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MALODOROUS DIMETHYLPOLYSULFIDES IN PERTH DRINKING WATER

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CONTENTS

ABSTRACT

The formation of an objectionable "swampy" odour in drinking water distribution systems in Perth, Western Australia, was first described by Wajon and co-authors in the mid-1980s (Wajon et al., 1985; Wajon et al., 1986; Wajon et al, 1988). These authors established that the odour, variously described as "swampy", "sewage" or "cooked vegetable" was caused by dimethyltrisulfide (DMTS) which has an odour threshold concentration of 10 nanograms per litre (ng/L). Investigations described in the present Thesis extend the work of Wajon and co-workers in attempting to establish the origin and cause of DMTS formation in Perth drinking water distribution systems.

The DMTS problem appeared to be confined to water originating from a particular type of groundwater, specifically groundwater sourced from shallow, unconfined aquifers, which contain relatively high concentrations of sulfide, dissolved natural organic matter (NOM) and dissolved iron. DMTS was not present in the groundwater, but only formed in the distribution system, after treatment of groundwater via alum coagulation-filtration and oxidation processes. One objective of the present work was to determine the reasons for the observed association between DMTS formation and this specific groundwater type. A primary focus was to investigate the chemistry and biochemistry of sulfur species and NOM which might act as precursors to DMTS. The work was driven by the view that increased understanding of the problem might lead to more effective and acceptable treatment solutions than those presently in use.

The observation that DMTS forms in distributed water that originates from groundwater, but not in water from surface sources has led to the hypothesis that groundwater NOM may contain precursor(s) to DMTS. For example, it was proposed that methyl esters and ethers within humic substances might be a source of methyl groups that could participate in DMTS formation in distributed water (Wajon and Heitz, 1995; Wajon and Wilmot, 1992). Further, comparison of levels of reduced sulfur with levels of dissolved organic carbon (DOC) in groundwaters feeding Wanneroo GWTP revealed that a positive

correlation between these two parameters existed. This observation provided further impetus to examine the nature of NOM in these groundwater systems. In the present study (discussed in Chapter 3), NOM from two Perth drinking water sources was isolated and characterised, with the aim of identifying major differences in structure and/or functional groups that might influence DMTS formation. NOM was isolated from water samples using ultrafiltration, and characterised using pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) and offline-thermochemolysis/methylation (TCM). Pyrolysis of groundwater NOM yielded a high proportion of organosulfur compounds, primarily methyl thiophenes and sulfur gases, but did not yield detectable amounts of methoxy-aromatic compounds. Analysis by TCM yielded sulfur compounds tentatively identified as the methyl esters of methylthio-propanoate and methylthiobutanoate, compounds that may arise as degradation products of dimethylsulfoniopropionate (DMSP), an algal osmoregulator. Compounds such as DMSP could potentially undergo reactions to form DMTS in distributed water.

The task of investigating the formation of nanogram-per-litre concentrations of DMTS demanded the development of new analytical procedures that could be used to determine similarly low concentrations of DMTS precursors. Evidence existed to suggest that inorganic polysulfides could be plausible precursor compounds, and since no technique existed to analyse and quantify individual polysulfide homologues a new technique needed to be developed and verified. The technique, first used in a semiquantitative manner by Wajon and Heitz (1995), utilizes methyl iodide to derivatise polysulfides in-situ. The technique was developed further and shown to be quantitative and specific for inorganic polysulfides. Further, a new procedure for the determination of dimethylpolysulfides (DMPSs; $CH_3S_nCH_3$, where n = 2-5), based on purge and trap was developed. In this new procedure analytes were trapped on a "Grob" activated charcoal tube, which was integrated into a commercially available, automated purge and trap instrument. Perdeuterated analogues of the DMPS analytes were synthesized and used as internal standards. These modifications resulted in a more rapid and robust procedure than the previously used procedures,

which were based on closed loop stripping analysis (CLSA). Validation of the precision, accuracy, linearity and robustness of the new procedures for both inorganic polysulfides and dimethylpolysulfides is described in Chapter 4.

Previous authors (Wajon and Heitz, 1995; Wajon and Wilmot, 1992; Wilmot and Wajon, 1997) hypothesized that DMTS could arise in the distribution system from residual polysulfides or other reduced sulfur compounds originating from groundwater. The latter authors showed that a small proportion of sulfide in the groundwater was not completely oxidised to sulfate during the water treatment process and proposed that this residual reduced sulfur fraction, which they referred to as non-sulfide reduced sulfur (NSRS) could contain precursors to DMTS. In a review of the chemistry of sulfide oxidation (Chapter 2) it was shown that the most likely forms of sulfur comprising the NSRS that enters the Wanneroo distribution system are organosulfur compounds and elemental sulfur, probably associated with organic matter in the form of a sulfur sol.

Analysis of inorganic polysulfides in treated water, using the newly described method in Chapter 4, revealed that small amounts of these compounds (20-80 ng/L) were occasionally present in some samples. However, it was concluded that, since inorganic polysulfides could not survive water treatment processes, these compounds probably arose from traces of biofilm or pipe sediment that may have entered the water during sampling. It was proposed that the presence of biofilm particulates in water samples probably also accounted for observations that DMTS appeared to form in some water samples during storage of the sample. These studies are discussed in Chapter 5.

The primary method of control of DMTS formation in the distribution system has been to maintain free chlorine residuals. However, the mechanisms by which this occurs have not been studied; the effectiveness of DMTS oxidation by chlorine, or how chlorine affects microbial processes that might form DMTS is not known. These issues are addressed in the final section of Chapter 5. Experiments to determine the effectiveness of oxidation of

dimethyldisulfide (DMDS) and DMTS (5 μg/L) by free chlorine (0.2 to 0.6 mg/L) in distributed water showed that these substances are rapidly and completely oxidised in water containing a chlorine residual of more than 0.4 mg/L. However, slow regeneration of traces of DMDS and DMTS after dissipation of free chlorine to non-detectable levels showed that these compounds were incompletely oxidised at the lower chlorine concentrations. This provides some rationale for field observations that DMTS occurs even where low, but measurable, chlorine residuals appear to exist (<0.2 mg/L).

As was established in a review of the chemistry of reduced sulfur compounds (Chapter 2), reducing conditions not present in the oxic bulk water are required for DMTS to form and to persist. It was therefore proposed that microbial reduction processes could generate anoxic microniches in the distribution system, within which DMTS production could occur. This hypothesis was investigated in Chapter 6; the new methods for analysis of organic and inorganic polysulfides were applied to the study of biofilms and deposits of colloidal material found in distribution pipes and storage reservoirs. The study demonstrated that these materials contained concentrations of methylated and inorganic polysulfides four to six orders of magnitude higher than those ever found in the bulk water phase. The results indicated that reducing conditions most probably exist within the biofilms and pipewall deposits, where these polysulfides were formed. The iron-rich pipe slimes appeared to protect the sulfur compounds against the oxidative effects of chlorine and dissolved oxygen. It was concluded that the organic and inorganic polysulfides most probably arise through microbial sulfate reduction processes that occur in anoxic microenvironments within the slimes and deposits.

Microbial processes that lead to the formation of polysulfides and dimethylpolysulfides under conditions approximately representative of those in distribution systems were investigated in work described in Chapter 7. The aim of this work was to investigate the role of biofilms in the formation of DMTS and to determine the nature of chemical precursors which might stimulate these processes. Biofilms, artificially generated on synthetic

supports within chambers filled with water from Wanneroo GWTP, were exposed to compounds thought to be potential DMTS precursors. The response of the systems in terms of production of methylated sulfur compounds was monitored. Conclusions of the study were that, under the test conditions, production of DMDS and DMTS could occur via several mechanisms and that these dimethyloligosulfides could be formed even without the addition of compounds containing sulfur or methyl moieties. DMTS did not form in the absence of biofilms and it was therefore concluded that minimisation of biofilm activity was a key in preventing DMTS formation. Outcomes of the work imply that environments within distribution systems are complex and dynamic, as perhaps manifested by the intermittent nature of the DMTS problem.

Finally, in Chapter 8 the conclusions to the present studies are summarised. It is shown how they underpin the rationale for proposed new treatment solutions aimed at preventing DMTS problems in the Wanneroo zone, primarily by minimising microbial activity and biofilm formation within distribution systems.

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ABBREVIATIONS

AAS	Atomic absorption spectrophotometry
AC:	Asbestos cement
AOC:	Assimilable organic carbon
BDOC:	Biodegradable organic carbon
BOM:	Biodegradable organic matter
CLSA:	Closed loop stripping analysis
DCM:	Dichloromethane
DMDS:	Dimethyldisulfide
DMF:	Dimethylformamide
DMPeS:	Dimethylpentasulfide
DMPS(s):	Dimethylpolysulfide(s) ($CH_3S_nCH_3$, where n = 2-5)
DMS:	Dimethylsulfide
DMSP:	Dimethylsulfoniopropionate
DMSO:	Dimethylsulfoxide
DMTS:	Dimethyltrisulfide
DMTeS:	Dimethyltetrasulfide
DO:	Dissolved oxygen
DOC:	Dissolved organic carbon
EDS:	Energy dispersive spectrum technique
EH:	Enhanced coagulation
EPS:	Extracellular polymeric substances
FTIR:	Fourier-transform infrared spectroscopy
GC:	Gas chromatography
GWTP:	Groundwater treatment plant
HPC:	Heterotrophic plate count
ICP-MS	Inductively coupled plasma-mass spectrometry
3-MPA:	3-Mercaptopropionic Acid
ML/d:	Megalitres per day
MSA:	Methanesulfonic acid
MT:	Methanethiol
NMR:	Nuclear magnetic resonance spectroscopy
NOM:	Natural organic matter

NSRS:	Non-sulfide reduced sulfur
OSC:	Organosulfur compounds
PAC:	Proanthocyanidin
PAH:	Polycyclic aromatic hydrocarbons
PTFE:	Polytetrafluoroethylene or Teflon
Py-GC-MS:	Pyrolysis-gas chromatography-mass spectrometry
RC:	Reinforced concrete
SEM:	Scanning electron microscopy
SIM:	Selected ion monitoring
SRB:	Sulfate reducing bacteria
TCM:	Thermochemolysis/methylation
TDS:	Total dissolved solids
TCA:	Trichloroanisole
TCP:	Trichlorophenol
1,2,4,5-TeMB:	1,2,4,5-Tetramethylbenzene
TMAH:	Tetramethylammonium hydroxide

LIST OF FIGURES

LIST OF TABLES

Publications in reviewed journals or books, arising from the present study.

Franzmann, P.D., Heitz, A., Zappia, L.R., Wajon, J.E. and Xanthis K. (2001) The formation of malodorous dimethyl oligosulphides in treated groundwater: the role of biofilms and potential precursors. *Water Research*, 35,(7), pp 1730-1738.

Heitz, A., Kagi, R.I. and Alexander, R. (2000) Polysulfide sulfur in pipewall biofilms: Its role in the formation of swampy odour in distribution systems. *Water Science and Technology*, 41, (4-5), pp. 271-278.

Heitz, A., Joll, C., Alexander, R. and Kagi, R.I. (2001) Characterisation of aquatic natural organic matter in some Western Australian drinking water sources. In *Understanding and Managing Organic Matter in Soils, Sediments and Waters*. Eds. R.S. Swift and K.M Spark, International Humic Substances Society, Adelaide, Australia, 2001, pp. 429-436.

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CHAPTER ONE

POTABLE WATER RESOURCES OF THE PERTH REGION

Potable water is supplied to residents of Perth, the capital city of the state of Western Australia (population of 1.4 million (Australian Bureau of Statistics, 2001)) by the Water Corporation, Western Australia's major water utility. Water is sourced approximately equally from surface catchments (50%) and groundwater (50%). Surface water is obtained from nine separate storage reservoirs on rivers in the Darling Range, and groundwater originates from extensive aquifers in the sands of the Swan Coastal Plain (Water Corporation, 2001). Surface water catchments are located in largely pristine, uninhabited Jarrah forest on predominantly nutrient-poor lateritic sediment, and water quality is usually excellent. However much of the groundwater requires treatment to comply with Australian drinking water guidelines (Water Corporation, 1999; NH&MRC, 1996). The present study is largely concerned with water originating from shallow aquifer sources and the following discussions therefore focus specifically on groundwater.

1.1 HYDROGEOLOGY OF GROUNDWATER RESOURCES

The Perth Region is an area of 4000 km^2, located largely within the Swan Coastal Plain and incorporating the Perth metropolitan area. It is bounded to the east by the Darling Scarp, to the north by the Gingin Brook, to the south by the South Dandalup River and to the west by the Indian Ocean (Figure 1.1). The important groundwater resources of the Region are the unconfined "superficial" aquifer, incorporating the Gnangara and Jandakot Mounds, and the semi-confined and confined Mirrabooka, Leederville and Yarragadee aquifers. The hydrogeology of groundwater in the Perth Region is described comprehensively by Davidson (1995). Aspects of Davidson's report which are applicable to the present study are summarised in the following paragraphs.

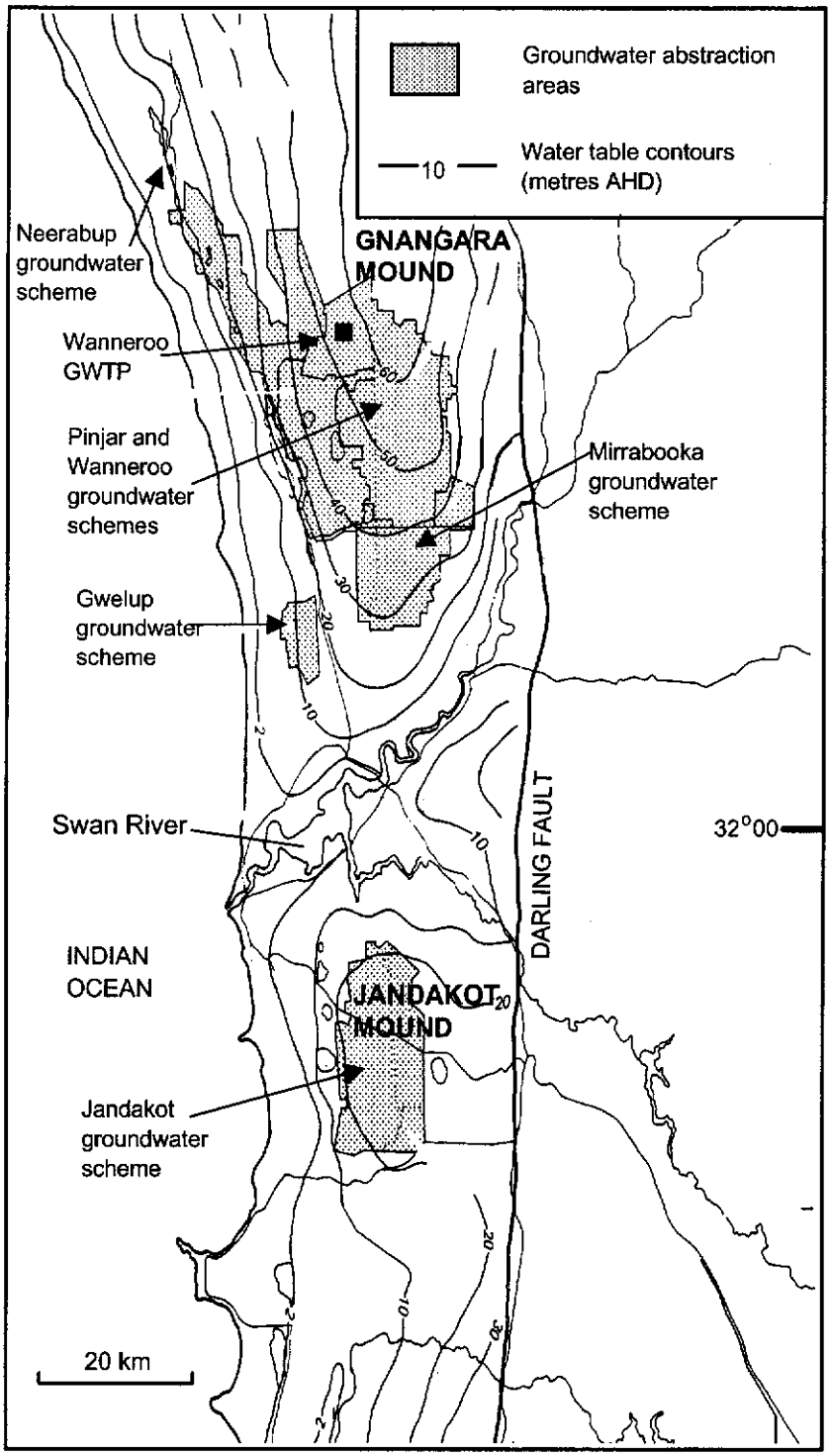


Figure 1.1: Location map showing Perth metropolitan region and six groundwater schemes. Surface water reservoirs are to the east of the Darling Fault (modified from Hirschberg, 1989).

