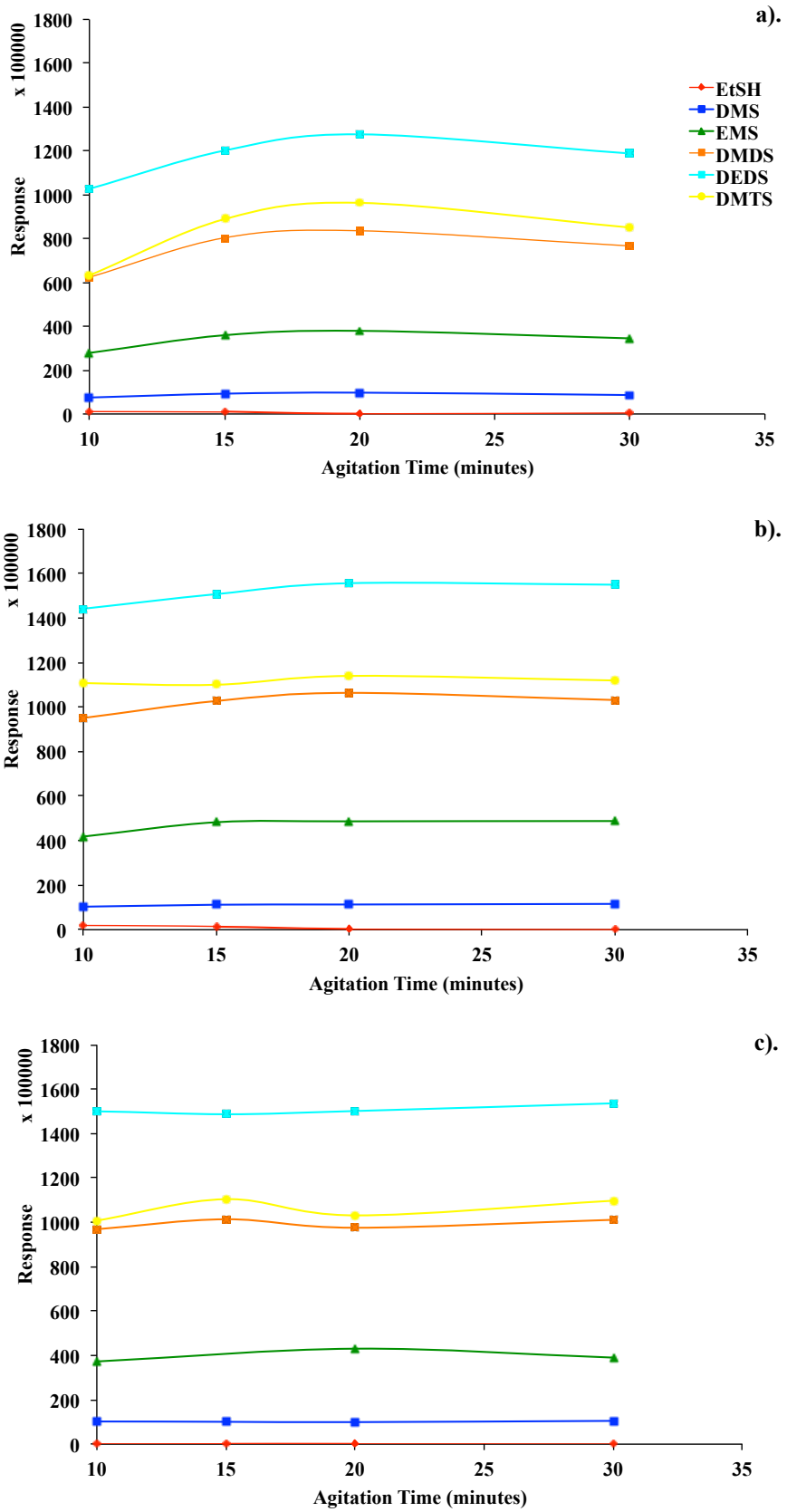


Figure 6.1: Responses of analytes using different SPME agitation times at different desorption temperatures. The water sample (10 mL; 500 ng L^{-1} each analyte), with 3 g of salt was agitated for 10, 15, 20 or 30 minutes at 40°C, followed by 10 minutes extraction and desorption at 200 °C (a), 220 °C (b) or 250 °C (c) and then analysed by GC-MS.

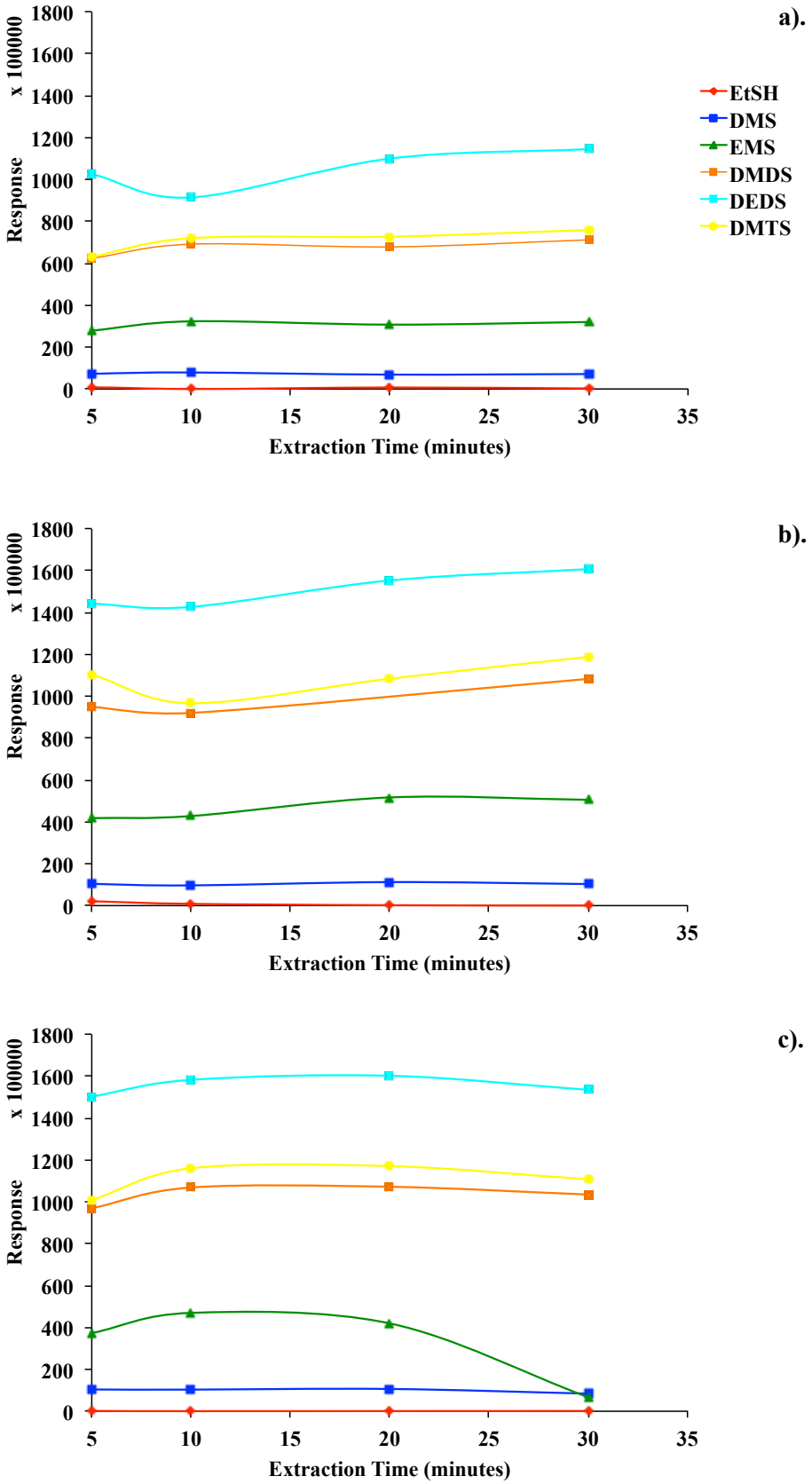


Figure 6.2: Responses of analytes with different extraction times. The water sample (10 mL; 500 ng L^{-1} each analyte), with 3 g of salt was agitated for 15 minutes at 40 °C, followed by 5, 10, 20 or 30 minutes extraction and desorption at 200 °C (a), 220 °C (b) or 250 °C (c) and then analysed by GC-MS.

6.3.1.2 Method Validation

6.3.1.2.1 Calibration of the Method

Calibration curves for the individual VSCs, excluding ethanethiol, were established by adding varying concentrations (in total) of each analyte, together with a fixed concentration (0.5 µg L^{-1}) of internal standard (dimethyl-d_6 disulfide) into MilliQ water and analysing using the optimised HS SPME/GC-MS method. A typical chromatogram is shown in Figure 6.3. Relative response factors for each standard were determined by dividing the total area of the standard by the area of the internal standard and these were then plotted versus the concentration of each standard. Linear calibration curves with high correlation coefficients (Figure 6.4) were achieved for each analyte (typical curves shown in Figure 6.4).

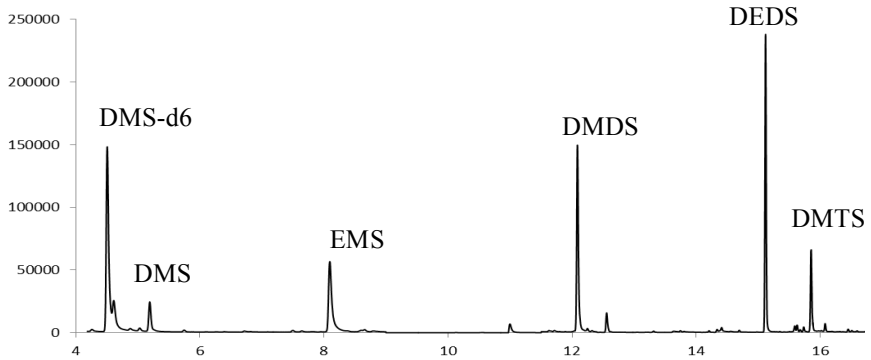


Figure 6.3: A sample chromatogram (GC-MS; SIM mode) of DMS, DES, DMDS, DEDS and DMTS (250 ng L^{-1} each analyte) with DMS-d6 as internal standard analysed by the optimised method.

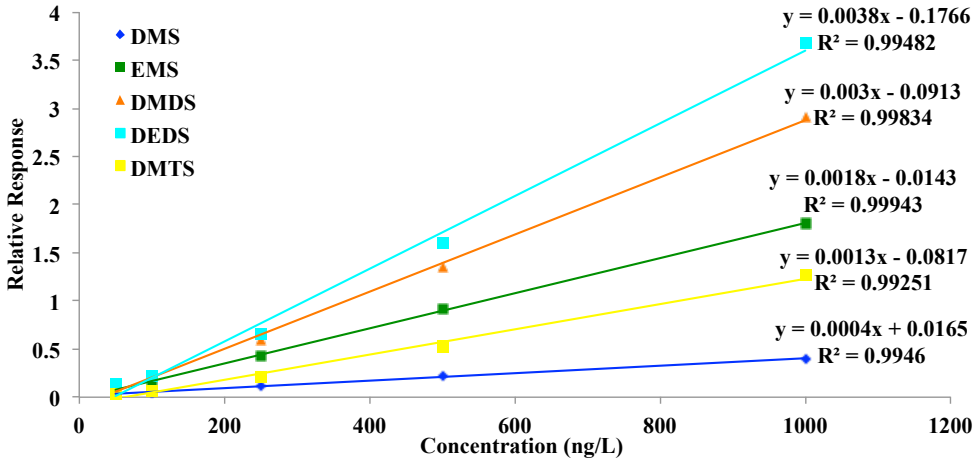


Figure 6.4: Calibration curves for the analysis of the VSCs by HS SPME/GC-MS by the optimised method.

6.3.1.2.2 Method Sensitivity

In order to determine the sensitivity of the method for each analyte, LODs, and LOQs were determined using a method described by Adams (2004). Six replicate blank analyses (MilliQ water and internal standard) were carried out. The spectrum noise over the retention time window of each analyte was integrated, the mean and standard deviation of these areas calculated, and then converted to equivalent concentrations of each respective analyte using the calibration curves. The LOD was calculated as the mean concentration of the blank analyses plus three times the standard deviation, and the LOQ was the mean concentration of the blank plus ten times the standard deviation.

LODs for all of the compounds were below 10 ng L^{-1}, which is less than the lowest odour threshold concentration (OTC) for the compounds where an OTC has been published (Table 6.5).

Table 6.5: Correlation coefficients (r^2), LODs and LOQs for analysis of VSCs using the optimised method. Odour threshold concentrations of the analytes in water where reported from literature are also shown.

Compound	**Linearity** (r^2)	***Sensitivity (ng L^{-1})***		**OTC (ng L^{-1})**
		LOD	**LOQ**	
DMS	0.995	10	22	300[a]
EMS	0.999	0.5	1	
DMDS	0.998	1	2	1200[b]
DEDS	0.995	0.2	0.4	
DMTS	0.993	0.6	1.5	10[b]

a: (Buttery *et al.*, 1990) b: (Buttery *et al.*, 1976)

6.3.1.2.3 Method Precision

The precision of the method was evaluated by determining the repeatability and the reproducibility of the method. The repeatability refers to 'run to run' precision, while reproducibility refers to 'day to day' precision. Three concentrations (10, 150 and 500 ng L^{-1}) of each analyte were spiked into MilliQ water and analysed using the optimized method. The relative standard deviation (RSD) for each concentration was calculated, with the %RSD being the standard deviation divided by the mean multiplied by 100 for each concentration sample set (Table 6.6). Repeatability was calculated on triplicate analyses across one run, while reproducibility was calculated on analyses repeated across three separate days.

Both repeatability and reproducibility were poor at the lowest concentration range, suggesting that the method LOQ should be greater than 10, and caution should be taken at these low levels. At the higher concentrations, good to moderate repeatability (4 – 17 %RSD) and reproducibility (6 – 26 %RSD) were achieved for all analytes. The highest %RSD values were observed for DMS, which is the most volatile of the compounds analysed.

Table 6.6: Repeatability and reproducibility for analysis of the VSCs using the optimised method.

Compound	***Repeatability***			***Reproducibility***		
	%RSD 10 ng L^{-1}	%RSD 150 ng L^{-1}	%RSD 500 ng L^{-1}	%RSD 10 ng L^{-1}	%RSD 150 ng L^{-1}	%RSD 500 ng L^{-1}
DMS	85	13	17	76	26	26
EMS	56	8	11	47	18	21
DMDS	50	6	11	40	12	15
DEDS	46	5	10	47	13	15
DMTS	25	4	10	33	6	14

6.3.1.3 Effect of Sample Matrix

The investigation of sample matrix was undertaken using a wastewater sample, where the analytes were spiked into the wastewater at two concentrations (A- 50 and B- 500 ng L^{-1}) and analysed in triplicate by the optimised method (Table 6.7). Analysis of the blank sample without any analytes (but with internal standard) identified the presence of several of the analytes of interest in the range of 35 – 177 ng L^{-1}. Accuracies (based upon the recovery of the analyte spiked into the sample) for each analyte were within a range of 67 – 140% at concentration A, and 71 – 114% at concentration B. The higher recoveries for DMDS and DMTS could be due to disproportionation of higher homologues of sulfur compounds which might have been present in the wastewater. The precision (based on repeatability) for the concentration A was 4 – 27 %RSD, while at the concentration B was 2 – 17 %RSD.

Table 6.7: Average percentage recoveries of VSCs from a wastewater sample. Concentrations of analytes present in sample shown in ng L^{-1}.

Spike Concentration (ng L^{-1})	Recovery of VSCs % (%RSD)				
	DMS	**EMS**	**DMDS**	**DEDS**	**DMTS**
Wastewater concentrations	35 (23)	0	177 (20)	0	107 (24)
50 ng L^{-1}	126 (4)	104 (27)	140 (35)	67 (20)	130 (16)
500 ng L^{-1}	79 (7)	102 (2)	114 (17)	71 (12)	109 (13)

Initial spiked concentrations were 50 and 500 ng L^{-1}. RSD [=(standard deviation/mean) x 100%] are shown in parentheses. Analyses were carried out in triplicate.

6.3.2 Analysis of Volatile Sulfur Compounds in Gaseous Samples

As discussed in Section 1.6.4, in order to be able to use SPME to quantitatively analyse air samples containing VSCs, suitable standards needed to be prepared. In order to keep the analysis as automated as possible, it was desirable to analyse standards in vials (20 mL) of the type that fitted into the autosampler tray. Two different methods (A and B) were trialled to determine the best procedure to achieve this.

Previous studies had prepared gaseous sulfur standards by injecting a minimal volume of a mixture of VSCs prepared in methanol into a vial, heating this to 100 °C for a fixed time period (from 30 seconds (Ras *et al.*, 2008b) up to one hour (Lestremau *et al.*, 2004) to volatilise the analytes and methanol, and then inserting the SPME fibre and extracting as per normal. We chose to investigate 30 seconds in this study in order to shorten the analysis time.

Standard mixtures of VSCs at concentrations of 50, 150, 250, 500 and 1000 ng L^{-1} in methanol were added directly to a 20 mL vial (without water in it), heated to 100 °C for 30 seconds and then left at room temperature until extraction by SPME (Method A). The optimised method used for aqueous samples was then employed for analysis, where each sample was agitated at 30 °C for 10 minutes, after which the SPME fibre was extracted for 10 minutes before being desorbed and analysed for the analytes of interest. Agitation was probably unnecessary, however this was inbuilt into the SPME sample preparation method.

The areas for each analyte were then plotted against concentration (internal standards were not used at this stage; Figure 6.5). This resulted in calibration curves that plateaued at higher concentration values and in some cases decreased slightly (*e.g.,* DEDS, EtSH). This could have been due to either too much analyte being present and competing for adsorption sites on the fibre, or because the analytes had not completely vaporised.

The samples were also analysed by heating utilising the agitator on the Gerstel, followed by SPME analysis immediately afterwards (Method B), rather than heating externally and allowing them to return to room temperature prior to extraction (Method A). Mixed standards of EtSH, DMS, EMS, DMDS, DEDS and DMTS at concentrations of 50, 150, 250, 500 and 1000 ng L^{-1} in methanol were added to a series of 20 mL vials. The vials were placed in the sample tray, from where each was transferred to the agitator which was set at 100 °C. The fibre was then introduced into the headspace of the vial for 30 seconds, after which it was immediately inserted into the GC injector for desorption and analysis. The vial was in the agitator for approximately 25 seconds prior to the fibre being introduced. The areas for each analyte were then plotted against concentration (Figure 6.6). Internal standards were not used at this stage.

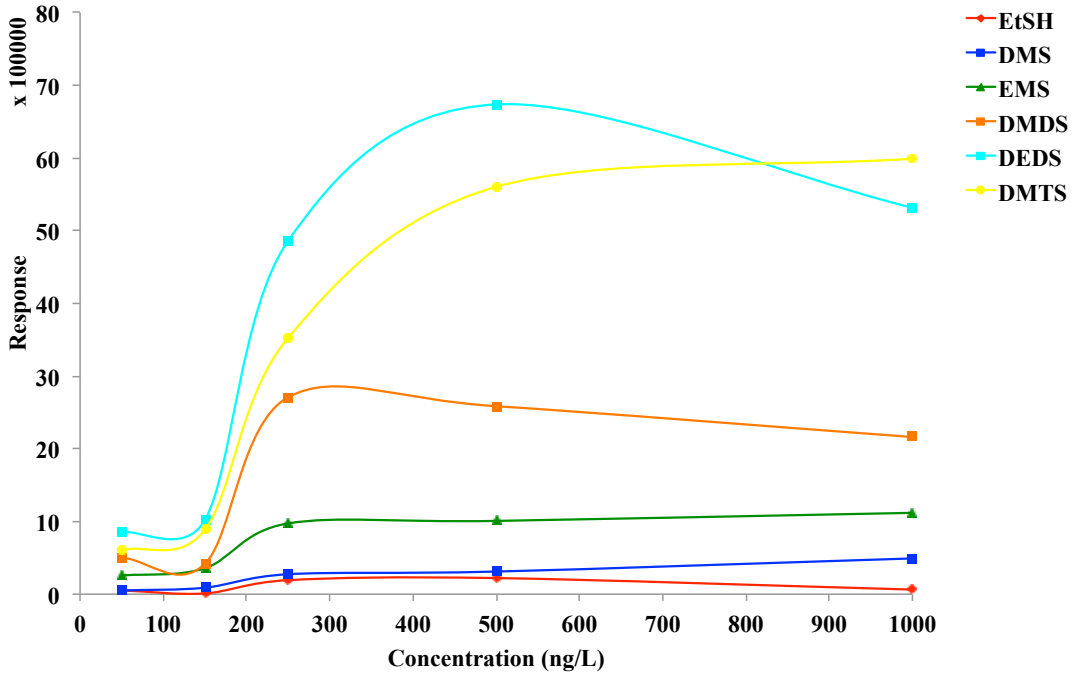


Figure 6.5: Area response versus concentration of VSCs volatilised from methanol (Method A).

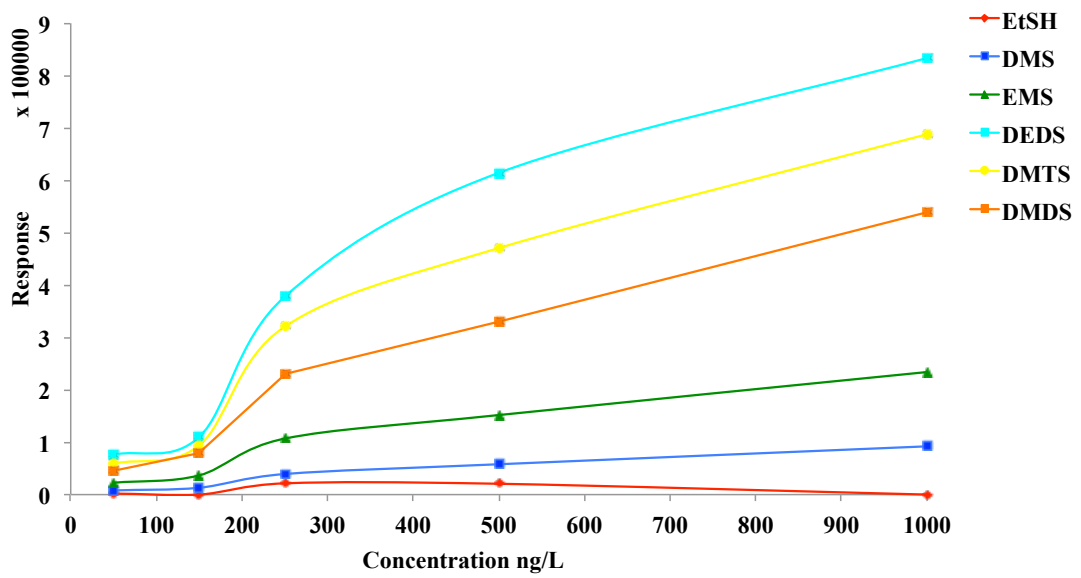


Figure 6.6: Comparison of responses from samples run immediately after being heated (Method B).