Superficial aquifer

The "Superficial Formations" aquifer, also referred to locally as the "shallow aquifer", "unconfined aquifer" or "Bassendean aquifer" (Allen 1976; Davidson, 1995) lies within the surficial Quaternary to late Tertiary sediments of the Swan Coastal Plain. These are sandy coastal sediments comprising the Safety Bay Sand, Becher Sand, Tamala Limestone, Bassendean Sand, Guildford Clay, Yoganup Formation and Ascot Formation. Their collective thickness is up to 110 m and they consist of largely sand, silt, clay and limestone in varying proportions. Generally, the sediments to the east contain more clay, those in the central area are predominantly sand, while those to the west comprise mainly limestone. The surface geology of the Swan Coastal Plain is shown in Figure 1.2 (a).

Major groundwater mounds, namely the Gnangara Mound North and South and the Jandakot Mound are formed because of variations in the aquifer thickness and hydraulic conductivities of the sediments. The mounds occur because the rates of vertical infiltration at the major points of recharge exceed the rate of horizontal flow through the sediments. Groundwater flows away from the crests of mounds and foothills of the Darling Plateau and terminates at the major drainages, which are the ocean, Gingin Brook, Swan River, Canning River, Serpentine River and the North Dandalup River and the South Dandalup River. Recharge occurs almost exclusively through rainfall, and significant seasonal variations in watertable are therefore observed. These range from a maximum of three metres in areas of clay adjacent to the Darling Fault, to about one metre in the central sandy area, and to less than 0.5 m in coastal limestone areas. Rates of groundwater flow through the aquifer vary considerably, ranging from 50 to 150 m/year in the central sandy area to up to 2000 m/year in parts of the Tamala Limestone to the west. Maximum aquifer thickness is 70 m and groundwater age ranges from the present at minimum depth, to about 2000 years at the aquifer base.

There are many permanent and ephemeral swamps and lakes which form the upper surface of the groundwater table. These are an important

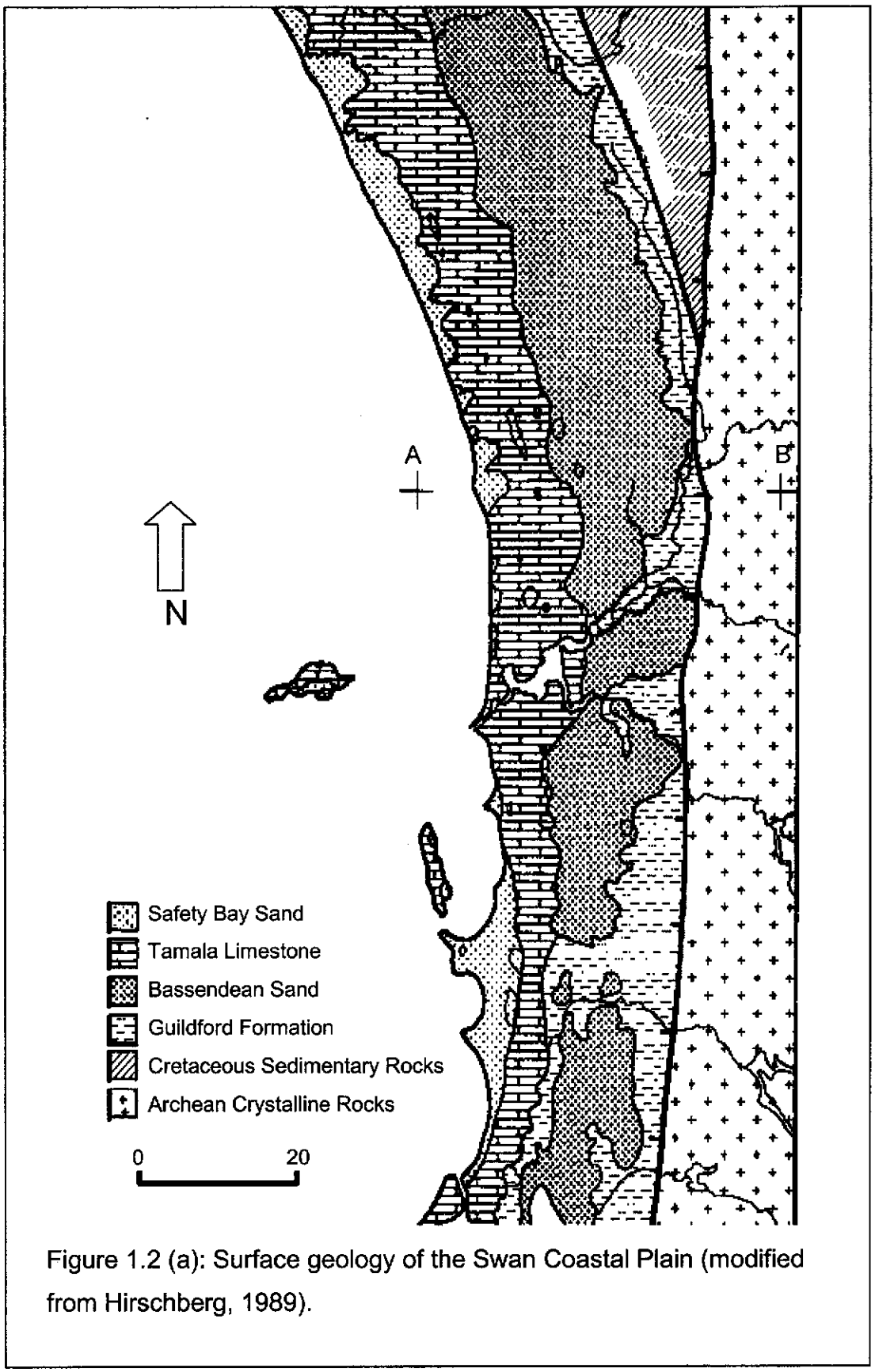


Figure 1.2 (a): Surface geology of the Swan Coastal Plain (modified from Hirschberg, 1989).

geomorphological feature of the Swan Coastal Plain and exist within dunal systems in interdunal swales and interbarrier depressions. Many are in hydraulic connection with the groundwater table, either seasonally or permanently. Lakes are defined as being permanently inundated (Davidson, 1995, p. 9) and many are fringed by vegetation including reeds and sedges. All contain sediments of biogenic origin, comprising peat, peaty sand, diatomite, calcareous clay (boglime) and freshwater marly limestone. Swamps are defined by Davidson (1995) as sumplands and damplands, being seasonally inundated or waterlogged, many only containing water during winter. Like lakes, most swamps contain peaty deposits at their base.

Most of these wetlands are evaporative basins, shallow in depth (0.5 to 3 m), and therefore of highly variable salinity. Salinity is influenced by the seasons, the water being freshest at the end of winter, but the groundwater flow system associated with the existence of the wetlands is also a factor. Many lakes and swamps are perched above impervious deposits such as clay, peat, ferruginous sandstones (coffee rock) or limestone. Seasonal evaporative losses lower the water levels and this may result in loss of hydraulic connectivity with the groundwater. However, "after heavy rainfall this more-saline water is flushed into the groundwater to form a brackish plume down hydraulic gradient from the lakes and swamps" (Davidson, 1995, p. 11). In some circumstances these effects can cause localised and temporary variations in groundwater salinity. Clearly these wetlands, since they are essentially surface expressions of the groundwater table, are of critical importance in the quality of groundwater within the unconfined aquifer. The relationships between groundwater of the superficial aquifer and the wetland systems are illustrated in a geological cross section of the northern Swan Coastal Plain (Figure 1.2 (b)).

Physical and chemical groundwater quality in the superficial aquifer varies with location, influenced mainly by the groundwater flow system relative to recharge and discharge. Davidson (1995, p. 91) classified groundwaters from confined and unconfined aquifers of the Perth Region into three types

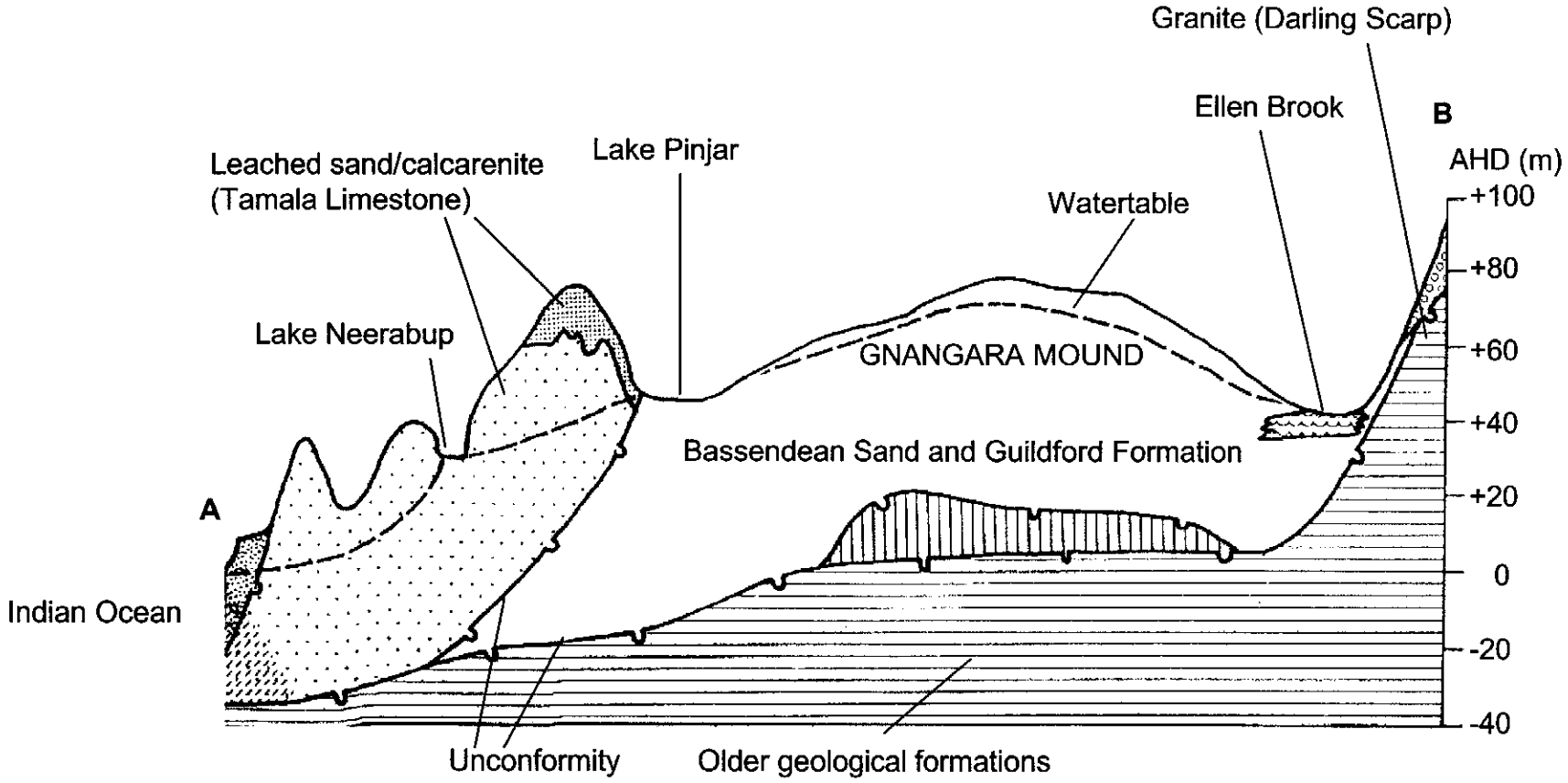


Figure 1.2 (b): Geological cross section of superficial formations on the northern Swan Coastal Plain, showing the raised water table of the Gnangara Mound and two of the lakes typical of this system (Lakes Pinjar and Neerabup). (Source: Webster, 1989)

- Sodium chloride-rich (mainly from the Bassendean Sand)
- Calcium and bicarbonate-rich. In the Tamala Limestone and Ascot Formation
- A mixture of sodium chloride and calcium and bicarbonate. At the base of the superficial aquifer where Tamala Limestone and Ascot Formation are absent and in the underlying confined aquifers.

Salinity in the superficial aquifer can vary from 130 to 12 000 mg/L total dissolved solids (TDS), but is usually less than 1000 mg/L. The least saline is near the recharge areas, with a progressive increase in salinity towards discharge zones. Factors such as hydraulic connectivity with saline wetland areas may have localised effects as discussed above. Sulfate concentrations are usually less than 100 mg/L, although they can be higher in localised areas of high salinity. Sufide is associated with peaty wetland deposits and areas of evaporative concentration. Humic substances, or natural organic matter (NOM) (measured as "colour"), originate from decaying vegetation and occur in highest concentration near swamps and wetlands, and nearer the groundwater table (up to 50 mg/L dissolved organic carbon (DOC)). Waters high in humic substances are associated with low pH (4.0 to 6.5), and low hardness (around 50 mg/L as $CaCO_3$). Water in the calcareous sediments, often closer to the coast, is generally of higher pH (around 6.5 to 8.0) and higher hardness (up to 500 mg/L as $CaCO_3$). Ferrous iron is present in groundwater at levels from 1 mg/L to more than 50 mg/L, and is thought to be derived from dissolution of iron minerals such as ilmenite grains in the Bassendean Sand, the goethite coating on sand granules, or pyritic palaeolake deposits. Infiltration of iron-rich groundwater from the underlying Leederville aquifer is also thought to contribute (Davidson, 1995, p. 89).

Confined aquifers

The superficial formations rest unconformably on Tertiary and Mesozoic formations, which form the confined aquifer systems, most significantly the Mirrabooka, Leederville and Yarragadee aquifers. Groundwater quality in

these aquifers, while variable, is quite different to that from the superficial aquifer, in that it is free from sulfide and low in humic substances.

The Mirrabooka aquifer is a semi-confined to confined aquifer, which is hydraulically connected to the superficial aquifer. Groundwater ranges in salinity from 130 to 350 mg/L TDS and pH from 5.0 to 7.7 and is a mixture of the sodium chloride and calcium and bicarbonate types, according to Davidson's classification. The aquifer lies within largely Cretaceous sediments, with groundwater age estimated at 2000 to 10 000 years. These sediments partially overlay those forming the Leederville aquifer, a major confined aquifer comprising the Cretaceous Osborne Formation and the Leederville Formation. These formations consist of discontinuous interbedded sandstones, siltstones and shales. Salinity in the Leederville aquifer ranges from 180 to 2500 mg/L in production bores and up to 10 000 mg/L in some monitoring bores. These extreme salinity variations are probably due to connate water being trapped between siltstones and shales during periods of marine deposition. Evidence exists that salt locked within the shales is slowly being released to the groundwater. Dissolved iron concentrations ranging from 0.42 to 18 mg/L, probably result from pyritic deposits in shale interbeds (Davidson, 1995, pp. 105-107). Aquifer thickness ranges from a maximum of 550 m to a minimum of 50 m (Davidson, 1995, p. 98), and groundwater age ranges from 1900 to 36 000 years (Davidson, 1995, p. 53).

The Yarragadee aquifer is a major confined aquifer, comprising interbedded sandstones, siltones and shales of the Cretaceous Gage and Parmelia Formations and the Jurassic Yarragadee Formation and Cattamarra Coal Measures. The aquifer is extensive; it underlies the entire Perth Region and its thickness exceeds 2000 m, with only the upper 500 m having been investigated by drilling. The aquifer is largely confined, but in some areas is in hydraulic connectivity with that of the superficial aquifer, and also with that of the Leederville aquifer (Davidson, 1995, p. 109). Groundwater salinity varies from around 140 mg /L to greater than 10 000 mg/L TDS and, the groundwater is largely of the sodium chloride type. In most parts of the

Yarragadee Formation, groundwater contains very low concentrations of dissolved iron, except in areas where water permeates from iron-rich overlying units of the Leederville Formation. Groundwater in the Yarragadee Formation is generally older than 36 000 years.

1.2 GROUNDWATER TREATMENT AND DISTRIBUTION

Groundwater supplying the Perth metropolitan area is treated at five major groundwater treatment plants (GWTPs) located at Wanneroo, Mirrabooka, Jandakot, Gwelup and Neerabup. Groundwater is obtained from six different groundwater schemes, as shown in Figure 1.1 (Water Corporation, 1999). More than 50% of groundwater is sourced from the Gnangara and Jandakot mounds, and this generally contains unacceptable concentrations of sulfide, iron, manganese and NOM (Water Corporation, 1999). This water type is characteristic of the unconfined water feeding GWTPs at Wanneroo (includes Pinjar groundwater scheme), Mirrabooka and Jandakot and is treated by conventional coagulation/filtration processes. Other unconfined aquifer sources are those supplying Gwelup and the northern regions including the Neerabup groundwater scheme, Two Rocks and Yanchep. This water type is sourced from limestone aquifers and contains generally higher alkalinity and hardness, but lower sulfide and NOM than the Wanneroo/Mirrabooka type. Increasing demand for water and decreasing availability of unconfined sources due to urbanisation and other pressures has lead to the artesian (confined) aquifers within the Yarragadee and Leederville formations becoming increasingly important sources. Water quality from these aquifers can vary from slightly saline to excellent, such that in some cases it is treated along with water from the unconfined sources and in others it is pumped directly into storage reservoirs.

Due to the large number of individual water sources, considerable complexity exists in the Perth water supply system, and this has important implications in the present study of the DMTS odour problem; the number of individual bores contributing to the water supply totals well over 150, and waters of highly variable quality are usually blended to obtain an acceptable product (in terms

of Australian Drinking Water Quality Guidelines (NHMRC, 1996)) in the most cost-effective manner. Water quality is therefore relatively stable in terms of major components, but the nature of components which are not individually measured, such as reduced sulfur compounds and organic matter, is unknown and might vary considerably (these substances are generally measured collectively as "bulk" parameters only). The recurrent nature of DMTS and other organoleptic water quality problems where a tainting agent is intermittently present in ultratrace concentrations (e.g. Heitz et al., 2001) suggests that this is probably the case. The high number of individual sources which can potentially change water quality in ways that are often unknown and difficult to measure has frustrated the study of these problems because the complexity of the water supply system has confounded attempts at correlating sources with taste-and-odour incidents.

Treatment and distribution of groundwater from the Wanneroo scheme

All of Perth's five major GWTPs receive mixtures of water of varying quality from both the shallow unconfined aquifers and from artesian aquifers. Water feeding Wanneroo, Mirrabooka and Jandakot GWTPs is of similar quality, and treatment processes at these three plants closely resemble each other, with only minor variations. However, the studies discussed in the present Thesis were conducted using water almost exclusively from Wanneroo GWTP. The present discussion therefore focuses on treatment and distribution processes at only this treatment plant.

Wanneroo GWTP is Perth's largest groundwater treatment facility, processing up to 225 megalitres per day (ML/d). Treatment is via a conventional clarification process which includes coagulation, sedimentation and filtration, and overall, the process is optimized to remove iron, sulfide, turbidity and NOM. A plant flow diagram is shown in Figure 1.3. As the water enters the treatment plant it is subject to spray aeration followed by chlorination (6-8 mg/L) to oxidise iron, much of which is complexed with NOM. Alum coagulant is typically dosed at around 60 mg/L, although in

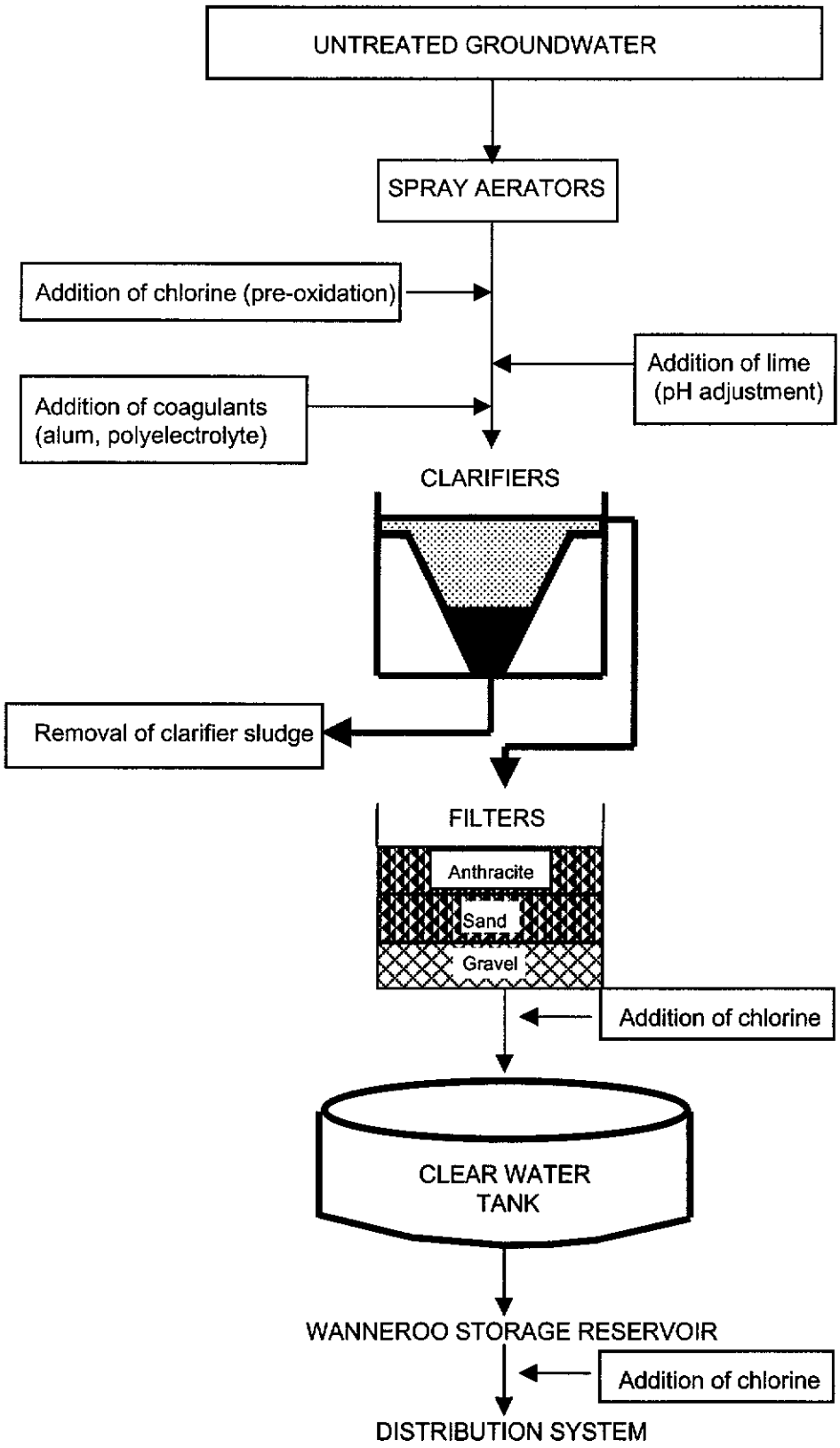


Figure 1.3: Flow diagram of water treatment process at Wanneroo GWTP.

December 1999 the plant commenced operating in "enhanced coagulation (EH)" mode and this was increased to around 90 mg/L.

Polyelectrolyte (0.6 mg/L, or 0.9 mg/L in EH mode) is dosed as the water enters one of three clarifiers typically operating at 3.5 m/h upflow velocity. Supernatant water from the clarifiers then flows through up to twelve concrete filters of sand (0.3 m) and anthracite coal (0.6 m) at a velocity of 15 m/h. Filters are periodically backwashed with chlorinated water. After filtration the water is post-chlorinated in the clear water tanks and then pumped to Wanneroo storage reservoir. Water is re-chlorinated at the outlet of the reservoir, to bring the total chlorine dose (including pre-chlorination) to around 13 mg/L. The aim is to maintain a free chlorine residual of around 1.0 mg/L at the reservoir outlet. This is higher than at other similar storage reservoirs and is maintained at this level to minimise formation of off-odours (i.e. DMTS) within distribution pipes (Cadee et al., 2000; Consulaqua Consultants, 1999).

1.3 SWAMPY ODOUR IN PERTH DRINKING WATER

The formation of an objectionable "swampy" odour in drinking water distribution systems in Perth, Western Australia, was first described by Wajon and co-authors in the mid-1980s (Wajon et al., 1985b; Wajon et al., 1986; Wajon et al, 1988). These authors established that the odour, variously described as "swampy", "sewage" or "cooked vegetable" was caused by dimethyltrisulfide (DMTS; $CH_3S_3CH_3$) which has an odour threshold concentration of 10 nanograms per litre (ng/L). The odour resulted in considerable consumer dissatisfaction and Perth's only water supply utility, the Water Authority of Western Australia (now Water Corporation), has been under continual pressure to prevent recurrences. Since the problem was first identified considerable resources have been invested into finding its cause and into preventative methods.

The occurrence of DMTS appeared to be strongly associated with poor quality, sulfidic groundwater which was sourced from the superficial

(unconfined) aquifer. DMTS formed almost exclusively in water supplied via the Wanneroo GWTP, and was very rarely reported in water from Mirrabooka GWTP and Jandakot GWTP, although these treated and supplied water of similar quality, sourced from the same aquifer. The odour-causing compound was not found in the raw groundwater nor at the outlet of the treatment plant, but appeared to form in the distribution system, occurring with the highest frequency and intensity at the extremities of the system and in "dead-ends". Water containing low concentrations of free chlorine was more prone to the problem but odour episodes generally occurred intermittently and unpredictably. Attempts to recreate odour formation in supply water in laboratory experiments were largely unsuccessful (Wajon and Heitz, 1995), and this, together with the intermittent nature of the problem and the low odour threshold of DMTS has made systematic study of the problem very difficult.

DMTS is oxidised by chlorine and to date the only method to control odour formation has been to increase chlorine residuals in the most affected areas. This method is however often not completely successful, firstly because it is not always possible to maintain free chlorine residuals at the extremities of the distribution system, and secondly because consumers close to re-chlorination points complain of chlorinous tastes and odours. Moreover, the increased chlorine doses required result in a significant cost increase to the water utility. The present study was, to some extent, motivated by the requirement for improved treatment processes to eliminate or minimise aesthetic water quality problems associated with DMTS formation.

1.3.1 Microbiological studies of Perth water distribution systems

Recognition that microbial processes might facilitate DMTS formation prompted several studies investigating the microbiological populations within Perth distribution systems. The most comprehensive of these was a survey of the microbiological quality of Perth drinking water conducted by Neil (1987). The study included microbiological examination of water samples taken before and after chlorination and sediment samples collected from

pipes, reservoirs, tanks and GWTPs. More recent studies (Barton, 1995 a,b,c) focussed on identification of sulfate reducing bacteria (SRB) in pipewall biofilms and sediments from Wanneroo reservoir and GWTP.

In the study by Neil (1987) it was found that while the microbiological quality of water was excellent in terms of coliform and indicator organisms, many non-pathogenic organisms were present. Heterotrophic bacteria (*Flavobacterium, Pseudomonas/Alcaligenes* and *Pseudomonas fluorescens*) were found in almost all of the 271 water samples tested, and their type and number were not dissimilar to results from comparable studies elsewhere (references in Wajon et al., 1988, p. 6). Algae were found in many samples, and were usually associated with unroofed service reservoirs. These reservoirs have subsequently been roofed and algal numbers should now have decreased to undetectable amounts. *Actinomycetes*, including *Streptomyces* and *Nocardia* were found in treated water samples from Wanneroo and Mirrabooka GWTPs, but not in any of the other treated groundwaters or surface waters. Fungi were found in water from groundwater sources, but not in water from surface water sources. Most common were *Fusarium* and *Penicillium*, with *Aspergillus, Epicoccus* and *Rhizopus* identified less commonly. Yeasts were rarely detected. Chemoautotrophic bacteria, such as sulfur oxidising bacteria, sulfate reducing bacteria and iron and manganese bacteria were not detected in samples of distributed water.

According to Neil (1987), "attached sediments" within the Perth distribution system contained far greater numbers and diversity of organisms than those found in the bulk water (10^2 to 10^5 times as many heterotrophic bacteria were found in sediments as could be detected in water samples (also discussed by Wajon et al., 1988)). Genera most commonly isolated from sediments were *Pseudomonas, Alcaligenes, Acinetobacter* and *Bacillus*. Significant numbers of coliform were also detected, despite the absence of these in the associated flowing water samples (Wajon et al., 1988, p. 11). Sediments contained greater relative numbers of facultative anaerobes (*Bacillus, Staphylococcus, Aeromonas/Vibrio* and *Enterobacter*) than water samples, presumably because anaerobic conditions existed within the thicker deposits

Wajon et al. (1988). Microorganisms that were rarely found in water, but occurred commonly in sediments included actinomycetes, especially *Streptomyces*, which was detected in sediments in areas fed by both surface waters and groundwaters. Viable diatoms and non-viable filamentous algae were also found, but numbers of these might have decreased since reservoirs were roofed. SRB were commonly found in areas supplied by treated groundwater, but only occasionally in those supplied by surface water. Iron bacteria and the sulfur-oxidising bacterium, *Beggiatoa*, were found only occasionally. Other organisms such as amoebae, nematodes and protozoa were also detected in sediments within pipes and reservoirs.

Every type of internal surface examined within the distribution system was coated with a sediment or biofilm, but biofilm thickness and other characteristics varied considerably. Biofilms were generally thickest closest to the source (i.e. GWTP or dam), decreasing in thickness towards the extremities of the distribution system. In general, sediment thickness was inversely related to chlorine concentration, such that the larger diameter pipes contained the thickest deposits. Maximum thickness was reported as 10 mm (Wajon et al., 1988, p. 10) in pipes greater than 1000 mm in diameter. Sediments were almost indiscernible in pipes with diameters less than 120 mm. Vertical zonations were noted in many of the sediments, especially those from the Mirrabooka and Wanneroo distribution systems. A black zone nearest the pipe surface was shown by culture studies to be an anaerobic zone containing high numbers of SRB. This internal zone also contained higher counts of actinomycetes and fungi than the external layer. However, overall the external layer contained higher total numbers of organisms than the black internal layer, possibly due to limitations of nutrient diffusion through the biofilm.

Barton (1995 a,b,c) examined biofilms within experimental pipe-loops set up at each of two sites; at (a) Wanneroo GWTP; and (b) Wanneroo reservoir. The pipe-loops were set up so that DMTS formation could be studied in the field, but even though they were in service for several years, DMTS was observed in only one of the systems (Wanneroo reservoir site) for a period of

a few months in 1995. In the initial studies by Barton (1995a,b), sections of pipes at each of the two sites were removed and examined for the presence of SRB. It was found that biofilm sampled from a pipe section from the Wanneroo GWTP pipe-loop (where DMTS had never been observed) contained sulfide-generating microorganisms, while biofilms collected from the Wanneroo reservoir pipe-loop (where DMTS had formed during 1995) did not contain sulfide-generating organisms. In a further study, several samples of sediment were taken from the base of Wanneroo reservoir, and all of the samples examined were found to contain anaerobic organisms capable of generating (inorganic) sulfide (Barton, 1995c).