Due to the limitations of the agitator on the Gerstel, it was not possible to cool the agitator down from 100 °C in a reasonable timeframe, nor was it possible to introduce the fibre into the sample anywhere except while it was in the agitator. To avoid potential breakdown or artefact formation from the analytes, the fibre was only introduced into the sample and extracted for 30 seconds during Method B, in comparison to Method A, where the sample was extracted for 10 minutes.

The difference in signal response from these two methods is shown in Figure 6.7, where the response from Method A is nearly a magnitude higher than Method B. The longer extraction time in Method A allowed for a greater amount of analyte to sorb onto the fibre and it is clear that Method B did not reach equilibrium.

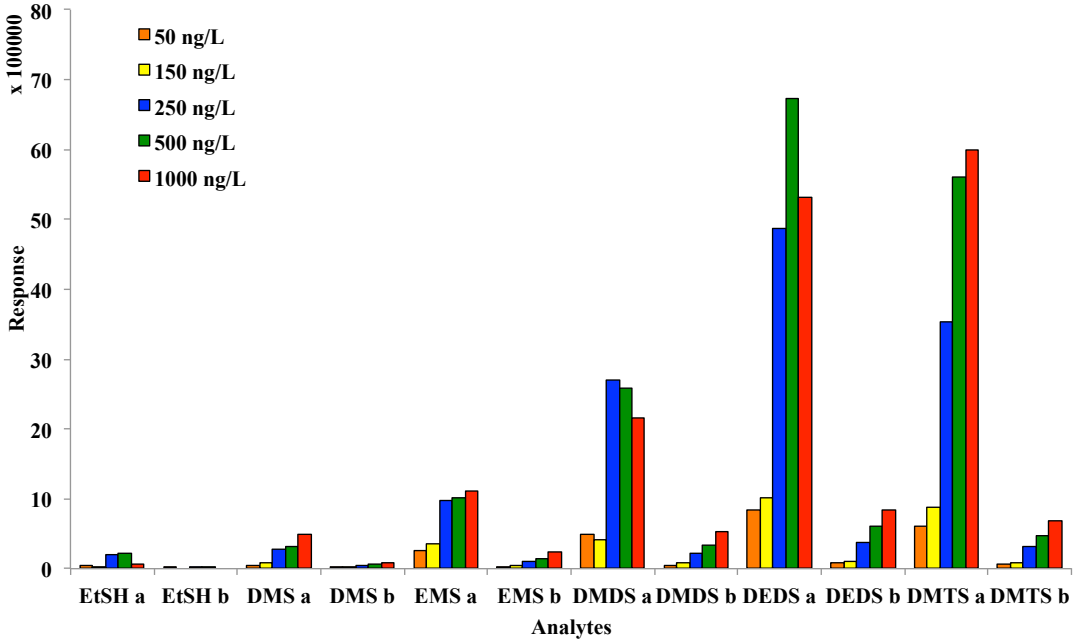


Figure 6.7: Comparison of response from standards preheated a) and standards heated in agitator b) and analysed immediately.

The plateau effect that was observed in the calibration of the mixed standards (as shown in Figure 6.5) was further investigated by analysing individual samples of each analyte at 50 and 100 ng L^{-1}. A comparison between the responses from the mixture and when the analyte was prepared separately is shown below in Figure 6.8.

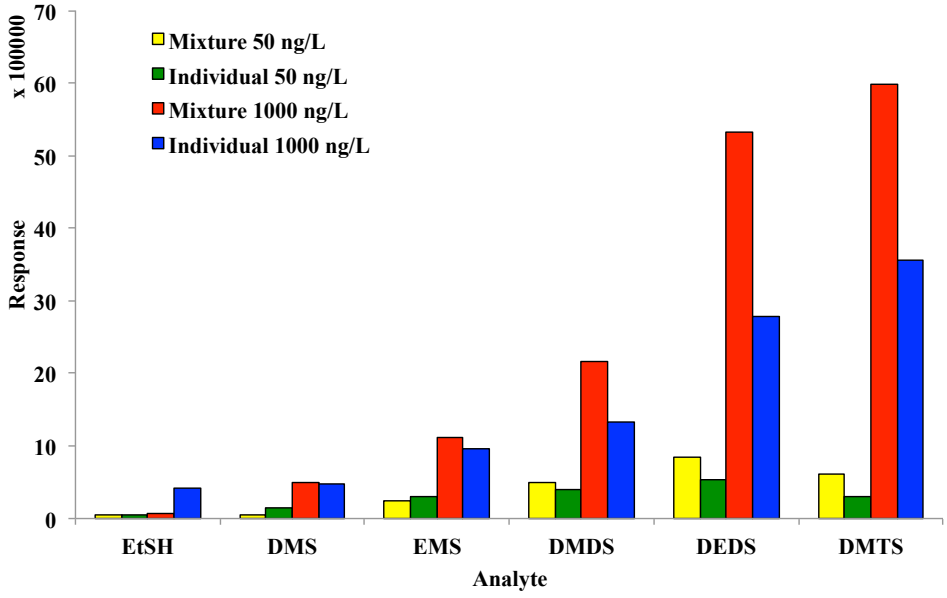


Figure 6.8: Comparison of responses from individual analyte preparation and within a mixture at two concentration levels.

In the cases of DMDS, DEDS and DMTS, a greater response was observed in the mixture. This could be due to the breakdown and reaction of EtSH, whose response was very low in both the individual and mixture analysis of 50 ng L^{-1} and the mixture at 1000 ng L^{-1} indicating that it is likely to have reacted to contribute to the concentrations of other VSCs. This behaviour is similar to what was observed during the aqueous method development. Yet how much it can account for is unknown. Based on the response, there appears to be twice as much DEDS and DMTS present in the mixture in comparison to the individual sample. This could be due to the sample preparation and the effect of the mixture, resulting in disproportionation and some sort of equilibrium forming within the mixture but not in the individual analyte samples.

Another method of standard preparation employed in several studies (Murray, 2001; Haberhauer-Troyer *et al.*, 1999) was then adapted and investigated to determine if a more reliable method of standard preparation could be utilised. This method was the addition of a known amount of the VSC standard to a vial, allowing it to vaporise by keeping it at a known temperature (30 °C) for a certain time period, and then diluting it to a working concentration. However, this was unsuccessful for the higher molecular weight compounds (such as DMDS and DMTS) which did not volatilise at all, and were not present in the chromatogram.

Based on the outcomes of these trials and time constraints within the study, it was decided to use the method of analysis of gas samples for qualitative purposes (to indicate the presence of a VSC), rather than quantitatively.

6.3.3 Artefact Formation and Compound Interaction

A number of sulfur compounds, namely VSCs, are known to be very volatile and reactive. They are subject to oxidation and breakdown readily to form other sulfurous compounds. For example, dimethyl polysulfides (*e.g.,* DMDS, DMTS) are susceptible to disproportionation and thermal degradation, with the disproportionation resulting in the formation of lower dimethyl polysulfide homologues and elemental sulfur (Kristiana *et al.*, 2010b; Wajon *et al.*, 1985b). In the suite of VSCs investigated here, the instability of EtSH was demonstrated.

Analysis of a nominally pure EtSH standard exhibited peaks associated with DEDS and DMTS. Whether these contaminants were present in the neat ETSH, or were produced as a result of interaction with the analytical instrumentation was difficult to ascertain. The analyte came into contact with a variety of different surfaces and parts during the analysis process. This included the glass vial, the fibre, the liner of the injector system and the phase of the column. Analysis of neat EtSH *via* GC-MS without SPME still requires similar interactions, therefore, this would be not useful in determining whether DEDS or DMTS were present in the EtSH standard. Studies by Lestremau *et al.* (2004) found that the metal in the fibre reacted with the sulfurous analytes such as thiols to form their corresponding dimer, however the presence of other analytes such as amines (which would be expected in a wastewater type sample) prevented any other reactions. This could explain the behaviour of EtSH in producing more DEDS.

The formation of such artefacts during analyses of VSCs has been reported (Gruchlik *et al.*, 2012; Lestremau *et al.*, 2004) and emphasises the importance of mixed standards for calibration purposes. Analysis of the individual analytes separately could possibly reduce the amount of artefact formation, however, a sample is likely to contain more than one of the analytes, and such interactions would be occurring within the sample, so the more representative a standard mixture is of a sample, the more valid it will be. On the other hand, standards containing only the single (individual) compound also need to be analysed to determine whether the analyte undergoes decomposition and/or recombination, as for EtSH.

6.3.4 Application of the Method to the Analysis of Volatile Organic Aromatic Compounds

The analytical method described in this Chapter was developed and optimised for the analysis of VSCs. Due to the success of previous studies in applying methods for a multiple range of compounds from different chemical classes the optimised method was applied to an aqueous sample containing toluene, ethylbenzene, styrene, *p*-cresol, indole and skatole. Each of the compounds was successfully identified from the method, however separate studies (Gruchlik *et al.*, 2012) found that a different fibre phase (65 μm PDMS-DVB) and longer (30 minute) extraction at a higher temperature (60 °C) was more suited to this suite of analytes. Due to constraints

associated with sample availability and time, it was decided that for the purposes of this study, qualitative analysis of these compounds would be suitable for further application of the developed method.

6.3.5 Application of the Method to the Analysis of Biosolid Samples

The analytical method developed in this Chapter for the analysis of aqueous samples was used to qualitatively analyse a biosolid sample which had been stored for a week prior to analysis. Figure 6.9 shows a chromatogram in SCAN mode, following HS SPME/GC-MS. The main odour compounds identified in the biosolid sample from the test site included DMS, DMDS, *a*-pinene and limonene, and are labelled in Figure 6.9. Other compounds identified in the sample include straight chain aliphatic hydrocarbons, toluene and methyl-substituted aromatic compounds. These compounds have also been identified by other researchers in biosolid samples (Kim *et al.*, 2005; Kim *et al.*, 2002a; Ramos *et al.*, 2002). The other major peaks present in the GC chromatogram from the GC-MS analysis of the biosolid sample appeared to be siloxane based, which can be attributed to the SPME fibre and also the GC column used.

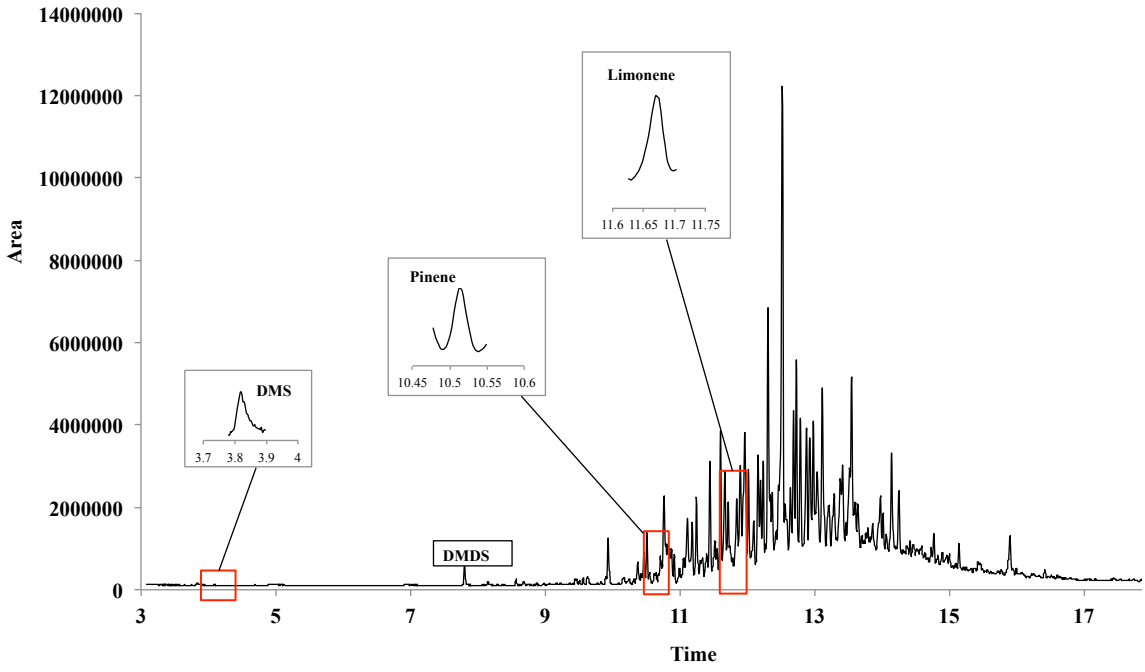


Figure 6.9: Chromatogram (GC-MS; SCAN mode) of a biosolid sample analysed by the optimised method.

6.4 Conclusions

A HS SPME/GC-MS method for the analysis of a range of aqueous volatile sulfur compounds was developed, optimised and validated. The method is rapid and simple, with good reproducibility and linearity. The precision of the method ranged from 4 to 26 %RSD, and detection limits less than the odour threshold concentrations of the compounds were achieved (0.2 to 10 ng L^{-1}). Analysis of a spiked wastewater sample indicated that the matrix of interest may provide some interferences when analysing actual samples, and that limits of quantification may need to be raised above 10 ng L^{-1} due to poor method precision at lower spike concentrations.

Adaptation of the method to analyse gas samples for the same analytes was only possible qualitatively. Determining whether a particular analyte was, in fact, present in the sample or whether it was an artefact was not always possible. Reactions of the analytes, such as ethanethiol with other analytes present in the sample or with the sampling equipment, such as the fibre, meant that it was difficult to determine whether an actual analyte was present in the sample or the result of a chemical reaction. As such, results should be treated with caution.

The difficulty in analysing volatile organic sulfur compounds was apparent. The formation of artefacts and breakdown by-products was evident in the analysis of EtSH. This highlighted the importance of a proper understanding of the potential interactions of the analytes of interest, and that it may not be possible to determine whether an analyte was actually present or formed through interactions of other compounds. The contribution of EtSH to the overall concentration of DEDS was evident, but the extent of transformation could not be determined during this study. It was assumed that if any EtSH was present in the sample, upon analysis it would be converted to DEDS, so any DEDS detected could have been originally present as EtSH or DEDS.

The method was also found to be suitable for the analysis of a range of other volatile organic compounds, including toluene, ethylbenzene, styrene, *p*-cresol, indole and skatole. While it was not optimised for these analytes, it was shown to be suitable for further studies for the identification of these analytes in real samples. Analysis of a

biosolid sample demonstrated the method's applicability to the detection of odorous compounds present in wastewater treatment processes.

This method was applied in Chapter 7 to examine the presence and formation of odorous compounds during wastewater treatment processes.

Chapter 7

Detection of Sulfur Compounds in Wastewater Systems Using Solid-Phase Microextraction

7.1 Introduction

Odour management at WWTPs and from sewer systems is of the utmost importance. A survey of nine Australian wastewater utilities, serving over 8.4 million people, was undertaken by Sivret and Stuetz (2012). The purpose of the survey was to summarise the current monitoring practices for assessment of odour abatement processes. They reported that most odour abatement processes were monitored through complaints from the surrounding community. H_2S was the dominant on-line and off-line monitoring parameter, and there was limited use of non-H_2S odourant analysis (Sivret and Stuetz, 2012). Based on this report, a long-term monitoring program in Sydney was conducted for a number of sewers to better understand the composition of sewer emissions, and ultimately, aid odour abatement process selection (Sivret *et al.*, 2013). This study reported the consistent identification of a number of reduced organosulfur compounds, including CS_2, MeSH, DMS, DMDS and DMTS in addition to H_2S in sewer emissions (Sivret *et al.*, 2013). A number of VOCs were also consistently emitted.

7.1.1 Odour Abatement Technologies

As identified in Section 5.5.2, if prevention or minimisation of odorous emissions is not possible, it is necessary to employ a range of odour abatement processes which fall into three categories: biological treatment, chemical treatment or physical treatment. Until the early 2000s, chemical and physical treatments were utilised, however due to high operating costs and the failure to remove some compounds, biological treatment processes have been more widely implemented (Cox *et al.*, 2002). A large number of biofilters have been installed throughout the world, e.g. in the United States in 2005, it was reported that the number of biofilters installed exceeded 300 (Iranpour *et al.*, 2005). At this stage, biotrickling filters were only new technology. In the past decade, the use of biotrickling filters for the control of odours has increased substantially (Johnson *et al.*, 2014). In Perth, WA, two biotrickling filters are currently in use for abatement of odours from municipal sewer systems.

7.1.1.1 Biotrickling Filters for the Removal of Odourous Emissions

Biotrickling filters utilise immobilised microbial cells that are attached to a medium inside the reactor (Figure 7.1), which then oxidise the odorous constituents to

odourless compounds (le Roux *et al.*, 2009). The odour compounds transfer from the gas to the liquid phase, and subsequently to the microbial film, or directly from the gas to the microbial film. As the main odorous compounds present in sewer emissions consist of both organic and inorganic compounds, there are two main groups of organisms active in biotrickling filters: autotrophic bacteria that are responsible for oxidising inorganic odours and heterotrophic bacteria that are responsible for oxidising organic odours (le Roux *et al.*, 2009).

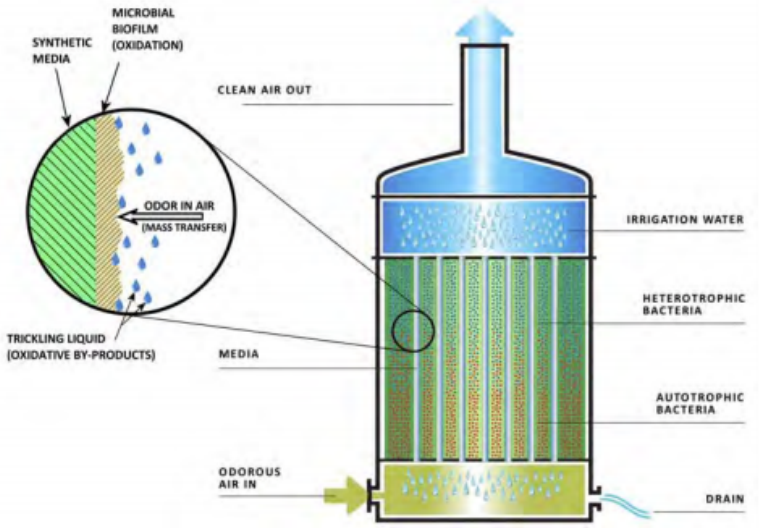


Figure 7.1: Schematic of the operation of a biotrickling filter. Reproduced from le Roux and Johnson (2010). Copyright WEF 2010.

A study of a water utility in the United States using biotrickling filters was undertaken by Johnson *et al.* (2014). The sewage collection system studied consists of two WWTPs and 40 pumping stations, of which several are located in close proximity to residential and commercial areas as well as busy roads. After complaints from nearby residents and commuters, the utility installed a number of biotrickling filters to remove odours from the two WWTP headworks, sludge tanks and also at three pumping stations in their sewer system. The installation of these biotrickling filter systems resulted in the elimination of odour complaints at both WWTPs and pumping stations (Johnson *et al.*, 2014). All of the systems also reduced the H_2S concentration to less than 0.05 ppmv, demonstrating the odour removal capacity of biotrickling filter units (Johnson *et al.*, 2014).

7.1.2 Odorous Emissions Sampling

In studies to determine the odour properties of in environmental air samples, the odorous air must be collected by either one of two ways so that it can be analysed. As discussed in Section 1.5.2, the sample may be collected either directly into a container, such as a bag or a canister; or is trapped or pre-concentrated by passing a volume of air through a filter onto an adsorbent or adsorbing solution.

7.1.2.1 Collection of Odorous Compounds in Sampling Bags

Traditionally, the use of Tedlar® and/or Nalophan® bags is the preferred method for odorous emissions sampling (Trabue *et al.*, 2006). However, it is well known that the bags themselves can impact on the integrity of the sampled gas (Polasek and Bullin, 1978).

Polasek and Bullin (1978) evaluated the use of a variety of bag materials, including Tedlar®, for the sampling of ambient air. The bags were filled with a calibration gas containing carbon monoxide, non methane hydrocarbons, and methane and then analysed after 24, 48 and 100 hours. The results showed that Tedlar® was inadequate for sample storage based on the decrease of calibration gas concentration over time (Polasek and Bullin, 1978).

McGarvey and Shorten (2000) examined the adsorption and desorption behavior of six different organic compounds in Tedlar® bags for the purpose of reusing bags. They demonstrated that methanol readily adsorbs to Tedlar® bags, and no amount of flushing could remove the methanol. They deemed it could be suitable to reuse bags for the analysis of higher molecular weight alcohols, and that these only displayed a recovery loss of 10% after five days. The bags were also found to be suitable for the collection and analysis of ethers, and bags could be reused following certain flushing procedures. In the case of substituted aromatic compounds, Tedlar® bags could not be reused with these compounds, and their analysis in new bags should be limited to short holding times. The authors recommended further investigation into other classes of compounds to fully understand whether Tedlar® bags could be reused for a wider variety of applications (McGarvey and Shorten, 2000). This study also highlighted the potential interaction that compounds may have with the bag surface.

Trabue *et al.* (2006) investigated the use of Tedlar® bags in the collection of air samples. They identified several background contaminants, including odorous compounds *N*,*N*-dimethyl acetamide, acetic acid and phenol in the Tedlar® bags when a sample of hydrocarbon-free air was collected in the bag. A recovery study for compounds typically found in a swine production facility, including volatile fatty acids, phenols, and indoles, also questioned the integrity of Tedlar® bags. The results indicated that the recovery of certain malodour compounds was dependent on residence time and the longer the sample was in the bag, the lower the recovery of the compound, with reported recoveries between 33 to 65% of analytes following 24 hours of storage (Trabue *et al.*, 2006).

7.1.2.1.1 The Effect of Sampling Bags on Volatile Sulfur Compounds

A range of studies on the suitability of sampling bags for the collection of air samples for the analysis of VSCs has been carried out (Le *et al.*, 2013; Mochalski *et al.*, 2009b; Trabue *et al.*, 2008; Nielsen and Jonsson, 2002a; Sulyok *et al.*, 2001; Lau, 1989). The majority of these studies found that Tedlar® bags are suitable for routine analysis, however are not suitable for the more volatile H_2S. The need to analyse the samples relatively soon after collection is also emphasised. In March 2009, DuPont® announced its plan to phase out Tedlar® film in the sample bag market, resulting in the adoption and investigation of other sample bag materials, such as Nalophan® and Mylar®. While in 2012, DuPont® made Tedlar® sample bags available again, their availability was limited (Coyne *et al.*, 2011).

Lau (1989) investigated the storage stability of a range of sulfur gases including H_2S, sulfur dioxide (SO_2), carbonyl sulfide (COS), CS_2, MeSH and EtSH in low parts per billion concentration in Tedlar® bags. Concentrations were measured over time, every 20 minutes for the first three hours and then periodically for the next 21 days. While the bags were found to be unsuitable for H_2S and SO_2, the stability of the remainder of compounds was good for two weeks, even at low concentrations (Lau, 1989).