Studies of DMTS-forming microorganisms

Wajon et al. (1988) isolated fifteen species of bacteria from swampy-smelling and non- swampy-smelling water samples and tested them individually for their ability to form DMTS. The isolated organisms were grown on culture media containing yeast and a variety of sulfur compounds, including cysteine, methionine, sulfate, sulfide and elemental sulfur. Production of DMDS, DMTS and DMTeS was monitored, although results were not reported quantitatively. The study focussed only on those species of bacteria found in highest abundance in water samples, particularly swampy-smelling samples. Although the microbes studied were also found in distribution system biofilms, the role of biofilms and sediments in DMTS formation was not investigated. These studies showed that every species of bacteria tested could produce DMTS from at least one sulfur substrate and some could do so from two or more substrates. It was therefore concluded that the ability of bacteria to produce DMTS was essentially ubiquitous, given the right substrate. These conclusions led to the hypothesis that DMTS formation in the distribution system depended on the presence of a crucial sulfur-containing precursor compound, possibly a sulfur amino acid or elemental sulfur (Wajon et al., 1988, p. 58).

1.3.2 The potential role of sulfur-containing precursors in DMTS formation

Formation of DMTS was largely confined to water originating from Wanneroo GWTP, although on rare occasions an odour event was reported in zones receiving water from Mirrabooka or Jandakot GWTP (Wajon et al., 1988). This observation led to the proposal that formation of the odour was somehow linked to the raw source water, which contains relatively high concentrations of sulfide (up to 1 mg/L) (Water Corporation, 1999). It was thought that the odorous organosulfur compound might form from sulfur precursors present in the groundwater. This hypothesis was examined by Wajon and Wilmot (1992) and Wilmot and Burkett (1992) who measured trace concentrations of reduced sulfur species in raw, treated, and distributed water, but found that these did not correlate with DMTS occurrences. The highest concentrations of total reduced sulfur and non-sulfide reduced sulfur (NSRS) were found in water from Jandakot, not Wanneroo, which was most prone to DMTS formation (Wilmot and Burkett, 1992). The aforementioned studies were hampered by the lack of techniques for measuring individual sulfur species; only sulfide was analysed individually and all other reduced sulfur species could only be detected and quantified as a bulk measurement. A primary aim of the present project was therefore to extend the work of Wajon and co-workers and to develop further insights into the reactions of reduced sulfur species in Perth groundwaters and water supply systems. It was the view that this would then lead to a greater understanding of the formation of highly odorous dimethylpolysulfides (DMPSs) in drinking water.

CHAPTER TWO

TRANSFORMATIONS OF SULFUR COMPOUNDS IN AQUATIC ENVIRONMENTS: A REVIEW

2.1 PRODUCTION OF SULFIDE IN SEDIMENTARY ENVIRONMENTS

Sulfide in aquatic and sedimentary systems arises primarily via biogenic dissimilatory sulfate reduction, and partly through organic sulfur compound catabolism by heterotrophic organisms and assimilatory sulfur metabolism (Kelly and Smith, 1990). (Generation of sulfide via geothermal processes is beyond the scope of the present discussion). In the process of dissimilatory sulfate reduction, sulfide is generated by bacterial anaerobic respiration, with sulfate (or sulfur, tetrathionate or thiosulfate) as the electron acceptor, and organic carbon, represented by CH_2O, as the electron donor (eq 2.1; Kelly and Smith, 1990; Appelo and Postma, 1993).

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3 \qquad ...[2.1]$$

Sulfate reducing bacteria (SRB) are obligate anaerobes (Hao et al., 1996) and bacterial sulfate reduction therefore occurs only in strictly anoxic environments. Kelly and Smith (1990) state that dissimilatory sulfate reduction probably occurs in "any organic-rich anaerobic environment on earth". The occurrence of sulfate reduction in apparently oxic environments has been observed, but in most cases this was shown to take place within anoxic microenvironments (Kühl and Jorgensen, 1992; Jorgensen 1977; discussed later in the present Section).

Although dissimilatory sulfate reduction typically occurs in marine or highly saline settings where sulfate is not limiting, the process can occur at appreciable rates in freshwater environments. For example, Smith and Klug (1981) detected concentrations of sulfate and sulfide at 0.05 and 0.2 mM, respectively, in freshwater lake sediments, and Hao et al. (1996) quoted 20 mg/L sulfate as

being the limiting concentration for sulfide production in wastewaters. SRB that grow on methoxy-aromatic acids (with concomitant formation of methylated sulfur compounds) have been isolated from anoxic freshwater mud samples (Bak and Finster, 1993; Bak et al., 1992). An acetate-oxidising species of SRB, *Desulfuromonas acetexigens*, which can reduce elemental sulfur to sulfide and polysulfide was isolated from freshwater sediments (Finster et al., 1994). Berner (1984) established that in environments where sulfate is present at concentrations greater than 500 mg/L sulfate-S (5 mM), sulfate reduction is limited by the availability of organic carbon.

Assimilatory sulfur reduction involves the uptake of sulfur, usually as sulfate, by living organisms, and its incorporation into biochemicals such as sulfolipids, amino acids (e.g. cysteine, glutathione, taurine and methionine), vitamins (e.g. biotin), iron-sulfur proteins (ferredoxins) and coenzymes (Fischer, 1989; Fischer 1986). Putrefaction of these compounds upon cell death can result in the formation of hydrogen sulfide and other sulfur compounds that are important in the global sulfur budget (Dunnette, 1989). Another major route to the formation of volatile reduced sulfur compounds such as hydrogen sulfide is via dimethylsulfoniopropionate (DMSP), an algal osmolyte produced extensively in the oceans. Hydrogen sulfide and DMS may be formed from DMSP, which in turn, is formed from microbial degradation of methionine (Taylor, 1993, and references therein).

Sulfate reduction in biofilms within oxic macroenvironments

Since sulfate reducing bacteria are obligate anaerobes (Hao et al., 1996), it is generally considered that the environments in which these organisms are found must be strictly oxygen-free. However, several studies have found that sulfate reduction occurs in systems that are apparently oxic, such as oxic sediments (Jorgensen, 1977; Canfield and Des Marais, 1991), within aerobic biofilms in trickling filters (Kühl and Jorgensen, 1992) and aerobic biofilm reactors and

activated sludge plants (Lens et al., 1995; Okabe et al., 1998; Santegoeds et al., 1998).

In most cases it was found that sulfate reduction occurred in anoxic microenvironments a few hundred micrometers below the surface of the biofilm or sediment studied. Vertical zonations of respiratory processes similar to the zonations found in sediments exist in biofilms only a few millimeters thick (Kühl and Jorgensen, 1992; Okabe et al., 1998; Santegoeds et al., 1998). The emergence of microelectrode technology has made it possible to study these systems at a spatial resolution of less than 100 μm. For example, Kühl and Jorgensen (1992) used microelectrodes to measure sulfide, oxygen and pH at high spatial resolution in biofilms sampled from a trickling filter: These are ostensibly aerobic biofilms in an oxic macro-environment. These workers found that oxygen penetrated only 0.4 mm into the biofilm, which had an average total thickness of 4.5 mm. Oxygen depletion was attributed to high rates of respiration in the top 0.1 mm of the biofilm where availability of the organic substrate was the greatest. Sulfide was produced by sulfate reducing bacteria in a zone 1.05 to 2.15 mm below the biofilm surface, and this was oxidised in a narrow zone 0.2 to 0.475 mm below the surface. In the section below 2.15 mm, the concentration of sulfide was constant, indicating that it was neither produced nor consumed. This was because sulfate did not fully penetrate the biofilm, being depleted within the sulfate reducing zone. These studies showed that diffusional resistance of reactants within the biomass layer allows a number of distinctive chemical and microbial oxidation and reduction processes to occur in layers less than a millimetre thick.

In another study Jorgensen (1977) demonstrated the existence of sulfate reducing bacteria in sediment pellets 50-200 μm in diameter. It was shown that sulfate reduction occurs in apparently oxidised surface sediments in a coastal marine setting. The interstitial waters had positive redox potentials and sulfide was therefore rapidly oxidised and not detectable, but even in these highly oxic

surface sediments (2 cm from the surface) with redox potentials up to 300 mV, significant sulfate reduction rates were measured (5 nmol sulfate/cm^3/day). The existence of sulfate reduction processes within microniches in the oxic sediment was also evident from the presence of sulfate reducing (*Desulfovibrio* spp.) and sulfide oxidizing bacteria (*Beggiatoa* spp.) and ferrous sulfide and pyrite.

In the studies described above, it was shown that sulfate reduction occurred in anoxic microenvironments within oxic macroenvironments. However, in a study by Canfield and Des Marais (1991), it appears that sulfate reduction occurred within actual aerobic zones of hypersaline bacterial mats, and not within anoxic microniches. These authors measured oxygen concentrations within a spatial resolution of 5 to 20 μm and did not observe anoxic microzones, yet sulfate reduction occurred at significant rates. At the time of their study, dissimilatory sulfate reduction had not been reported in the presence of free oxygen in pure cultures. To explain their observations they proposed that novel, as yet undiscovered, biochemical pathways for sulfate reduction, or complex microbial interractions, the details of which were unknown, might exist.

2.2 THE CHEMISTRY OF REDUCED SULFUR COMPOUNDS

Hydrogen sulfide is a weak diprotic acid, existing in solution as aqueous hydrogen sulfide (H_2S), bisulfide ion (HS^-) and sulfide ion (S^{2-}) (O'Brien and Birkner, 1977). The equilibrium distribution of these species between pH 1-13 is shown in Figure 2.1 (adapted from Pasiuk-Bronikowska et al., 1992). In the pH range of natural waters (5-8) hydrogen sulfide and bisulfide are the predominant species.

2.2.1 The chemistry of sulfide oxidation

Sulfide (S^{-II}) formed in sediments through assimilatory and dissimilatory sulfate reduction is re-oxidized progressively on contact with oxidants such as dissolved

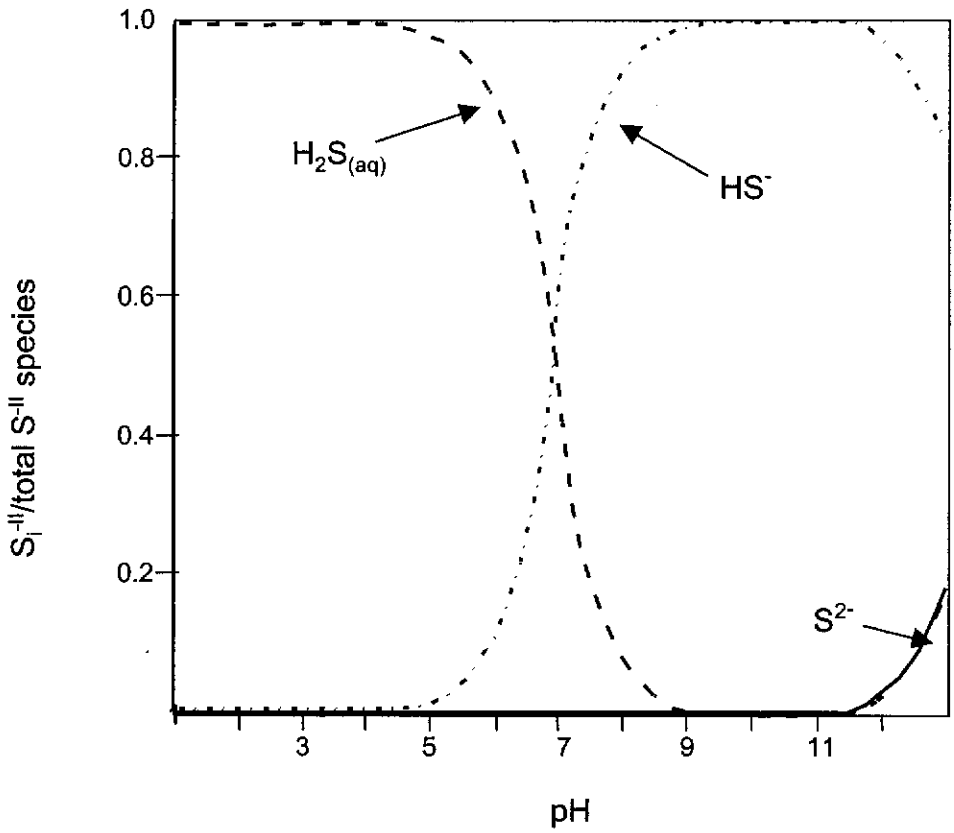
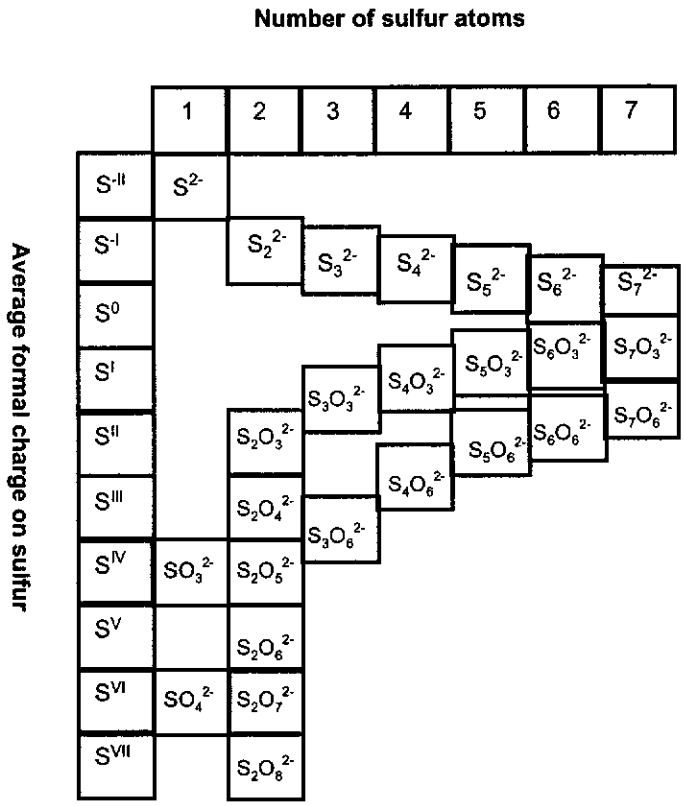


Figure 2.1: Distribution of hydrogen sulfide, bisulfide ion and sulfide ion species (S_i^{-II}) in aqueous media (adapted from Pasiuk-Bronikowska et al., 1992) .

and atmospheric oxygen, iron oxy-hydroxides, manganese dioxide and nitrate (Luther and Church, 1992 and references therein; Zhang and Millero, 1994; Yao and Millero, 1995). Oxidation of sulfide (S^{-II}) to sulfate (S^{VI}) is an eight-electron transfer reaction occurring via a series of steps, with each step involving the transfer of only one or two electrons. According to the octet rule, sulfide and sulfate are stable because they both have fully-occupied outermost atomic orbitals; the species formed from each successive oxidation step are considered to be metastable intermediates because they must gain or lose electrons to achieve complete stability (Williamson and Rimstidt, 1992). The major groups of metastable sulfur intermediates associated with sulfide oxidation in aqueous systems are polysulfides, elemental sulfur (S^0) and the sulfoxyanions, thiosulfate, sulfite (S^{IV}) and polythionates (Moses et al., 1984; Williamson and Rimstidt, 1992). Their molecular formulae and the formal oxidation states of sulfur in each of these groups are shown in Figures 2.2(a) and (b).

The ability of sulfur to form catenated and mixed-valent species enables the formation of many compounds of mixed oxidation state, where the average formal oxidation state of sulfur is a non-integer, or an intermediate value, as shown in Figure 2.2(a). Polythionates and polysulfides are the most stable examples of catenated inorganic sulfur species, in which each of the two terminal sulfur atoms in the chain possesses equal formal charge (Pasiuk-Bronikowska et al., 1992, p. 66, 71; Williamson and Rimstidt, 1992). In the case of polysulfides, the terminal sulfur atoms exist as sulfenyl sulfur, each with one formal negative charge, and the sulfur atoms in the chain are formally zero-valent. All of the sulfur-sulfur bonds are shorter than 2.08 Å, the value calculated on the basis of *p*-bonding only (Clarke et al., 1994). This suggests that the di-negative charge is distributed along the chain through resonance effects, with a partial negative charge localised at each end (Clarke et al., 1994; Williamson and Rimstidt, 1992). Polysulfides are unique in that the terminal sulfur atoms are nucleophilic, while the central uncharged sulfur atom(s) is/are electrophilic (Luther , 1991).