A study on the suitability of various sampling containers (including standard Tedlar® sample bags, black/clear layered Tedlar® sample bags and Silcosteel sample cylinders) for the analysis of a multicomponent gas standard containing MeSH,

EtSH, DMS, EMS, diethyl sulfide (DES) and several branched thiols was carried out by Sulyok *et al.* (2001). They found the standard Tedlar® sample bags were the most suitable sampling containers studied with respect to the stability of the analytes and their recovery. The recovery of MeSH after one week was approximately 90%. The use of black/clear bags resulted in much lower recoveries, thought to be a result of adsorption of the VSCs to the black carbon filled layer. While the Silcosteel sample cylinders were found to be suitable in terms of analyte stability, recoveries exceeded 100% for the higher boiling compounds, which they thought to be as a result of enrichment effects on parts of the sampling system, but required further investigation. The economic advantage of using the cheaper standard Tedlar® bags was not offset by the slightly lower stability of analytes in Tedlar® bags in comparison to the Silcosteel cylinders, and Tedlar® bags were decided to be the best choice for routine analysis (Sulyok *et al.*, 2001).

Trabue *et al.* (2008) reviewed the use of Tedlar® bags for the sampling of VSCs and reported that, while they might be useful for holding VSCs in dry environments, there was a greater risk for some analytes to sorb and potentially degrade when collected in a Tedlar® bag from a humid environment. While the air could potentially be dried using dessicants to improve results with Tedlar® bags, it was found to be short term solution due to diffusion of humidity into the bag during storage. Nielsen and Jonsson (2002a) found that while the samples were stable in the Tedlar® sampling bags over the storage period of 12 hours, water did permeate through the film and into the bags within a few hours of storage.

Investigation into the suitability of five different polymer sampling containers was carried out by Mochalski *et al.* (2009b) for sampling and storage of six VSCs (H_2S, MeSH, EtSH, COS, DMS and CS_2) relevant to breath analysis. The materials investigated included Nalophan®, standard Tedlar®, black layered Tedlar® and Flexfoil®. The suitability of the sampling bag was determined based on several factors *i.e.,* analyte recovery, background contamination, influence of light and ageing effects. It should be noted, that none of the examined bags were found to be suitable for long term storage of samples. The Flexfoil® material was found to be most suitable in terms of a 90% recovery with a storage time of 24 hours. If a shorter storage time was employed, *i.e.* six to eight hours, transparent Tedlar® was a suitable

alternative as it provided acceptable background contamination and good stability of all compounds. Nalophan® bags showed no background contamination, but exhibited significant degrees of adsorption, and subsequent loss of recovery of most compounds after 24 hours storage. After only six hours storage, COS, DMS and CS_2 were depleted (Mochalski *et al.*, 2009b).

A recent study by Le *et al.* (2013) explored the stability of VSCs in three sampling bag materials (Tedlar®, Mylar® and Nalophan®) at three different storage temperatures (5, 20 and 30 °C). The range of compounds studied consisted of H_2S, MeSH, EtSH, DMS, EMS, DMDS, DEDS, DMTS, *tert*-butanethiol and 1-butanethiol. This study reported that recoveries of DMS, EMS, DMDS and DEDS were fairly stable with little dependence on temperature at 24 hours storage across all three bag materials. DMTS appeared to be the least affected by temperature, however even for this compound a recovery of 74 - 84% was obtained, indicating a consistent loss. Higher losses, and lower recoveries for H_2S and the other thiols at 30 °C was observed across all three bag materials, in comparison to the lower temperatures studied. The authors proposed that VSC samples should be analysed within 24 hours and storage be at temperatures less than 20 °C (Le *et al.*, 2013).

7.1.2.2 Analysis of Volatile Sulfur Compounds collected in Sampling Bags using Solid-Phase Microextraction

As discussed in Section 6.1.1, SPME has been used for the analysis of VSCs across a range of different matrices. Accordingly, a number of studies have utilised SPME as an extraction technique for the analysis of whole air samples collected in bags for the detection and quantification of a range of VSCs. The limitations and issues that can arise as a result of SPME and sample bag use is investigated in some of these studies and discussed below.

A comprehensive study carried out by Nielsen and Jonsson (2002a) assessed procedures for the quantification of nine VSCs found in samples collected from a sewage treatment plant, utilising SPME as the extraction technique. The samples were collected in 40 L Tedlar® sampling bags and during transport and storage were kept at room temperature and stored in the dark. To try and overcome the potential interference from humidity, the samples were dried over calcium chloride. The

samples were found to be stable in the Tedlar® bags over the storage period, which did not exceed 12 hours, and further investigation found they were stable for up to 20 hours. However, water did permeate into the bags after a few hours. Matrix effects occurred during the SPME analysis using the 75 μm CAR-PDMS fibre, which included fibre saturation by MeSH and saturation by competitive adsorption by interfering compounds present in the matrix. However, by using the standard addition procedure for calibration, quantification was possible, with compounds present in the sample ranging from non-detectable to 820 ppbv (Nielsen and Jonsson, 2002a).

SPME was found to be a fast and simple pre-concentration technique which was assessed for its suitability for the analysis of human breath-gas for VSCs by Mochalski *et al.* (2009a). Standard gaseous mixtures containing H_2S, COS, DMS, MeSH, EtSH and CS_2 were injected into Tedlar® bags. These compounds have been previously identified as of interest in human breath. The samples were extracted using an 85 μm CAR-PDMS fibre and then analysed using GC-MS. Loss of both EtSH and MeSH from samples was observed and attributed to the oxidation of these compounds in the GC injector at high temperatures. Humidification of the test samples was carried out to mimic conditions in actual breath samples, resulting in a loss of analytes in comparison to the standards prepared with dry air. These findings led to the conclusion that it was necessary to dry breath samples prior to analysis. The Tedlar® bags were found suitable for the storage of samples up to 24 hours. While the SPME technique was found to have some disadvantages in the case of EtSH and MeSH analysis, the low sample volume requirement and simplicity make it a suitable technique (Mochalski *et al.*, 2009a).

7.1.3 Scope of Work in Chapter 7 of this Study

The benefit of identifying a wider range of compounds present in odorous emissions from sewer networks to help evaluate and assess the effectiveness of odour abatement processes was emphasised by Sivret and Stuetz (2012). Therefore, the aim of the study described in this Chapter was to assess the range of compounds present in a Perth sewer system and the effectiveness of a biotrickling filter to remove them. This study utilises the SPME method developed in Chapter 6 for the extraction and qualitative analysis of the odorous compounds from air samples collected in

Nalophan® bags from the local sewer network. The focus technique of this Thesis is SPME, and accordingly another aim of using this technique was to evaluate the use of SPME as a simple, cheap, semi-quantitative method to determine the efficacy of the biotrickling filter to remove the odorous compounds.

7.2 Experimental

7.2.1 Collection of Air Samples

Four gas samples were collected from Yokine pumping station within the sewer network in Perth, WA. Duplicate samples were collected from the inlet and outlet of the biotrickling filter treatment point. The samples were collected in 5 L Nalophan® bags using a lung sampler at a constant flow rate of 2 L minute^{-1} for 10 minutes. Due to instrument availability, the samples were only able to be analysed a week after collection. It is possible that some analytes may have deteriorated over this time. Subsequent samples were unable to be collected due to lack of availability of access to the site. The original samples were stored in the dark at ambient temperature at 22 °C (in an airconditioned laboratory) prior to analysis.

7.2.2 Analysis of Air Samples

The gas samples were analysed *via* manual HS SPME, using a 50/30 μm DVB-CAR-PDMS fibre, followed by GC-MS analysis. The fibre was introduced directly into the sampling bag of the sample for 30 minutes and then transferred to the GC injector for thermal desorption of analytes. The fibre was conditioned in between each sample analysis by desorbing it at 200 °C for 10 minutes in the injector port of the GC to ensure that no carryover occurred.

The SPME fibre was desorbed at 200 °C for 3 minutes in the injector port of the GC. The analytes were introduced *via* a splitless injection (100 mL min^{-1} with a 1 min. purge time) onto a fused silica 5% phenyl – 95% dimethylpolysiloxane capillary column (30 m x 0.25 mm id, column phase thickness of 1 μm (ZB-5; Phenomonex)). Helium was used as the carrier gas operating at a flow of 1 mL min^{-1}. The oven temperature was initially set at 0 °C for 2 minutes prior to being increased at a rate of 5 °C min^{-1} until 35 °C, then 15 °C min^{-1} to a temperature of 315 °C with a final hold

time of 10 minutes. The samples were analysed by mass spectrometry in the SCAN mode.

7.3 Results and Discussion

Gas samples were collected from the inlet and outlet of the biotrickling filter at the Yokine pump station in the sewer network system in Perth, WA (Figure 7.2). Duplicate samples were collected from both the inlet and the outlet of the biotrickling filter. The samples were collected *via* a lung sampler (Figure 7.3) into 5 L Nalophan® bags and stored until they were analysed *via* GC-MS after extraction by SPME. Prior to sample collection, the suitability of the bags and SPME fibre were investigated in regards to as whether any background contamination could occur.

Figure 7.2: Yokine Pumping Station, Creswell Street, Yokine, Western Australia

Figure 7.3: Lung sampling device used to collect gas samples from Yokine Pumping Station

7.3.1 Investigation of Sampling Bags and Solid-Phase Microextraction Fibre Prior to their Use for Sample Collection

As has been widely reported, sampling bags used for the collection of air samples can contain contaminants. For samples collected in this study, Nalophan® sampling bags were used. This material was chosen for its availability. Nalophan® bags have also previously been reported for their use in collecting samples containing VSCs and while they might not have been the optimal bag in terms of analyte stability, they were deemed suitable. In order to determine whether the sampling bags were introducing any contaminants into the samples collected, analysis of a blank sample bag was undertaken. The SPME fibre was introduced into the sampling bag and exposed for 30 minutes, after which it was desorbed into the injector port of a

GC-MS and qualitative analysis was undertaken. Analysis of the blank sample bag revealed some volatile contaminants that may be of interest in this study appeared to originate from the actual bag. A chromatogram of the analysis with the major compounds identified and labeled is shown in Figure 7.4. These included, but were not limited to, toluene, phenol, and acetone (peak not shown). However, these compounds appeared to be quite low in abundance (based on peak area response) overall. The major compounds present in the analysis of the sample bag were a range of siloxane compounds, but these are generally attributed to the fibre itself or the column used for GC. The contaminants detected appeared consistently in these bags and were also reported in other studies (Sivret *et al.*, 2013). The presence of these contaminants highlighted the importance of conducting "blank" analyses and of interpreting the source of analytes with caution. Based on these results, it was determined that the Nalophan® bags would be suitable for collection of samples from the inlet and outlet of the biotrickling filter.

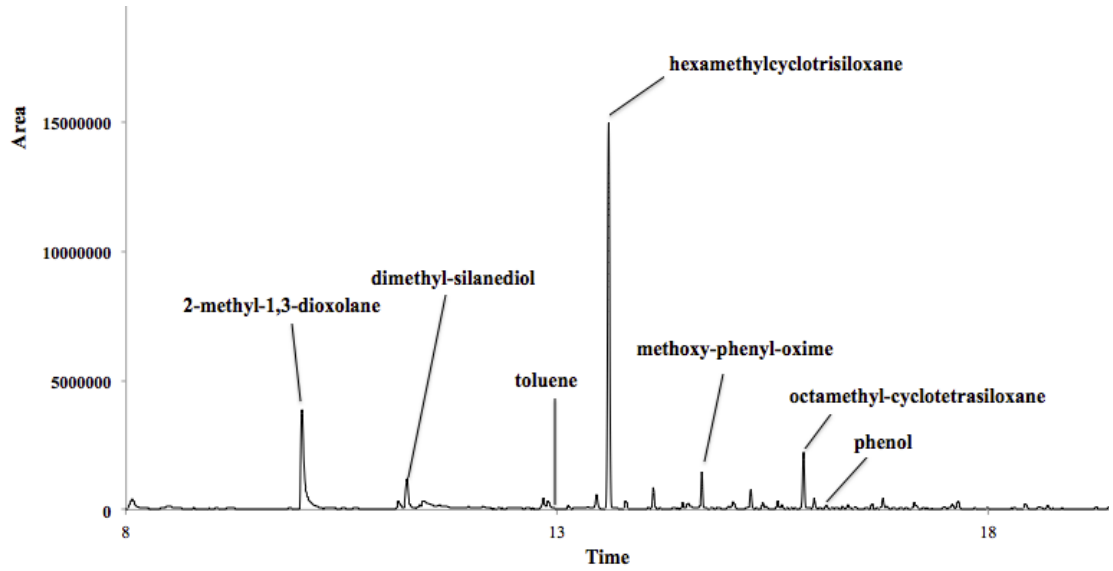


Figure 7.4: Chromatogram (GC-MS; SCAN mode) of empty Nalophan® bag.

Analysis of the SPME fibre prior to its use for the analysis of collected samples was undertaken to confirm that no analytes were already sorbed onto the fibre. The fibre was also conditioned between each sample run to ensure that it was clean prior to its use. Once again, siloxane compounds were present, but were the only major analytes observed (Figure 7.5). The peak area responses, which correspond to abundance, were also much less than in the blank bag analysis. This also confirms the suitability of the use of this fibre in terms of no contaminants of interest being present.

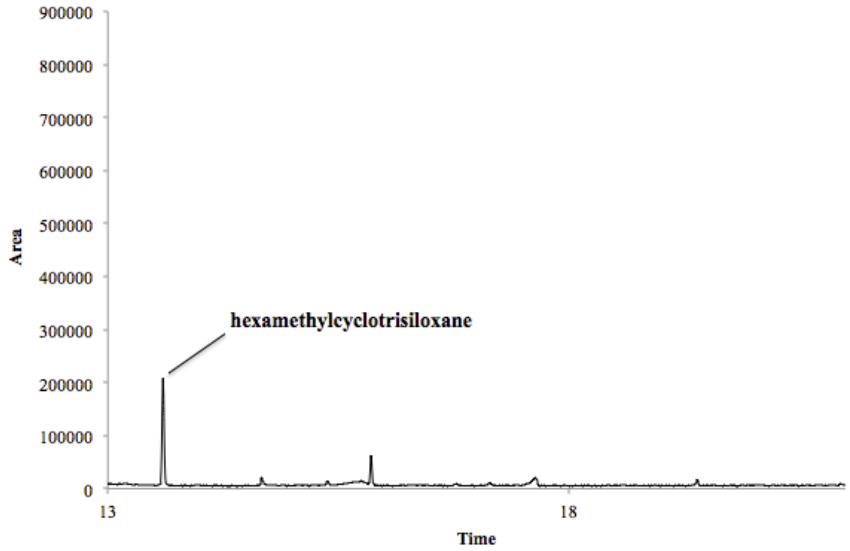


Figure 7.5: Chromatogram (GC-MS; SCAN mode) of blank fibre (DVB-CAR-PDMS).

7.3.2 Analysis of Samples Collected from the Yokine Pump Station

The samples collected from the inlet and outlet of the biotrickling filter treatment step at the pumping station were analysed qualitatively, *via* GC-MS after extraction by SPME for VSCs and also VOCs. While no concentrations can be reported, relative abundances have been estimated based on the peak area responses.

7.3.2.1 Analysis of the Sample Collected from the Inlet to the Biotrickling Filter at the Yokine Pump Station

A wide range of volatile organic compounds were detected at the inlet of the biotrickling filter, some of which were associated with odours. A full scan chromatogram from the analysis of the sample collected at the inlet to the biotrickling filter unit is shown in Figure 7.6. Only the peaks of interest in this study, as well as those with the greatest area responses, have been labelled. The compounds were tentatively identified based on library matching of mass spectra. The following VSCs were identified: SO_2, MeSH, CS_2, DMS, propenethiol, propanethiol, DMDS and, DMTS. Similar compounds were also identified in air samples from a sewage management plant in Spain using SPME followed by GC-MS (Ras *et al.*, 2008a). Another study carried out in two major Australian cities-Melbourne and Sydney also detected similar compounds, but instead utilised an Air Server (CIA 8, Markes International, UK), and pre-concentrated the compounds onto

a specialised cold trap, and then analysed them using a GC with a specialised sulfur detector (Wang *et al.*, 2014).

The detection of SO_2 in the sample collected from the inlet may not actually reflect its presence in the sample, as this compound has been shown to be an analytical artefact. While SO_2 was not identified in the blank analysis of the DVB-CAR-PDMS fibre used in this study, it could still have been produced from the fibre and not be present in the sample. Lestremau *et al.* (2004) reported that an SO_2 peak was sometimes found in blank analysis of a CAR-PDMS fibre. Carboxen (a trademark of the Supelco company) is made by pyrolysis of polysulfonated polymers. The pyrolysis removes most of the non-organic material to form the porous structure, but the reaction is incomplete, and typically Carboxen coatings contain a few percent of sulfur material (Lestremau *et al.*, 2004). Therefore, it can be assumed that SO_2 may be formed by the oxidation of the elemental sulfur present in Carboxen.

Hydrogen sulfide was not detected in the present study, however this is likely to be due to the analytical methodology employed. The method used in this analysis was not optimised for this compound, and due to its volatility it is likely to have eluted early, been lost during the injection process, or not have even sorbed on the SPME fibre.

A number of non-sulfur VOCs were also identified, including but not limited to limonene, eucalyptol and *a*-pinene, which are all odorous compounds. These terpenic compounds can be associated with aroma compounds present in powder detergents, liquid detergents and cleaners and fabric conditioners (Escalas *et al.*, 2003a). It has been reported that powder detergents can contain 0.6 – 1.2 mg kg^{-1} fragrance additives, with 25% of them being terpenic compounds (Escalas *et al.*, 2003a). A range of alkyl benzenes and straight chain hydrocarbons were also identified. These findings are also consistent with other studies reported by Sivret *et al.* (2013). They reported that sewer air contained a wide variety of VOCs including the above mentioned compounds (Sivret *et al.*, 2013).

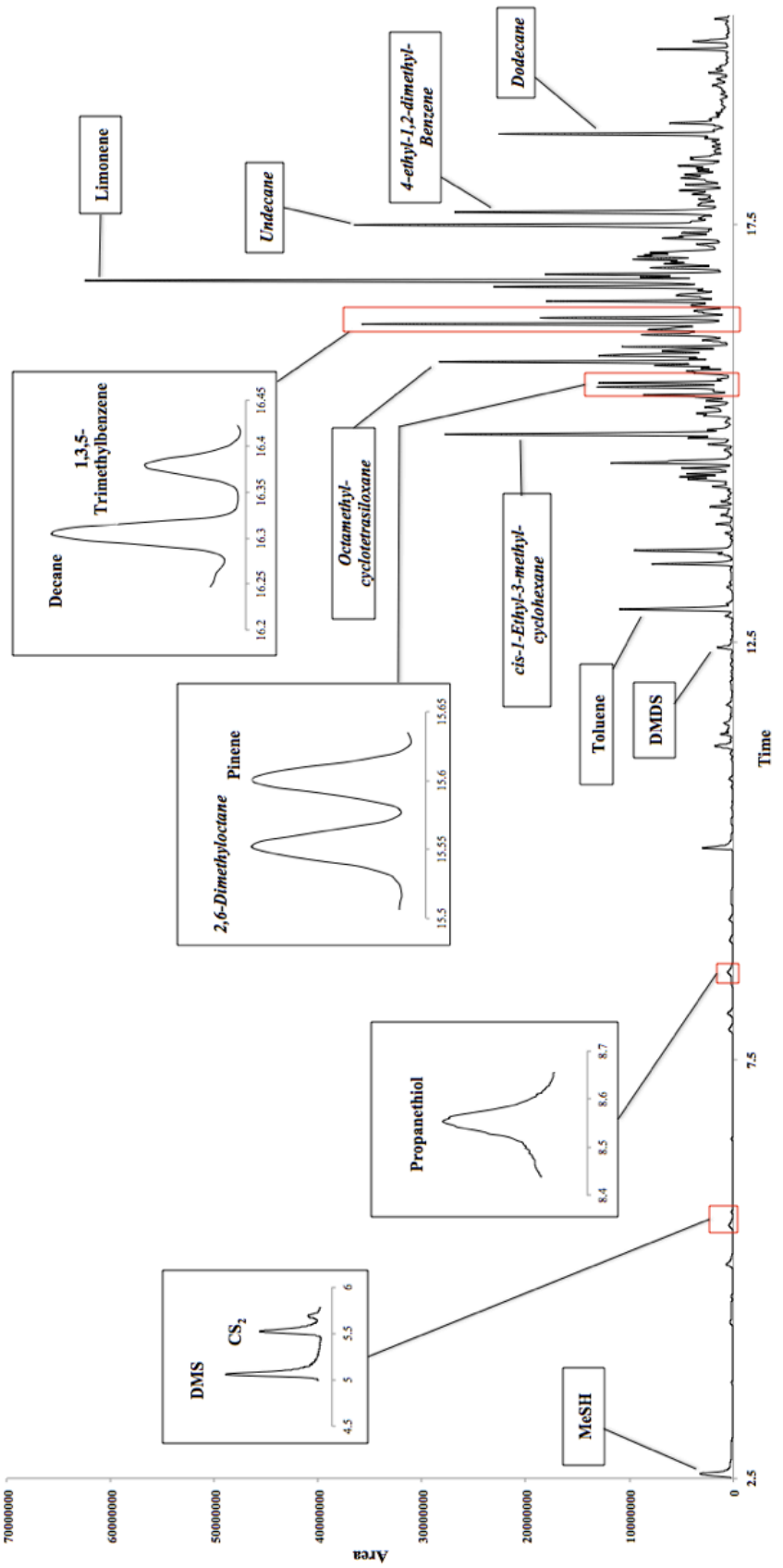


Figure 7.6: Chromatogram (GC-MS; SCAN mode) of Yokine Inlet Sample, collected 12/03/2012.

Also identified were a number of halogenated disinfection by-products, specifically trihalomethanes. These compounds may have originated from chlorination of drinking water which now comprises the wastewater stream.

Peak identification based on library matching of mass spectra to those in a published database (Wiley) is presented in Table 7.1, with the corresponding retention times, quality of the compound match (as a percentage) to the reference database and peak area. As quantitation was not possible, peak areas between compounds should be compared with caution, as different compounds would have different affinities for the fibre. For example, a larger peak area may not necessarily indicate a greater concentration, especially for compounds of different chemical classes.

Table 7.1: Compound identification from a sample collected from the inlet of the biotrickling filter at the Yokine Pump Station.