Figures 2.2. (a) and (b)(next page): Sulfur species that are thermodynamically stable, or have significant metastable persistence in aqueous solution.

(a) Average formal charge on sulfur versus number of sulfur atoms, and molecular formula for each species. Illustrates how the average formal charge on sulfur tends towards zero with increasing sulfur chain length (from Williamson and Rimstidt, 1992).

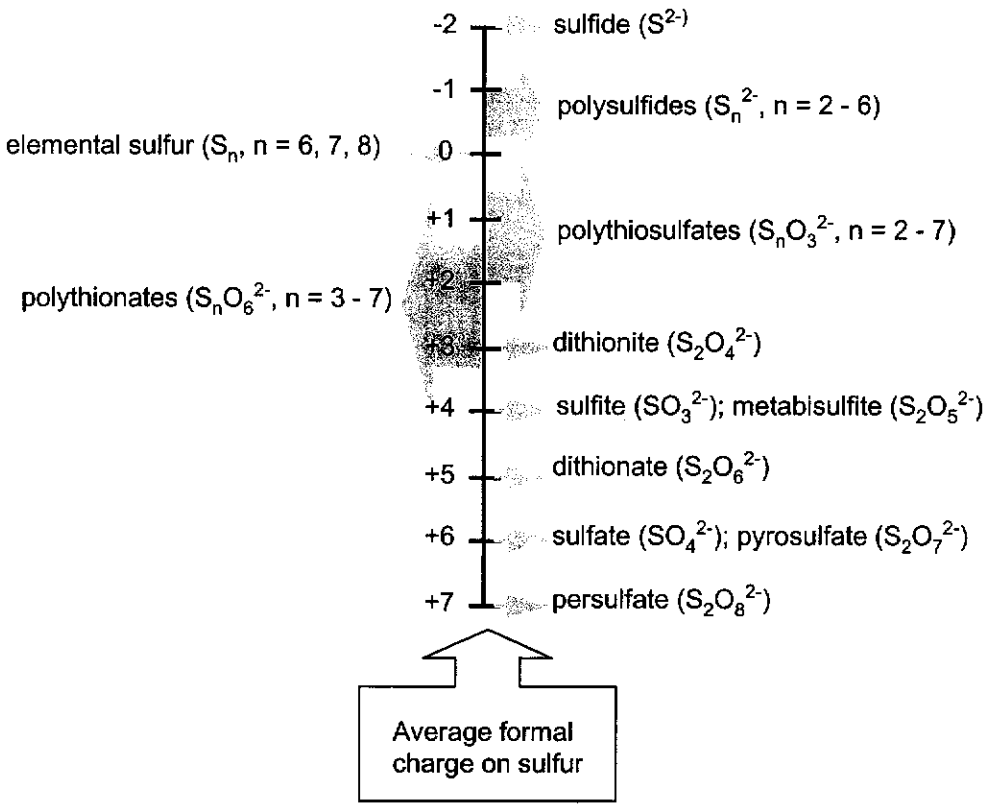


Figure 2.2 (b) Average formal charge on sulfur for each group of sulfur species. Illustrates more precisely than in Figure (a), the range of average formal charges possible for each group.

According to Luther (1991), the order of nucleophilicity is as follows:

$$S^{2-} > S_5^{2-} > S_4^{2-} > HS^- > FeS_2 \sim S_3^{2-} > H_2S$$

In the case of polythionates the sulfur atoms in the chain are formally in the zero-valent state, while the terminal groups exist as sulfonyl moieties (SO_3^{2-}), with sulfur as S^V (Figure 2.2 (a)). Williamson and Rimstidt (1992) contend that since S^V is not known to exist in the aquatic environment, polythionates should be considered as resonance structures where the oxidation state of the sulfur atom in each of the two terminal sulfonyl groups varies from S^{IV} to S^{VI}.

Factors affecting the distribution of sulfide oxidation products

Mechanisms of sulfide oxidation are highly complex and poorly understood; overall, the process results in production of sulfate, the most thermodynamically stable product, but numerous pathways and steps are possible. The distribution of metastable intermediate products depends on many factors, including the availability of oxygen (Chen and Morris, 1972; O'Brien and Birkner, 1977), pH, temperature (Giggenbach 1974a), and the presence of metal ions (Steudel 1996, Zhang and Millero, 1993). A high sulfide-to-oxygen ratio results in formation of polysulfide/elemental sulfur, while a low sulfide to oxygen ratio results in the formation of sulfoxyanions at neutral pH (Chen and Morris, 1972). Existing literature on the occurrence and chemistry of metastable sulfide oxidation products, especially polysulfides, is contentious and confusing and it is therefore appropriate to discuss the topic in some detail. Zhang and Millero (1993) have summarized the major studies and a summary Table from their paper is reproduced in Table 2.1.

Polysulfides were detected as intermediates in sulfide oxidation in only one out of the nine studies listed: that by Chen and Morris (1972). These authors found that the formation of polysulfides occured only at high sulfide to oxygen ratios, with total sulfide at mM levels between pH 6.3 and 7.0. Polysulfides were

probably not detected in other studies because they were not conducted in this pH range. Zhang and Millero (1993) did not detect polysulfides in their studies at various pH values but admit that this could be attributed to the lack of sensitivity of their analytical technique (measurement of absorbance at 290 nm), rather than to the lack of polysulfide.

Table 2.1: Summary of products of hydrogen sulfide oxidation observed by various workers (reference: Zhang and Millero, 1993)

Medium	T (^{o}C)	pH	H_2S init conc. (μM)	O_2 init conc. (μM)	$t_{1/2}$ (h)	Products	Ref
water	25	11-13	1000	1000		SO_4^{2-}; $S_2O_3^{2-}$	a
water	25	7.94	100	800	50	SO_3^{2-}; $S_2O_3^{2-}$	b
water	25	8.75	100	800	50	SO_3^{2-}; $S_2O_3^{2-}$	b
water	25	6.9	3000	800		S_n^{2-}; SO_3^{2-}; $S_2O_3^{2-}$	c
water	25	7.55	1070	1000	24	SO_4^{2-}; SO_3^{2-}; $S_2O_3^{2-}$	d
water	8.2	8.2	25	183	21	SO_4^{2-}; SO_3^{2-}; $S_2O_3^{2-}$	g
seawater	9.8	7.5-7.8	60	240	16	SO_4^{2-}; SO_3^{2-}; $S_2O_3^{2-}$	e
seawater	15	8.5	32	75	65	SO_3^{2-}; $S_2O_3^{2-}$	f
seawater	25	8.1	25	200	25	SO_4^{2-}; SO_3^{2-}; $S_2O_3^{2-}$	g

a) Avrahami and Golding (1968)
b) Chen and Morris (1971)
c) Chen and Morris (1972)
d) O'Brien and Birkner (1977)
e) Cline and Richards (1969)
f) Skopintsev et al. (1964)
g) Zhang and Millero (1993)

Chen and Morris (1972) proposed that oxidation of sulfide produces elemental sulfur, which is then attacked by the nucleophilic bisulfide ion to give polysulfide. Further oxidation produces thiosulfate and sulfite, which is finally oxidised to sulfate. According to Steudel et al. (1986) polysulfides undergo rapid

autoxidation upon contact with molecular oxygen, forming thiosulfate and elemental sulfur.

The auto-oxidation of sulfide is a free radical process, which may be catalysed by one-electron oxidants, typically free or chelated transition metal ions, for example Fe^{III}, Cu^{II}, V^{V} (Steudel, 1996; Pasiuk-Bronikowska et al., 1992; Zhang and Millero, 1994; Kotronarou and Hoffman, 1991), or organic redox couples such as the quinone-hydroquinone system (Pasiuk-Bronikowska et al., 1992, p. 73). The first of the oxidation steps is autoxidation of sulfide to form polysulfides via a series of free radical reactions. The processes of sulfide auto-oxidation to form polysulfides are described in detail by Pasiuk-Bronikowska et al. (1992, Chapter 3) and Steudel (1996) and are summarised as follows:

The initiator in the free radical autoxidation of sulfide is thought to be a form of polysulfide or polythionate species, as shown in Scheme 2.1.

Scheme 2.1: Proposed mechanism of formation of radical initiator formed during sulfide autoxidation (Pasiuk-Bronikowska et al., 1992, p. 68).

$$S_nS^{2-} + O_2 \rightleftharpoons S_nS^{2-}.O_2 \quad \text{...[2.2 (a)]}$$

$$S_nS^{2-}.O_2 + S_nS^{2-} \rightleftharpoons 2(S_nS^{2-}.O)^{\bullet} \quad \text{...[2.2 (b)]}$$

$$(S_nS^{2-}.O)^{\bullet} + S_nS^{2-} \longrightarrow (S_nS^{2-}.O.\ S_nS^{2-})^{\bullet} \quad \text{...[2.2 (c)]}$$

$$2(S_nS^{2-}.O.\ S_nS^{2-})^{\bullet} \longrightarrow \text{inert products} \quad \text{...[2.2 (d)]}$$

$$(S_nS^{2-}.O.\ S_nS^{2-})^{\bullet} + (S_nS^{2-}.O_2) \xrightarrow{\text{slow}} (S_nS^{2-}.O.\ S_nS^{2-}.O_2.\ S_nS^{2-})^{\bullet} \quad \text{...[2.2 (e)]}$$

The structures of the intermediate products in equations 2.2 (a) to (d) and of the initiator (product of eq 2.2 (e)) are not known. The final step (eq 2.2 (e)) is slow, accounting for the induction period observed in sulfide oxidation reactions (Chen and Morris, 1972).

The radical initiator formed in eq. 2.2 (e) then reacts with bisulfide anion to form polysulfide anions via the following radical transfer reactions:

Bisulfide anion is oxidised to the bisulfide radical ($HS^{\bullet}$) by a one-electron transfer process according to eq 2.3. (a) (Steudel, 1996).

$$\text{Initiator} + HS^{-} \rightarrow HS^{\bullet} + e^{-} \qquad \ldots[2.3\ (a)]$$

It is thought that the pK_a of $HS^{\bullet}$ is less than 7, and therefore, the major reactive species at pH > 7 would be the sulfide radical ($S^{\bullet-}$) (Steudel 1996):

$$HS^{\bullet} \rightleftharpoons S^{\bullet-} + H^{+} \qquad \ldots[2.3\ (b)]$$

Dimerization of this radical results in termination of the reaction, and formation of disulfide anion (eq 1.3 (c)).

$$2S^{\bullet-} \rightarrow S_2^{2-} \qquad \ldots[2.3\ (c)]$$

Disulfide anion may then react with other species to form disulfide radical anion.

$$S_2^{2-} + HS^{\bullet} \rightleftharpoons S_2^{\bullet-} + HS^{-} \qquad \ldots[2.3\ (d)]$$

$$S_2^{2-} + S^{\bullet-} + H^{+} \rightleftharpoons S_2^{\bullet-} + HS^{-} \qquad \ldots[2.3\ (e)]$$

Dimerization of disulfide radical anion and combination with sulfide radical anion results in the formation of trisulfide and tetrasulfides, and theoretically, these may react further via analogous radical transfer reactions to produce hexa-, hepta- and octa-sulfide anions (Steudel, 1996).

$$HS^{\bullet} + S_2^{\bullet-} \rightleftharpoons S_3^{2-} + H^{+} \qquad \ldots[2.3\ (f)]$$

$$2S_2^{\bullet-} \rightleftharpoons S_4^{2-} \qquad \ldots[2.3\ (g)]$$

To explain the formation of elemental sulfur upon acidification of polysulfide solutions, Steudel (1996) proposed that *cyclo*-hexasulfur, *cyclo*-heptasulfur and *cyclo*-octasulfur form from disproportionation of heptasulfide, octasulfide and nonasulfide anions (eqs 2.3 (h-j)).

$$S_7^{2-} + H^+ \rightleftharpoons HS_7^- \rightleftharpoons HS^- + S_6 \quad \text{...[2.3 (h)]}$$

$$S_8^{2-} + H^+ \rightleftharpoons HS_8^- \rightleftharpoons HS^- + S_7 \quad \text{... [2.3 (i)]}$$

$$S_9^{2-} + H^+ \rightleftharpoons HS_9^- \rightleftharpoons HS^- + S_8 \quad \text{...[2.3 (j)]}$$

However, the presence of polysulfides with chain lengths of six or more sulfur atoms has never been demonstrated in solution, and the sulfur homocycles could also form from disproportionation and recombination of trisulfide and tetrasulfide anions (Pasiuk-Bronikowska et al., 1992, p. 64; Steudel, 1996).

Steudel (1996) and Pasiuk-Bronikowska et al. (1992) attributed the pH dependence of sulfide oxidation partly to the sulfide radical reactions discussed above. At pH < 6 the rate is extremely slow; the composite rate constant, k_{com} increases from $<0.003 \times 10^{-3}$ $(mol^{-0.9}\ dm^{-3})\ s^{-1}$ at pH 6.00 to 4.74×10^{-3} $(mol^{-0.9}\ dm^{-3})\ s^{-1}$ at pH 12.50 (Chen and Morris, 1972; Pasiuk-Bronikowska et al., 1992, p 71). This is possibly because at the lower pH, the dominant species is hydrogen sulfide (H_2S), which does not form the reactive radical anions ($HS^{\bullet}$ and $S^{\bullet-}$) in appreciable quantity (Steudel, 1996; Pasiuk-Bronikowska et al., 1992, p. 63).

Formation of products of further oxidation are dependent on the availability of oxygen, temperature and pH. At high oxygen concentrations, the major products of polysulfide oxidation are the sulfoxyanions thiosulfate, sulfite and polythionates (Chen and Morris, 1972). At low oxygen concentrations and pH <7, elemental sulfur is formed, and at high pH and high temperature, polysulfides eventually disproportionate to thiosulfate and sulfide (Giggenbach, 1974a). Kinetic and thermodynamic data describing sulfide oxidation under

various conditions of temperature, pH, and ionic strength, and in the presence of metal catalysts are listed in references listed in Table 2.1 and in the following: Pasiuk- Bronikowska et al., 1992; Gun et al., 2000; Millero et al., 1987; Zhang and Millero, 1994; Hoffmann and Lim, 1979; Kotronarou and Hoffmann, 1991.

2.2.2 The chemistry of inorganic polysulfides

Inorganic polysulfides are formed as intermediates during auto-oxidation of sulfide, as described above and on reaction of elemental sulfur with hydrosulfide anion (Steudel et al., 1986). In aqueous solution polysulfides exist as equilibrium mixtures of dianionic chains of sulfur atoms, S_n^{2-}. Aqueous solutions containing a single polysulfide homologue apparently do not exist, except for solutions of extremely high pH (>14), (Giggenbach, 1972), or, according to Gun et al. (2000), at very low concentrations. In both cases, disulfide is expected to be the only homologue. Dissolution of the di-alkali salt of an individual homologue invariably results in rapid redistribution into higher and/or lower polysulfides (Schwarzenbach and Fischer, 1960). Raman studies of polycrystalline, molten and aqueous disodium tetrasulfide confirmed that the penta- and di-sulfides were formed almost immediately upon dissolution in water (Janz et al., 1976). Whereas the trisulfide was consistently absent from the equilibrium mixture formed from dissolution of disodium tetrasulfide (Schwarzenbach and Fischer, 1960; Gun et al., 2000), solutions prepared from the dipotassium salts resulted in mixtures containing all of the homologues from n = 2 - 6 (Schwarzenbach and Fischer, 1960).