Retention Time	Compound	Quality	Peak Area
2.55	Methanethiol	91	10449418
5.06	Dimethyl sulfide	97	2222607
5.52	Carbon disulfide	83	1486203
7.87	Thiirane, methyl-	94	1417816
8.07	Hexane	90	1610982
8.55	1-Propanethiol	87	1447273
8.94	Methane, bromochloro-	97	1066492
10.04	1,3-Dioxolane, 2-methyl-	87	8046037
11.40	Heptane	95	1931834
11.53	Methane, dibromo-	95	1784745
11.75	Methane, bromodichloro-	94	1193317
12.44	Disulfide, dimethyl	96	2978095
12.81	Heptane, 2-methyl-	96	1460330
12.89	Toluene	94	21507733
13.16	Cyclohexane, 1,3-dimethyl-, cis-	94	1120464
13.44	Octane	91	12001909
13.56	Methane, dibromochloro-	99	2748834
13.60	Cyclotrisiloxane, hexamethyl-	91	16406121
13.92	Heptane, 2,6-dimethyl-	94	2019279
14.02	Heptane, 2,5-dimethyl-	94	1749306
14.12	Cyclohexane, ethyl-	95	3192221
14.18	Cyclohexane, 1,1,3-trimethyl-	94	1838475
14.36	Heptane, 2,3-dimethyl-	95	2901287
14.48	Octane, 2-methyl-	90	5999097
14.51	Ethylbenzene	94	5522849

Retention Time	Compound	Quality	Peak Area
14.58	Octane, 3-methyl-	94	6874403
14.65	p-Xylene	97	25021694
14.86	Cyclopentane, 1-methyl-2-propyl-	95	4050332
14.94	cis-1-Ethyl-3-methyl-cyclohexane	95	5381343
14.99	Nonane	94	50437164
15.04	Methane, tribromo-	99	3555743
15.24	1-Ethyl-4-methylcyclohexane	87	3526160
15.31	Octane, 2,5-dimethyl-	91	4350996
15.45	Octane, 2,6-dimethyl-	94	14055031
15.60	1S-.alpha.-Pinene	96	16648324
15.78	Nonane, 5-methyl-	87	2158226
15.81	Nonane, 4-methyl-	91	8289488
15.85	Cyclotetrasiloxane, octamethyl-	90	42372124
15.93	Benzene, 1-ethyl-3-methyl-	94	21933404
15.98	Benzene, 1-ethyl-2-methyl-	87	8323290
16.03	Benzene, 1,2,3-trimethyl-	95	14199178
16.30	Decane	97	53321139
16.38	Benzene, 1,3,5-trimethyl-	95	27151986
16.71	Benzene, 1,3-dichloro-	96	1509730
16.75	Benzene, 1-ethyl-2,3-dimethyl-	93	31104689
16.82	D-Limonene	94	105365171
16.87	Cyclohexane, butyl-	90	5551974
16.90	Eucalyptol	99	24527613
17.02	Decane, 4-methyl-	87	6242188
17.08	Decane, 2-methyl-	94	5396741
17.11	Benzene, 2-ethyl-1,4-dimethyl-	97	6384282
17.16	Decane, 3-methyl-	93	9730520
17.33	Naphthalene, decahydro-, trans-	95	8809987
17.40	Benzene, 1-ethyl-2,4-dimethyl-	94	3255127
17.49	Undecane	96	63318914
17.73	Benzene, 4-ethyl-1,2-dimethyl-	90	4112804
17.79	Decane, 3,7-dimethyl-	81	1909883
17.86	Benzene, 1,2,4,5-tetramethyl-	93	2952553
17.90	Benzene, 1,2,4,5-tetramethyl-	94	5612468
17.97	trans-Decalin, 2-methyl-	98	5323751
18.05	Cyclohexane, pentyl-	87	8122246
18.09	Undecane, 5-methyl-	86	3658359
18.17	1-Methyldecahydronaphthalene	96	1224867
18.20	Undecane, 2-methyl-	91	6938066
18.27	Undecane, 3-methyl-	93	3820273
18.53	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	91	1825962
18.58	Dodecane	96	28668475
19.23	Undecane, 2,10-dimethyl-	87	2191197

Retention Time	Compound	Quality	Peak Area
19.31	Dodecane, 4,6-dimethyl-	95	2703179
19.59	Tridecane	94	8862507
19.68	Cyclohexanol, 2-(1,1-dimethylethyl)-	80	6686798
19.96	Nonane, 2,2,4,4,6,8,8-heptamethyl-	86	2074524

7.3.2.2 Analysis of the Sample Collected from the Outlet to the Biotrickling Filter at the Yokine Pump Station

A smaller range of compounds were identified in the sample collected from the outlet of the biotrickling filter than those found at the inlet. This included compounds identified at the inlet but generally at lower abundances, with some compounds removed to levels that could not be detected using the current method. Some additional compounds that had not been detected at the inlet were found at the outlet, which had formed within the biotrickling filter, presumably through the microbial processes within the filter. The VSCs that were identified included DMS, DMDS and DMTS. In terms of VOCs, a smaller range of alkyl benzene compounds were identified, in comparison to those found in the inlet sample. Limonene and *a*-pinene were also present again, but in apparently lower abundance in comparison to the inlet sample. A chromatogram (GC-MS in full scan mode) of the outlet sample is shown in Figure 7.7, with larger peaks and compounds of interest labelled. Other significant peaks in terms of peak area, which were not labeled, included other siloxane-type compounds which were believed to be artefacts of analysis and not actually present in the sample. Bromoform, chloroform and dibromochloromethane were also present. Peak identification based on a library match to a database is presented in Table 7.2, with the corresponding retention times and quality of the compound match (as a percentage) to the reference database listed.

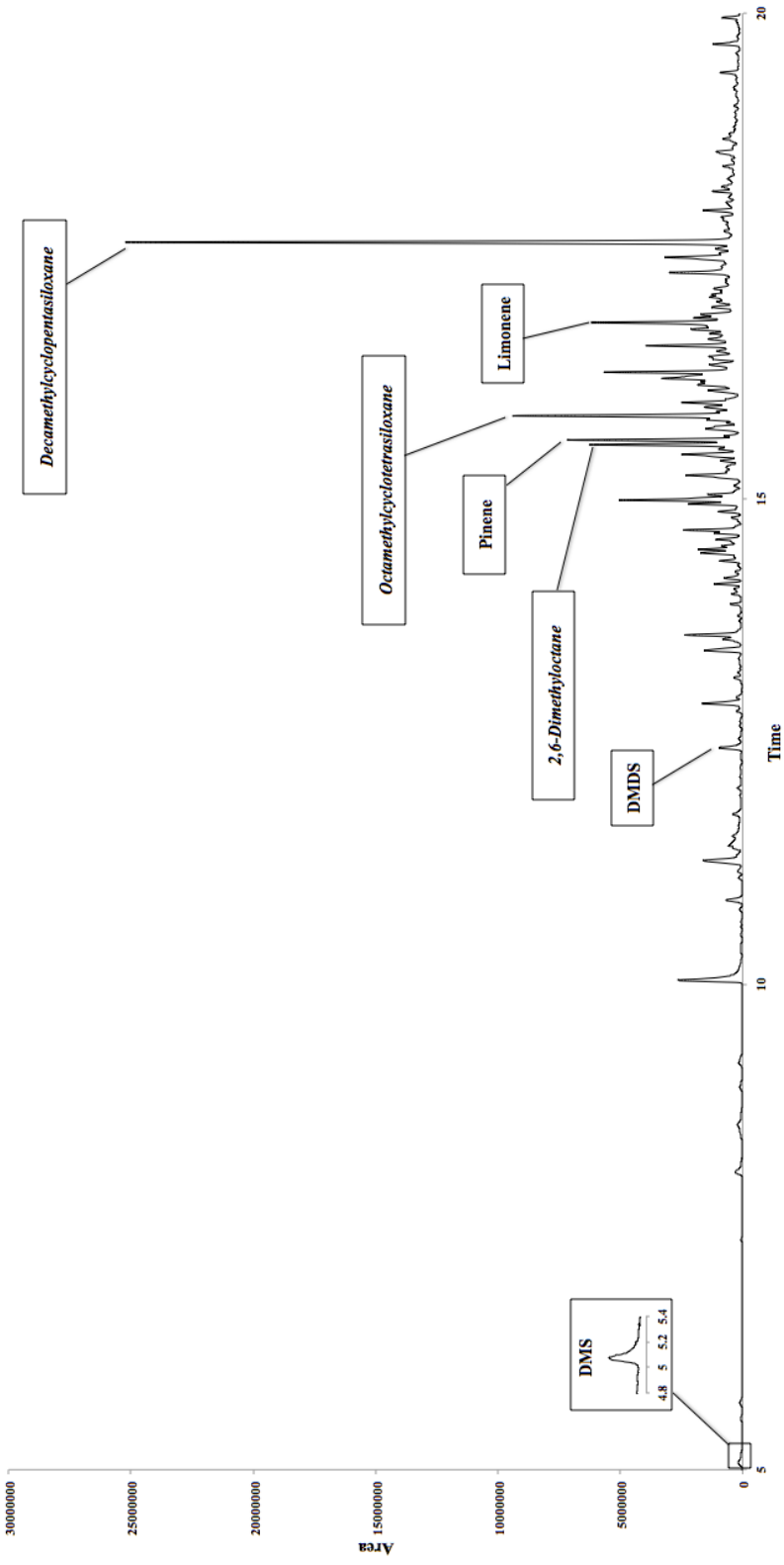


Figure 7.7: Chromatogram (GC-MS; SCAN mode) of Yokine Outlet Sample, collected 12/03/2012.

Table 7.2: Compound identification from a sample collected from the outlet of the biotrickling filter at the Yokine Pump Station.

Retention Time	Compound	Quality	Peak Area
5.07	Dimethyl sulfide	97	665075
5.69	Methylene Chloride	95	319214
8.07	Hexane	90	1162687
8.55	Acetic acid	80	1135045
8.94	Methane, bromochloro-	96	278419
9.19	Trichloromethane	95	573554
10.04	1,3-Dioxolane, 2-methyl-	91	7815550
10.86	Disiloxane, hexamethyl-	90	1456895
11.16	1,3,5-Trioxane	90	447457
12.02	Cyclohexane, methyl-	95	299653
12.44	Disulfide, dimethyl	97	1957080
12.81	Heptane, 2-methyl-	93	467381
12.89	Toluene	94	3236886
13.16	Cyclohexane, 1,3-dimethyl-, cis-	95	541368
13.44	Octane	93	3152645
13.56	Methane, dibromochloro-	99	1524088
13.60	Cyclotrisiloxane, hexamethyl-	91	4162233
13.91	Heptane, 2,6-dimethyl-	83	675136
14.02	Heptane, 3,5-dimethyl-	90	695889
14.12	Cyclohexane, ethyl-	94	1647097
14.18	Cyclohexane, 1,1,3-trimethyl-	95	1267000
14.36	Heptane, 2,3-dimethyl-	94	1249419
14.51	Ethylbenzene	86	595005
14.58	Octane, 3-methyl-	87	1802065
14.65	o-Xylene	97	1483070
14.68	Oxime-, methoxy-phenyl-_	87	3732982
14.80	Cyclohexane, 1,2,3-trimethyl-, (1.alpha.,2.beta.,3.alpha.)-	97	566083
14.87	Cyclopentane, 1-methyl-2-propyl-	94	1625777
14.95	1-Ethyl-4-methylcyclohexane	91	2828166
14.99	Nonane	97	7391441
15.04	Methane, tribromo-	98	2316602
15.24	Cyclohexane, 1-ethyl-2-methyl-, trans-	80	5122814
15.46	Octane, 2,6-dimethyl-	89	4104833
15.61	1S-.alpha.-Pinene	96	9029761
15.85	Cyclotetrasiloxane, octamethyl-	91	12820314
15.94	Nonane, 3-methyl-	92	1571615
16.18	Dimethyl trisulfide	83	2515260
16.24	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	89	6414931
16.30	Decane	95	6996646
16.38	Benzene, 1,2,3-trimethyl-	97	2113522

Retention Time	Compound	Quality	Peak Area
16.58	3-Carene	86	4571974
16.70	Benzene, 1,4-dichloro-	96	529122
16.81	D-Limonene	96	7694222
16.86	Cyclohexane, butyl-	87	1241916
17.33	Naphthalene, decahydro-, trans-	96	4359948
17.49	Undecane	91	4698726
17.64	Cyclopentasiloxane, decamethyl-	94	37622565
17.97	Naphthalene, decahydro-2-methyl-	97	1789910
18.16	Naphthalene, decahydro-2-methyl-	98	1119626
19.59	Tridecane	94	257634
19.96	Nonane, 2,2,4,4,6,8,8-heptamethyl-	86	1020582
21.10	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]-	99	433766

7.3.2.3 Assessment of the Performance of the Biotrickling Filter at the Yokine Pump Station

The Yokine pump station is located in a residential area and is one of two pump stations in the Perth metropolitan area that has a biotrickling filter installed. Due to the proximity to housing, a park and a busy road, it is important that odorous emissions are limited at this pumping station to minimise public concerns and nuisance.