Average polysulfide chain length (ñ)

There exists some contradiction in the literature regarding the average chain length of sulfur atoms in polysulfides, partly due to the difficulties associated with direct measurement of individual polysulfide homologues. The average value of n, ($\tilde{n}$), has been reported variously as 3.5 (Giggenbach, 1974a), 3.7 and 4.4 at 25°C, and 4.0 and 5.0 at 50°C and 80°C respectively (Arnston et al., 1958;

Teder, 1971). O'Brien and Birkner (1977) cited a study where pentasulfide was detected as the only species in neutral and slightly acidic or alkaline solutions (Pringle, 1967). More recent studies using laser desorption-mass spectrometry have shown the trisulfide to be the major species in solutions formed from absorption of hydrogen sulfide into chelated ferric iron (Clarke et al., 1994). The proportions of polysulfide homologues decreased in the order,

$$S_3^{2-} > S_4^{2-} > S_2^{2-} > S_5^{2-}$$

and the value of *ñ* was estimated at 3.1, constant over the pH range 7.0 to 8.5. Only minor quantities of pentasulfide were detected.

The effect of pH, temperature and concentration of total dissolved sulfur on ñ

The disparities in *ñ* between the various reports are probably due to the large differences in concentrations, pH and temperature used (Giggenbach, 1974a), and by the serious technical difficulties involved with the experimental determination of chain length. Chain lengths vary according to pH. According to Giggenbach (1972) and Pasiuk-Bronikowska et al. (1992, p. 64), shorter chains predominate at higher pH values, such that at pH values around 14 disulfide is the major species, and as pH decreases, *ñ* increases. The concentration of pentasulfide anion reaches a maximum not exceeding the tetrasulfide concentration at near-neutral pH (Giggenbach, 1972). However, in an apparent contradiction, Chen and Morris (1972) calculated that tetrasulfide and pentasulfide are the dominant species from pH 6 and up, comprising more than 90% of the solution at pH >7.3. The situation is further complicated by the finding that *ñ* increases with increasing starting pH, in concentrated solutions in contact with elemental sulfur (Giggenbach, 1974a). Giggenbach (1972 and 1974a) predicted that in dilute solutions at near-neutral pH and ambient temperature, tetrasulfide is the major polysulfide homologue, and that it is unlikely that *ñ* would exceed 3.6. The work of Gun et al. (2000) offers some explanation for these contradictory reports. These authors showed that the observed variations in the distribution of polysulfide species of different chain

length could be rationalised on the basis of changes in concentration of total reduced sulfur. They calculated the theoretical distribution of polysulfide chain length from equilibrium constants and determined that disulfide is the dominant species at low concentrations (0.05 mg/L) and that the average chain length increases with increasing concentration of total reduced sulfur (up to 10 mg/L).

Maximum chain length of polysulfides

Based on equilibrium calculations, the maximum chain length of sulfur atoms in polysulfide solutions is assumed to be six (Cloke, 1963; Schwarzenbach and Fischer, 1960). Chen and Morris (1972) stated that chain lengths of more than five sulfur atoms are unlikely, and that this is well established, but did not cite studies, nor show experimental evidence to support this. Theoretically, longer chain polysulfides (n>7) are possible, but these are so unstable that they are undetectable with methods presently available and have never been observed in aqueous solution (Pasiuk-Bronikowska et al., 1992, p. 64). However, crystalline polysulfides with chains comprising up to eight sulfur atoms have been isolated, from non-aqueous solutions (Steudel et al., 1986 and references therein). Also, a species thought to be S_8^- was observed in dimethylsulfoxide solution, upon electrochemical reduction of elemental sulfur (Merritt and Sawyer, 1970).

Polysulfide stability: Effect of pH and temperature

Polysulfides are relatively stable at high pH in the absence of oxygen, but disproportionate rapidly to elemental sulfur and hydrogen sulfide under neutral and acidic conditions (eq. 2.4) (Giggenbach, 1974a; Steudel, 1996; Schwarzenbach and Fischer, 1960).

$$^-SS_xS^- + 2H^+ \longrightarrow S_n + H_2S \qquad ...[2.4]$$

(x=*n*-1)

The pH and temperature dependence of the sulfide-elemental sulfur-polysulfide equilibria (0.1M S^{2-} and 0.01M S^0) have been investigated by Giggenbach, (1974a). At high temperature (>100°C), polysulfides in solution disproportionate to give thiosulfate and sulfide (H_2S + HS^-), with the rate and extent of disproportionation increasing with increasing pH. Theoretically, polysulfide solutions are thermodynamically unstable at room temperature, but Giggenbach (1974b) found that there was no significant reaction in solutions containing predominantly disulfide, trisulfide and tetrasulfide at pH 9 even after heating to 100°C. This suggests that the higher polysulfides (n>4) are more susceptible to disproportionation than those with n<4, and provides some explanation for the variation in values of $\tilde{n}$ observed in polysulfide solutions under different conditions. Aqueous polysulfide solutions are thermodynamically unstable, and eventually decompose to give thiosulfate and sulfide (Licht and Davis, 1997); however, for practical purposes, in the absence of oxygen, solutions of polysulfides can be considered stable for around one year at ambient temperature (Roberts et al., 1992).

Polysulfide stability: Effect of oxygen

Upon contact with oxygen, polysulfides are oxidised to thiosulfate and sulfite, probably via polysulfane monosulfonates (eq 2.5) (Pasiuk Bronikowska, 1992 p. 66). These intermediate products are eventually oxidised to sulfate, and elemental sulfur may form as a side reaction.

$$^{-}SS_nS^{-} + {}^{3}/_{2}O_2 \longrightarrow S_nSO_3^{2-} \qquad \ldots[2.5]$$

Apparently polysulfides are much more susceptible to auto-oxidation than sulfide (or bisulfide) ions and catalyze the oxidation of monosulfide (Steudel et al., 1986).

Polysulfanes

Diprotonated forms of polysulfides, termed polysulfanes cannot be prepared as dilute aqueous solutions, due to their extreme reactivity with water (Giggenbach, 1974; Schwarzenbach and Fischer, 1960). The diprotic forms are prepared by pouring a concentrated polysulfide solution into cold concentrated hydrochloric acid with vigorous stirring. Immediate disproportionation to elemental sulfur and hydrogen sulfide occurs if this is done in reverse, testament to the incompatibility of the polysulfanes with water. The yellow 'oil' formed can be dissolved in only very dry organic solvents. Even trace amounts of basic substances on surfaces of glass storage vessels catalyse rapid disproportionation to elemental sulfur and hydrogen sulfide (Schwarzenbach and Fischer, 1960).

Monosulfanes, or hydro-polysulfide ions, where only one of the terminal sulfur atoms is protonated, are very shortlived in aqueous solution, decomposing rapidly to bisulfide and elemental sulfur. They were not observed during the disproportionation of polysulfide upon reaction with acid (eq 2.6 (a)), nor in the reaction of bisulfide with elemental sulfur at near neutral pH (eq 2.6 (b)), in which they are theoretical intermediates (Giggenbach, 1974a).

$$S_n^{2-} + H^+ \longrightarrow [HS_n^-] + H^+ \longrightarrow H_2S + S_8 \qquad \text{...[2.6 (a)]}$$

$$HS^- + S_8 \longrightarrow [HS_n^-] \longrightarrow S_n^{2-} \qquad \text{...[2.6 (b)]}$$

The theoretical concentration ([a]based on calculated equilibrium constants) of hydro-polysulfide at pH 7 and 25 °C is approximately 10% of the concentration of tetra- and penta-sulfide anions which could exist under those conditions. At lower pH values, polysulfide concentrations decrease, and in moderately acidic solutions hydro-polysulfide anions are probably the dominant zero-valent sulfur-containing species in solution, although these would be present at extremely low relative concentrations (Giggenbach, 1974a). Monoprotonated forms of organic

[a] See Steudel (1996) for pKa values of individual dihydro-polysulfides and hydro-polysulfides.

polysulfanes (alkyldisulfanes, RS_nH) have been observed in the gas phase as decomposition products of dialkylpolysulfides, especially in RS_nR where n>4 (Clark and Oriakhi, 1992).

2.3 ENVIRONMENTAL SIGNIFICANCE OF SULFIDE AND POLYSULFIDES

Sulfide and polysulfides are strong nucleophiles and reducing agents, and because of this reactivity, can participate in many environmentally significant processes. Even under mild conditions, these sulfur nucleophiles participate in addition and substitution reactions with organic functionalities such as halides, aldehydes, ketones and activated double bonds. Reactions of sulfur with sedimentary organic matter is thought to be a key process in generation of oil source rocks. Reactions with halogenated organic compounds can facilitate transformation of toxic and persistent industrial pollutants. The great affinity of sulfide for transition metal ions, particularly iron, has generated deposits of insoluble sulfide minerals. These are important, from an environmental viewpoint, as a sink for sulfur in sedimentary systems and from an economic viewpoint, as sources of metals such as gold, nickel, zinc and copper. The complexity of the interrelationships and transformations of sulfur species in sedimentary systems is illustrated by the intricacies of processes shown in Figure 2.3 (Luther and Church, 1992). Excellent reviews of sulfur transformations and interrelationships in the environment are given by Luther and Church (1992) and Taylor (1993).

Incorporation of sulfur into sedimentary organic matter

The incorporation of sulfur into sedimentary organic matter during early diagenesis has been shown to be a key process in organic matter preservation in ancient sediments. Krein and Aizenshtat (1993, 1994) showed that polysulfides could react with α,β-unsaturated aldehydes and ketones under

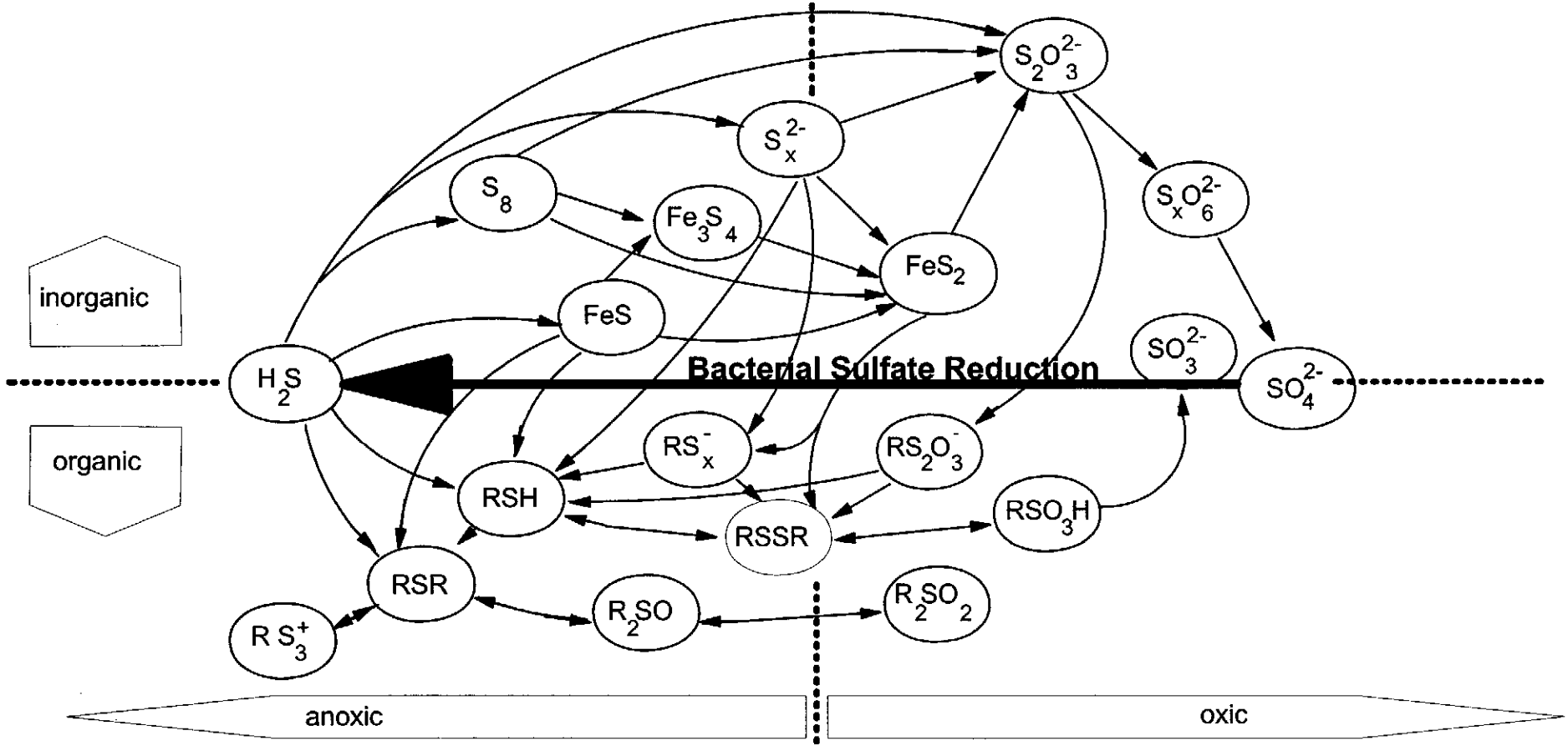


Figure 2.3: Illustration of some of the transformations and interrelationships that occur between sulfur species in sedimentary systems. The Figure is divided into four quarters, approximately illustrating organic and inorganic species and oxic and anoxic compartments. The process of bacterial sulfate reduction (sulfate to sulfide) is highlighted as a major process. (Modified from Luther and Church, 1992).

phase-transfer-catalysis conditions at ambient temperature in the laboratory.

The products were dimers and polymers cross-linked via disulfide and polysulfide bridges. Similarly, Schouten et al. (1994) found that polysulfide was substituted for the oxo group in simple unconjugated aldehydes and ketones under mild conditions, giving sulfide-linked dimers and polymers. In the same study, phytenal, an $\alpha\beta$-unsaturated aldehyde formed cyclic disulfides and trisulfides, resulting from intramolecular incorporation of sulfur. Acid and alcohol moieties give similar products, but are generally far less reactive than conjugated aldehydes and ketones. De Graaf et al (1992) found that reactions of phytol gave similar products to those of phytenal, but in much lower yield and Schouten et al. (1994) reported that acids and alcohols were essentially unreactive in their experiments. It is thought that substitution of oxygen atoms with sulfur leads to cross-linking of molecules, and in sedimentary environments, to eventually form geomacromolecules which are resistant to degradation. Several authors have proposed that this process of organic matter preservation might be a key in the formation of kerogen and oil source rocks (Kohnen et al., 1991; Krein and Aizenstaht, 1993; 1994; Schouten et al., 1994).

In other reactions where sulfur is incorporated into natural organic matter, sulfide and polysulfide react with activated double bonds resulting in sulfur addition. Vairavamurthy and Mopper (1987) demonstrated the addition of sulfide to the terminal double bond in acrylic acid to give 3-mercaptopropionic acid. Sinninge Damste and de Leeuw (1990) showed how sulfur can be incorporated into isoprenoid dienes and polyenes by addition across 1,4-double bonds to give thiolanes. Further reaction of thiolanes is thought to result in the formation of thiophenes and this mechanism explains the high abundance of these compounds in oil source rocks originating from marine or hypersaline environments. Kohnen et al. (1991) demonstrated the formation of isopreniod thiolanes and cyclic and acyclic disulfides and polysulfides through Markovnikov addition of sulfide and polysulfide onto conjugated double bond systems.

The incorporation of sulfide and polysulfides into humic substances has been discussed by several authors. Vairavamurthy et al. (1997) showed that the thiol, 3-mercaptopropionic acid (3-MPA) was bound to sedimentary humic substances through disulfide and polysulfide linkages. Anaerobic microbial degradation and turn-over of free 3-MPA occurs rapidly in sediments, but degradation was inhibited in the humic-bound thiol. Binding to sedimentary humic substances thus resulted in preservation of the thiol. Francois (1987) proposed that changes in carbon-sulfur ratios in humic acids extracted from near-shore sediments were indicative of sulfur addition to the humic matrix in early diagenesis. Enrichment of sulfur with respect to carbon increased with depth to levels that strongly suggested sulfur incorporation into humic substances. Similarly, Ferdelman et al. (1991) showed that sulfur bound to humic substances isolated from salt marsh sediments represented up to 51% of the total sedimentary sulfur. These authors used x-ray photoelectron spectroscopy to show that the humic sulfur consisted of sulfoxides or sulfones and organic sulfides and/or polysulfides. From mass balance calculations it was concluded that at least some of the sulfur originated from reaction of reduced sulfur compounds with organic matter. The authors note that humic sulfur formation is enhanced by the lack of availability of iron.