A large range of compounds were identified in gas samples collected from the sewer network at the both the inlet and the outlet of the biotrickling filter at the Yokine pumping station. These include a broad range of compounds, extending well beyond H_2S, which is the only compound that is routinely monitored. Far fewer compounds were detected in the sample collected from the outlet of the biotrickling filter in comparison to the inlet, and most compounds at the outlet were in apparent lower abundance. This is an indication that the biotrickling filter was successfully removing compounds from the sewer gas. This is demonstrated in Figure 7.8 in GC-MS chromatograms for the inlet and outlet samples which are overlain for direct comparison. The inlet sample has much greater peak area responses for equivalent compounds. Peak areas for each compound are also shown in Table 7.1 and 7.2, and are much smaller for corresponding analytes in the outlet sample when compared to the inlet sample, indicating that the concentration is most likely less in the outlet sample.

A smaller range of alkyl benzene compounds were identified in the outlet sample, suggesting that this filter is removing these compounds. Also, a smaller range of VSCs also indicates that the filter is removing some of these odorous compounds. MeSH, CS_2 and propanethiol were identified in the sample prior to its treatment by the filter and did not appear in the sample collected after the filter. These compounds may have been removed, or were significantly decreased in concentration and over the storage time of the sample broken down or volatilised so they were not present. Whichever the case, it still suggests the filters successful performance at removing such compounds. A reduction in the peak area response of other sulfur compounds, such as DMS and DMDS, indicated it was likely that there was a lower concentration of these present in the sample after it had been treated. Limonene and *a*-pinene were also less abundant in the treated sample.

Even though it was not possible to provide quantitative data, based on the peak area response it can be assumed that overall, the biotrickling filter seems to perform quite well in the removal of odorous compounds from samples generated from the sewer system. It removes a large variety of compounds from different chemical classes, including, but not limited to, VSCs and VOCs.

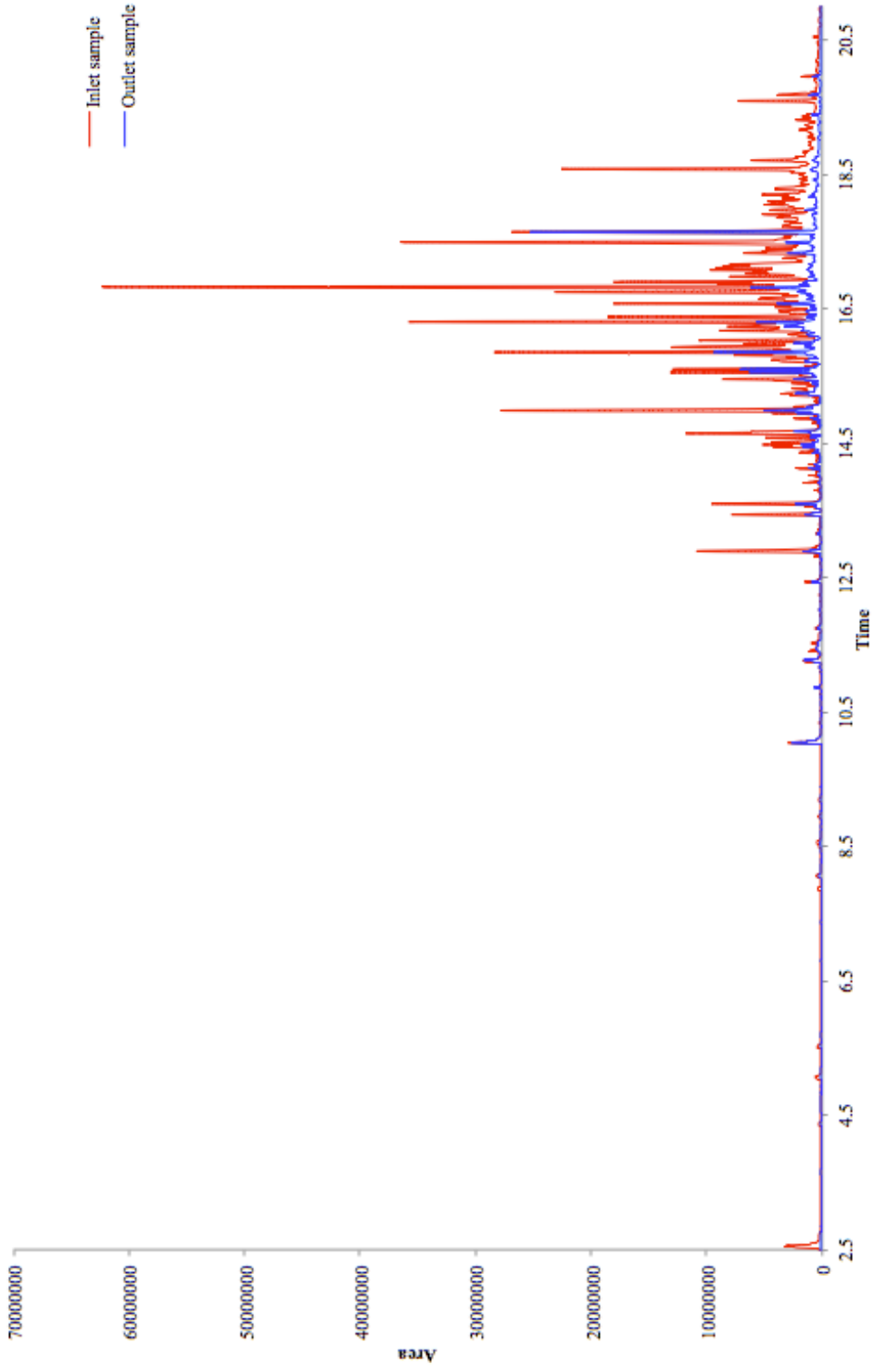


Figure 7.8: Chromatograms (GC-MS; SCAN mode) of Yokine Inlet and Outlet Sample, overlaid, collected 12/03/2012.

7.4 Conclusions

The findings from this study confirmed that the use of SPME as an extraction technique could enable successful identification of compounds associated with sewer odours. These compounds included the VSCs: MeSH, CS_2, DMS, and DMDS; as well a range of other odorous compounds, such as limonene, *a*-pinene, and eucalyptol. A range of alkyl benzenes and other straight chain hydrocarbons were also identified, as well as some disinfection by-products. Consistent with previous studies that used alternative methods, this work showed that sewer air is a highly complex matrix composed of a large range of odorous compounds, that could be contributing to potential odours, well beyond H_2S. This study supports previous studies showing that SPME is a suitable technique for the analysis of odorous air samples and that it would be particularly useful for rapid screening purposes. Further work should be conducted to evaluate the extent of quantitation that can be done using the method to further increase its usefulness as an alternative sampling technique.

Through comparison of the analysis of samples collected from the inlet and then outlet of a biotrickling filter, this treatment process was demonstrated to work effectively. The reduction of compounds present and apparent lesser abundance, based on peak area response in the sample collected from the outlet of the biotrickling filter, demonstrates the effectiveness of this as an odour abatement process. The biotrickling filter installed at the Yokine Pumping Station appears to be successfully removing odorous compounds, which in turn should reduce the odour of the sample and reduce complaints in the area. Once again, further refinement of the method to allow for quantitation will be able to provide an estimate of how effective the filter is in terms of reduction of compounds.

Chapter 8
Conclusions

Headspace solid-phase microextraction (HS SPME) was demonstrated to be a suitable technique for the extraction and pre-concentration of odorous analytes present in water samples. A simple method for the analysis of aldehydes and nitriles, which may be present in drinking water samples, was developed. The method did not require the use of a derivatising agent, making it rapid and easy, while still maintaining good reproducibility and linearity. Appropriate detection limits, which were were less than the odour threshold concentrations for the analytes, were acheived. This method was then applied to a study on the formation of odorous by-products as a result of the presence of amino acids in drinking water.

The presence of amino acids in drinking water has the potential to lead to formation of odorous by-products, which could be described as chlorinous, upon chlorination. Treatment of water samples containing amino acids with chlorine leads to the formation of aldehydes and nitriles. Analysis of these reaction mixtures by an Odour Panel confirmed these by-products are odorous, with a range of different descriptors including chlorinous and chemical. pH was investigated and found to be a factor affecting by-product formation. At pH 9, lower concentrations of both aldehydes and nitriles were observed in comparison to pH 7. The presence of bromide ion was also found to impact the formation of these by-products, with an increase in formation potential if bromide ion was present. A kinetic study of the chlorination reactions of three of the amino acids indicated that another intermediate by-product (potentially the dichloramino acid species) was being formed, with this by-product also having the potential to be odorous. Consideration of removal of the amino acid precursors in the treatment of drinking water may aid in the reduction of formation of chlorinous odours in the distribution system.

HS SPME was also found to be suitable for the analysis of odorous compounds associated with wastewater systems. A range of volatile sulfur compounds (VSCs) were able to be successfully quantified in water samples. The method has good potential to be used as a rapid screening tool. While limitations to the HS SPME extraction of VSCs was demonstrated by the transformation of ethanethiol to diethyl disulfide and careful interpretation of results was required to determine whether an analyte was actually present, or was a resulting artefact, the use of deteurated standards should overcome this. Adaptation of this method for quantitative analysis

of air samples was attempted but unsuccessful. Qualitative analysis allowed for the detection of a range of VSCs and other odorous compounds, including a range of terpenes and volatile organic compounds from air samples taken at both the inlet and outlet areas of a biotrickling filter, a treatment step used to remove odorous compounds within the wastewater treatment process. The range of compounds identified in these air samples highlights the wide ranging odour compounds present in air associated with wastewater treatment, a range which extends well beyond hydrogen sulfide, which is most commonly monitored. While analysis was not possible quantitatively, using abundance as an indicator of concentration showed that these compounds were present in lower concentrations in the biotrickling filter outlet compared to the inlet, indicating that reduction in the concentration of odour compounds present in the sewer system was achieved by the biofilter. This outcome is an important confirmation of the usefulness of biofilters for wastewater odour reduction, particularly necessary when wastewater treatment plants are located in suburban areas with nearby housing. The range of compounds identified also shows similarity to other studies which utilised different pre-concentration or extraction techniques, highlighting that HS SPME is a suitable technique for the analysis of such compounds. Further development of the method to be quantitative would allow for a comparative analytical technique that is simple and cost effective and which could be used as a rapid screening tool for the identification of odorous compounds and also assessment of the efficiency of the treatment process in removal of odorous compounds.

In summary, this Thesis has demonstrated the applicability of the HS SPME technique for analysis of odorous compounds in both drinking waters and wastewaters. The HS SPME technique has been successfully combined with gas chromatography-mass spectrometry for identification of a range of compounds. The drinking water related study demonstrated that the presence of amino acids in drinking water has the potential to lead to odorous by-products, which could be described as chlorinous, upon chlorination. Removal of such precursors could lead to a reduction in formation potential of chlorinous odour compounds. The wastewater study identified a range of volatile sulfur compounds and other volatile compounds associated with off-flavours at a wastewater treatment plant. The HS SPME technique shows promise for demonstrating odour reduction in odour abatement

processes implemented in wastewater treatment plants and potentially offers a more simple sampling device for odour screening.

Chapter 9

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