Reaction with halogenated organic contaminants

The reactivity of sulfur nucleophiles is also important because of their effect on the fate of halogenated organic contaminants in polluted groundwaters. Primary alkyl halides (Barbash and Reinhard 1989a,b; Schwarzenbach et al., 1985) and *gem*-dihalides (Roberts et al., 1992) undergo dehalogenation via nucleophilic substitution reactions with sulfide and polysulfide, even at the low concentrations typical of many aquatic environments. More recent work has shown that reductive dehalogenation of polyhaloalkyl species such as hexachloroethane and pentachloroethane is promoted by reduced sulfur species, predominantly polysulfides (Miller et al., 1998; Curtis and Reinhard, 1994). The mechanisms of

dehalogenation are not fully understood, but probably occur via free radical processes or nucleophilic elimination reactions, in which polysulfides are likely to be particularly important (Miller et al., 1998). Sulfide may take part indirectly in dehalogenation processes by acting as a bulk reductant to redox mediators such as hydroquinone/quinone redox couples and humic substances (Curtis and Reinhard, 1994; Dunnivant et al., 1992), or in association with mineral surfaces such as biotite and vermiculite (Kriegman-King and Reinhard, 1992).

Formation of sulfide minerals

A major sink for sulfide in sedimentary systems is the reaction with iron to form insoluble sulfide minerals such as pyrite (FeS_2), marcasite (FeS_2), pyrrhotite ($Fe_{1-x}S$) (Berner, 1984). Pyrite is the thermodynamically stable end-product of sedimentary reactions of sulfide with iron, but its formation is a complex multistep process, proceeding through various iron-sulfide intermediates (Luther, 1991; Schoonen and Barnes, 1991a,b; Murowchick and Barnes, 1986). In sedimentary systems the precipitation of iron sulfide minerals is thought to occur in preference to sulfur incorporation into organic matter (Ferdelman et al., 1991). Aspects of reactions of iron with sulfide and polysulfide that are relevant to the present study will be discussed further in Chapter 6.

2.4 BIOGENIC PRODUCTION AND TRANSFORMATIONS OF METHYLATED SULFUR COMPOUNDS

Methyl sulfur compounds are significant in a wide variety of industrial and environmental settings. They are important, if sometimes undesirable flavour and odour components of a great variety of foods and beverages, including brassica vegetables (Di Pentima et al., 1995; Chin and Lindsay, 1993), garlic (Rapior et al., 1997), crab meat (Chung and Cadawaller, 1993), potato chips (Wagner and Grosch, 1997), coffee (Semmelroch and Grosch, 1995) and cognac (Nedjema and Hoffman, 1996). Off-odours in sewage and industrial discharge, particularly those from the pulp and paper industries and abbatoir

and agricultural effluents have been attributed to methyl sulfur compounds both in aquatic and in atmospheric compartments (Campbell et al., 1994, Van Langenhove et al., 1982). Much of the recent research on biogenic production of methylated sulfur compounds has been stimulated by evidence of links between global climate cycles and dimethylsulfide (DMS) production (Charlson et al., 1987; Taylor, 1993 and references therein). DMS in the troposphere is oxidised within a few days to methylsulfonic acid (MSA) and sulfuric acid. These "non-sea-salt-sulphate" aerosols act as cloud condensation nuclei, and play an important role in cloud formation and consequently, world climatic behavior (Charlson et al., 1987). Interestingly, DMS, rather than H_2S is thought to be the most quantitatively important biogenic input of sulfur to the atmosphere (Kelly and Smith, 1990).

In the natural environment organic and inorganic sulfur compounds are part of a cycle of complex bacterial and chemical interreactions where compounds are continuously subject to reworking and transformation (Taylor, 1993; Cook and Kelly, 1992; Kelly and Smith, 1990). Whereas the present work concerns itself largely with DMTS, in nature this compound almost always co-occurs with the disulfide, and at times, other methyl sulfur compounds such as methanethiol (MT) and DMS (Ginzburg et al, 1999, Roberts et al., 1993, Rapior et al., 1997). It is therefore appropriate to review the current state of knowledge of the occurrence and interreactions of the important C_1-organo-sulfur compounds that may be directly or indirectly related to DMTS production.

Volatile methyl-sulfur compounds from dimethylsulfoniopropionate

The most significant natural source of DMS is from the breakdown of dimethylsulfoniopropionate (DMSP), an osmolyte synthesized by marine phytoplankton, macroalgae and marsh plants (Taylor, 1993 and references therein). Until recently the consensus had been that DMSP occurs exclusively in saline environments (Caron and Kramer, 1994; Taylor, 1993), but a study by Ginzburg et al. (1998) showed that *Peridinium gatunense*, a freshwater

dinoflagellate produces and stores a considerable amount of DMSP. Biosynthesis of DMSP by bacteria has not been reported (Taylor, 1993), although some bacteria are able to utilise exogenous DMSP for growth and osmotic purposes (Diaz and Taylor, 1992).
DMS is produced via bacterial decomposition of DMSP after its release from marine plants. Enzymatic cleavage of DMSP by bacteria possessing DMSP-lyase results in production of DMS, acrylate and a proton, as shown in eq 2.7 (Taylor, 1993 and references therein).

$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow (CH_3)_2S + CH_2{=}CHCOO^- + H^+ \quad ...[2.7]$$

Species of bacteria which are able to effect this reaction have been isolated from aerobic and anaerobic environments including coastal and open ocean seawater, marine sediments and river mud (Taylor, 1993 and references therein). In an alternative route for DMSP catabolism, MT, rather than DMS is produced (Taylor and Gilchrist, 1991). Demethylation of DMSP forms methylmercaptopropionate (MMPA) which then liberates MT. In a side-reaction a small amount of 3-mercaptopropionate (MPA) is also produced via demethylation of MMPA.

In high pH environments DMSP is hydrolysed abiotically to produce DMS and acrylate. The reaction is thought to proceed via a Hoffman elimination, with the abstraction of a proton from the α-carbon (Dacey and Blough, 1987). Indeed, treatment of sediment and water samples with sodium hydroxide, followed by analysis of the released DMS is the basis for the most commonly used technique for the analysis of DMSP (Caron and Kramer, 1994).

Volatile methyl-sulfur compounds from decomposition of methionine

Several studies have found that the primary methyl sulfur compounds released from the decomposition of methionine are DMS and MT. Segal and Starkey

(1969) found that the S-methyl group of methionine was converted to MT by a variety of bacterial cultures, with small amounts of DMS also produced. Similarly, Zinder and Brock (1978) observed that MT was the predominant sulfur compound released from methionine in anoxic lake sediments, with DMS a minor product. DMDS is also often found when MT is released, but this is probably formed from oxidation of MT (Kiene and Visscher, 1987; Visscher and van Gemerden, 1993).

Keine and Visscher (1987) observed the demethiolation of methionine to give MT by sulfate reducing bacteria in anoxic salt marsh sediment slurries. Since a small amount of MT was still being produced when the activity of sulfate reducing bacteria was inhibited, they concluded that anaerobic fermentative organisms may also play a role in MT production from methionine. These authors did not observe the production of DMDS and suggested that this was because the sediments in their study were highly anoxic and DMDS would be rapidly reduced back to MT.

Wajon et al (1988, pp. 42-51) discussed the microbial production of DMTS from methionine and other sulfur amino acids. In their review they reported that the production of MT, DMS and DMDS from methanethiol-containing compounds was almost ubiquitous in the wide range of microbes studied. In a study of bacterial cultures isolated from drinking water, these authors reported that fourteen out of fifteen bacterial cultures grown on methionine were able to produce DMDS and DMTS. The isolates studied included several species of *Pseudomonas, Flavobacterium, Moxarella, Aeromonas and Alcaligenes*. It therefore appears that volatile methylsulfur compounds can be generated from microbial transformations of methionine and other compounds possessing ω-methanethiol moieties, and that these transformations are widespread in nature.

Microbially mediated transmethylation reactions

Biomethylation is a biologically mediated transmethylation reaction defined by Fatoki (1997) as "a process whereby living organisms cause a methyl group to become directly bonded through a carbon atom to one or more heavy elements". Elements known to undergo biomethylation are the metal(loid) atoms of arsenic, mercury, lead, tin, selenium, tellurium, germanium and antimony and the non-metals chlorine, sulfur and phosphorus (Fatoki, 1997). True metals are methylated almost exclusively by anaerobic microorganisms, but elements such as arsenic, selenium and tellurium can be methylated by higher organisms, including man. Phosphorus can be methylated by soil organisms, and halogens by fungi, plants and marine kelps (Fatoki, 1997 and references therein).

Biomethylation appears to be a detoxifying process initiated by micro-organisms to modify the physico-chemical properties of a toxicant, and thereby promote its removal. For example, the most toxic form of arsenic is arsenious acid ($As(OH)_3$), or its salts or esters. Methylation of arsenious acid significantly decreases its toxicity and facilitates its removal by converting it to the water soluble methylarsonic acid ($CH_3AsO(OH)_2$) or cacodylic acid ($(CH_3)_2AsOOH$), or the volatile methylarsine, dimethylarsine or trimethylarsine (Fatoki, 1997). Similarly, selenium is detoxified by conversion to volatile dimethylselenides [$(CH_3)_2Se$, $(CH_3)_2Se_2$, and $(CH_3)_2SeO_2$] and the water-soluble trimethylselenonium ion [$(CH_3)_3Se^+$] (Tanzer and Heumann, 1991).

The most important methylating agent in biomethylation reactions is S-adenosylmethionine (SAM), a sulfonium salt (see Figure 2.4 (a)), which facilitates methylation by donating a methyl cation (Fatoki, 1997 and references therein; Harper, 1993). Methyl cations would therefore react rapidly to methylate strong nucleophiles such as sulfide and polysulfides. Many species of fungi are capable of producing copious quantities of methyl halides, and the involvement of SAM in these methylations has been proposed (Harper, 1993 and references

therein). It has been established that SAM is the methylating reagent for methylation of arsenic and selenium, and the same probably applies to tellurium, phosphorus, tin and antimony (Fatoki, 1997).

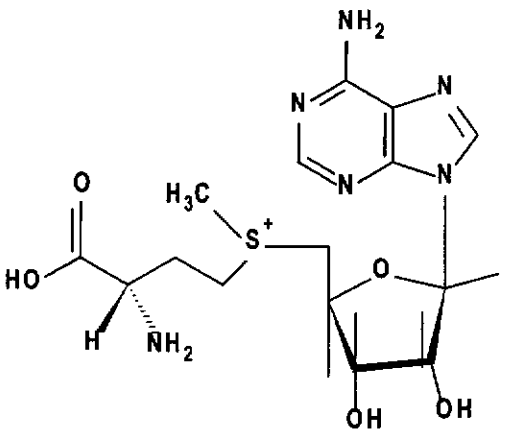


Figure 2.4 (a) S-adenosylmethionine

Another important methylating agent is methylcobalamin, a derivative of vitamin B_{12} (structure is shown in Figure 2.4 (b)). Unlike SAM, this reagent donates a methyl anion, and the methylated species must therefore be electrophilic. Evidence exists that mercury is methylated by this compound (Fatoki, 1997 and references therein), and it may also be involved in methylation of the higher valence states of germanium, tin, lead and antimony. Other methylating reagents are co-enzyme F-430, which forms a methylnickel species and selenoadenosylmethionine, the selenium analogue of SAM. According to Brinckman et al. (1985) dimethylsulfoniopropionate (DMSP) can methylate iodide to produce methyl iodide. Kadota and Ishida (1972) observed demethylation of DMSP with concomittant formation of methionine from homocysteine mediated by *Gyrodinium cohnii*.

Other transmethylation reactions involve the methylation of sulfur nucleophiles via transfer of a methyl group from methoxyaromatic compounds. Certain strains of sulfate reducers are able to facilitate these reactions whereby the methoxy-methyl group from aromatic compounds such as syringic acid or methoxybenzoate is transferred to sulfide, forming MT or DMS. The organisms that mediate these processes are obligate anaerobes and were isolated from both freshwater and marine habitats (Bak et al., 1992; Bak and Finster, 1993).

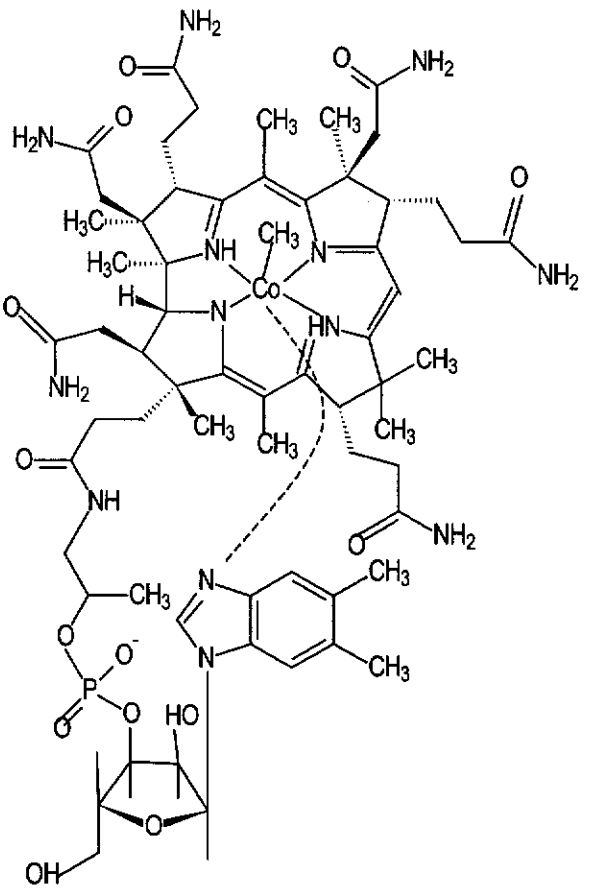


Figure 2.4 (b) Methylcobalamin

Occurrence of DMTS in natural waters and drinking water

Dimethyltrisulfide has been observed in natural waters, including those used for drinking water purposes by several workers. Trace concentrations of DMDS, DMTS and DMTeS have been found in Massachusetts coastal seawater samples (Schwarzenbach et al., 1978; Gschwend et al, 1982) and in lake water samples (Grob and Grob, 1974; Ginzburg et al, 1998). However, DMTS has been found in drinking water distribution systems in only one case, apart from the Perth experiences described in the present study. Krasner et al. (1986) found that DMDS and DMTS formed in drinking water in the water supply system of Los Angeles after the disinfection regime was switched from chlorination to chloramination. It was found that DMTS was associated with hydrogen sulfide formation in the bottom waters of a stratified source water reservoir. It was thought that methylated sulfur compounds were formed as bacterial decomposition products of blue-green algae. DMTS was also formed during recycling of reclaimed washwater at a treatment plant. Treatment of water with chlorine at 2 mg/L for a one hour contact time removed off-tastes and odours caused by DMTS and other other sulfur- and nitrogen-containing compounds. The situation described by Krasner et al (1986) is unlike that in Perth, in that in the latter case DMTS forms within the distribution system and not at the source.

CHAPTER THREE

NATURAL ORGANIC MATTER (NOM) IN GROUNDWATER AND ITS POTENTIAL ROLE IN DIMETHYLTRISULFIDE FORMATION

ABSTRACT

Natural organic matter (NOM) from two Perth drinking water sources was isolated and characterised, with the aim of identifying major differences in structure and/or functional groups that might influence DMTS formation. The two water types studied were (a) groundwater which is prone to DMTS problems after treatment and distribution and (b) surface water in which DMTS has never been detected, neither before, nor after distribution. One possible reason that DMTS does not form in distributed water from surface sources but forms in distributed water from the Wanneroo groundwater scheme is that the latter may contain precursor(s) to DMTS. For example, in previous studies it has been proposed that methyl esters and ethers within humic substances might be a source of methyl groups that could participate in DMTS formation in distributed water (Wajon and Heitz, 1995; Wajon and Wilmot, 1992). A primary aim of the present study was to determine whether NOM in groundwater contained moieties that could potentially act as precursors to DMTS in treated water.

Comparison of levels of reduced sulfur with levels of dissolved organic carbon (DOC) in groundwaters feeding Wanneroo GWTP revealed the existence of a positive correlation between these two parameters. The association between carbon and reduced sulfur was attributed to the natural processes responsible for sulfide generation in sedimentary systems, which rely strongly on the availability of organic carbon. Since reduced sulfur species are potentially important as precursors to DMTS, the above observation provided further impetus to examine the nature of NOM in these groundwater systems.

NOM was isolated from water samples using ultrafiltration, and characterised using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and

offline-thermochemolysis/methylation (TCM) using tetramethylammonium hydroxide (TMAH). Sludge taken from clarifiers at Wanneroo GWTP was also included in the study. Methoxy-aromatic moieties have previously been identified in humic substances isolated from swamp water using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) (Sihombing et al., 1996), and this method was applied to the present samples to determine whether these sub-units might be present. Pyrolysis of groundwater NOM yielded a high proportion of organosulfur compounds, primarily methyl thiophenes and sulfur gases, but did not yield detectable amounts of methoxy-aromatic compounds, suggesting that methyl ethers such as these were not a significant factor in the DMTS problem. Analysis by TCM yielded sulfur compounds identified as the methyl esters of methylthiopropanoate and methylthiobutanoate. Methylthiopropanoate could originate from 3-mercaptopropionic acid, a degradation product of dimethylsulfoniopropionate (DMSP), which is produced by algae as an osmoregulator. Compounds such as DMSP could potentially undergo reactions to form DMTS in distributed water. Surface water NOM exhibited a strong carbohydrate character compared with groundwater NOM, as shown by the relatively high abundance of carbohydrate-derived pyrolysis products, and the presence of permethylated monosaccharides in extracts obtained by TCM. The presence of dimethoxybenzene and trimethoxybenzene isomers in the product mixture obtained from TCM was attributed to input from condensed tannins.

3.1 INTRODUCTION

The nature of natural organic matter (NOM) in groundwater treated at Wanneroo GWTP is of potential importance in whether or not DMTS will form in treated and distributed water. Approximately 70-80 % of DOC is removed in the treatment plant but the nature and structure of the remaining 20-30 % (3-4 mg/L organic carbon) entering the distribution system is unknown. In the present Chapter it is shown that a positive correlation exists between reduced sulfur compounds and DOC in groundwater sourced from the superficial aquifer. As discussed in Chapter 5, the fraction of residual reduced sulfur in water at the outlet of the GWTP probably consists of

elemental sulfur and sulfur associated with organic matter; it is proposed (in Chapter 5) that the latter could exist either as organosulfur compounds (OSC) or as a sol formed by coprecipitation of elemental sulfur and NOM. It therefore appears that if reduced sulfur compounds originating from groundwater contribute to DMTS formation, as proposed previously by Wajon and Wilmot (1992), these would be largely associated with organic matter. Furthermore, it has been proposed that methoxyl moieties that might exist within humic substances in groundwater could provide methyl precursors to DMTS (Wajon and Wilmot, 1992; and Wajon and Heitz 1995).

The chemistry and nature of NOM in groundwaters in the Perth Region has not been previously studied. NOM samples isolated from two water sources, one prone to DMTS problems (Wanneroo groundwater) and the other not DMTS-prone (surface water from Mundaring Weir) were compared using a combination of two analytical techniques, pyrolysis-GC-MS and thermochemolysis. The aims of the study were to (a) obtain information on broad characteristics of NOM from these two sources, within the limitations of the analytical techniques as discussed below; and (b) compare NOM from the two sources in terms of organosulfur and methoxyl moieties and other factors that might influence DMTS formation.

3.1.1 Procedures for the analysis of humic substances

Aquatic NOM is an ill-defined and heterogeneous mixture of naturally occurring organic molecules originating from decomposition of the remains of living organisms in natural systems. It consists largely of macromolecular water-soluble and colloidal material that falls into the categories of humic and fulvic acids (Frimmel and Christmann, 1988), being the remnants of biopolymers such as lignin, cellulose and protein which have been altered by humification processes. The structural elements of the material depend, to some extent, on the nature of the original biological materials which have contributed to it and also on the environmental conditions during deposition. The chemical properties of NOM from different environments therefore vary

considerably, depending on geological and environmental conditions at the source (e.g. Del Rio et al., 1998; De Leeuw and Largeau, 1993).

Isolation and separation of NOM from water

Separation of NOM from water and salts is generally carried out by adsorption onto hydrophobic resins, or using membranes that effect separations based on molecular weight differences. Resins are usually hydrophobic methacrylate cross-linked polymers of the XAD type (Gaffney et al., 1996; Hayes, 1998 and references therein). Membrane separations are carried out by reverse osmosis, dialysis bags or ultrafiltration using hollow fibres, flat membranes with tangential flow or in stirred cells (Buesseler et al. 1996; Hayes, 1998 and references therein). Combinations of resin adsorption and membrane filtration are often used; organic matter is first adsorbed onto resin, then desorbed (with hydroxide) and acidified to yield the humic and fulvic fractions, before desalting using membrane technology (Gaffney et al., 1996). The use of strong acids and bases in preparation of humic and fulvic fractions is not favoured by some workers (Gaffney et al., 1996) since it can promote degradation, decarboxylation, oxidation and condensation reactions. Alternative reagents that have been used are sodium pyrophosphate and sodium fluoride (Gaffney et al., 1996; Hayes, 1998 and references therein). Separations based on molecular weight have also been carried out using preparative size exclusion chromatography (Sihombing et al., 1996).

Membrane separations of NOM based on molecular weight differences overcome the problems associated with the use of strong acids and bases, but assumptions regarding the molecular weight of the isolated substances must also be treated with caution. Humic substances are generally considered to be macromolecular, but may also be associated with lower molecular weight compounds (<1000 D). For example, it has been proposed that small molecules bind to macromolecules either by physical entrapment within voids, or by hydrogen bonding (Schulten, 1996; Smeulders et al., 2000). Changes in the concentration of amphiphilic molecules in solution can

potentially alter their apparent molecular weight through aggregation and colloid formation (Guetzloff and Rice, 1996). A considerable proportion of the molecules comprising aquatic NOM are likely to exhibit this behaviour (Guetzloff and Rice, 1996; Mills et al., 1996), and for this reason, assumptions of molecular weight distributions based on membrane, or chromatographic separations are not always accurate. In the present study, NOM was desalted and concentrated using tangential-flow ultrafiltration. Materials with a nominal molecular weight greater than 1000 Daltons were thus isolated for analysis by instrumental methods and no attempts were made to further separate this fraction.

Methods of characterisation

At present no single analytical technique exists that can provide information for absolute characterisation of pseudo-macromolecular NOM. In order to interpret the complexities associated with the composition and properties of these substances, a combination of several techniques must be used, and their results compared and confirmed. Techniques that have been used in the study of humic substances include chemical, physical, degradative and non-degradative methods and these are discussed briefly below.

Non-degradative instrumental techniques have been used to determine the nature of functional groups within humic substances. These include techniques such as Fourier Transform Infrared Spectroscopy (FTIR) (e.g. Marley et al., 1996), ^{13}C and proton solution and solid-state nuclear magnetic resonance spectroscopy (NMR) (e.g. Bortiatynski et al., 1996) and electron spin resonance (ESR) (e.g. Cheshire et al., 1997). These methods are non-destructive to the sample and results yield parameters that are valuable tools when comparing sample types and determining broad sample characteristics. For example, using ^{13}C-NMR, integrated values of aromatic carbons (110-150 ppm) and aliphatic carbons (0-40 ppm) can be used to calculate the percent aliphaticity and aromaticity of a humic material (Gaffney et al., 1996). A disadvantage of the methods is that detailed information is difficult to obtain. For example, due to the heterogeneity of the substances under study,

infra-red spectra yield very broad bands with significant overlap that often cannot be resolved, with consequent loss of detailed information. Band broadening can also occur with NMR techniques, due to the presence of free radicals in the humic structure. Recent advances in internal reflectance techniques promise improved resolution in infra-red analyses and the possibility to obtain results from humic substances in aqueous phase (Gaffney et al., 1996).

Thermal degradative techniques that have been employed in the study of humic substances include pyrolysis gas chromatography-mass spectrometry (Py-GC-MS), pyrolysis-mass spectrometry (Py-MS)(Schulten, 1996), pyrolysis-FTIR and thermochemolysis/methylation (TCM) (del Rio and Hatcher, 1996; Challinor, 1991). Chemical degradative methods include (a) reduction using metals, hydrogen or metal hydrides; (b) oxidation using copper oxide, permanganate, or chlorine; and (c) alkaline hydrolysis. (Gaffney et al., 1996, and references therein). In the analysis of organic macromolecular substances using degradative techniques, the macromolecular structure is broken down into simpler units that can be identified using conventional methods, most commonly GC-MS. These simple compounds are then related back to the structure of the original material.

Disadvantages of pyrolytic and chemical degradative methods are that the yield in terms of analysable low-molecular-weight compounds is often low; usually less than 25% of the original sample is recovered as analysed products (Gaffney et al., 1996). In pyrolytic methods carboxyl units are converted to carbon dioxide, but this is either lost or present as a contaminant and it is therefore difficult to estimate the carboxylate content of samples (Saiz-Jimenez, 1994). Pyrolytic results, in particular, must be interpreted with caution since very reactive species can be produced, which can result in unexpected transformations (Saiz Jimenez, 1994). However, when methods are carefully researched and developed, they can provide precise and quantitative information on specific moieties or subunits within

macromolecular organic matter (e.g. Kralert et al., 1991, 1995; Sihombing et al, 1996; del Rio et al, 1998)

In the present study two techniques, offline-thermochemolysis/methylation (TCM) using tetramethylammonium hydroxide (TMAH) and Py-GC-MS were used to compare NOM from two water sources, Wanneroo groundwater and surface water from Mundaring Weir. Groundwater NOM from three bores at Wanneroo was compared with organic matter removed during the ground-water treatment process (i.e. clarifier sludge) and with surface water NOM. In order to determine possible origins of some of the products obtained by TCM analysis, water-soluble organic matter extracted from two species of indigenous eucalypts was also analysed.

3.2 EXPERIMENTAL

3.2.1 Samples

Groundwater samples were obtained from the Wanneroo production borefield drawing from the Gnangara mound, a shallow unconfined aquifer in sandy Quaternary sediments which are in hydraulic contact with wetlands in dune systems on the Swan Coastal Plain (Davidson, 1995). Groundwater samples for NOM analysis were taken from three bores W50, W90 and W110 (Figure 3.1). Surface water was collected from Mundaring Weir. Vegetation within this catchment consists primarily of indigenous jarrah and peripheral wandoo forest, and the soil type is predominantly lateritic sediment. Typical DOC and sulfide levels in Mundaring Weir water are 5 mg/L and <0.1 mg/L respectively.

Preparation of water samples and isolation of NOM

Water samples were collected in medium density polyethylene containers that had been cleaned by pre-soaking in hydrochloric acid (1%) for 24 hours, then twice, for 24 hours each time, in deionised water (Barnstead, Milli-Q).

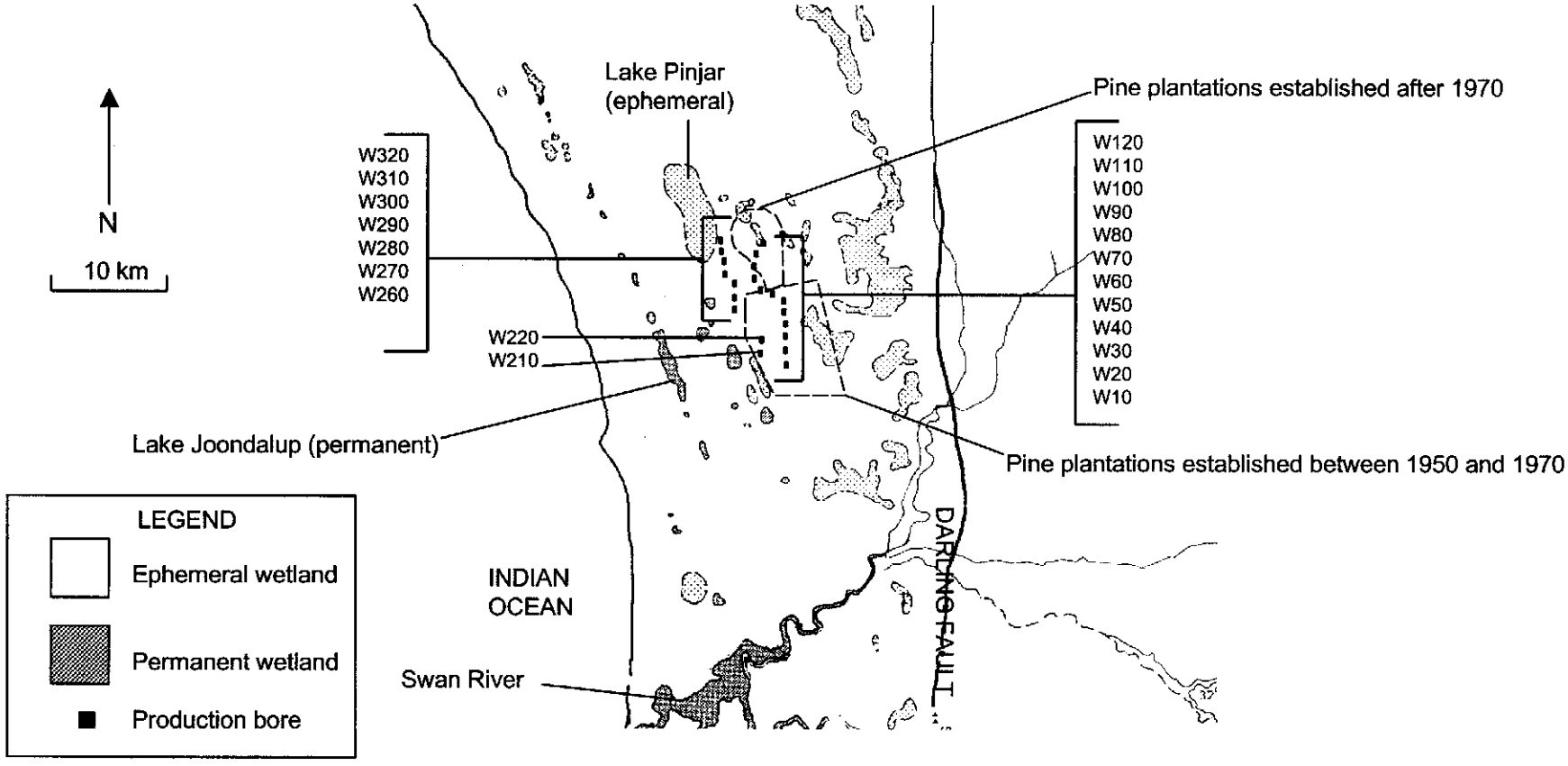


Figure 3.1: Location of Wanneroo production bores drawing water from superficial aquifer (W10 to W320), pine plantations and permanent and ephemeral wetlands on the northern Swan Coastal Plain. In the map area groundwater flows in a south-westerly direction (Davidson, 1995; CALM, 1986; Hirschberg, 1989).

Aquatic NOM (soluble and colloidal) was desalted and concentrated using tangential flow ultrafiltration. The ultrafiltration system used was a Millipore Pellicon system, fitted with polysulfone membranes with a cut-off of 1000 D nominal molecular weight. Sample volumes were 20 L for groundwater and 100 L for surface water. Water remaining in the retentate (~500 mL) was removed by freeze drying and permeate water was discarded. The freeze-dried sample materials were extracted by ultrasonication with dichloromethane (2 x 30 minutes) to remove any elemental sulfur and sorbed low molecular weight organic matter, before analysis by Py-GC-MS and TCM.

Preparation of clarifier sludge samples

Sludge was collected in a pre-annealed glass Schott bottle from clarifier #2 at Wanneroo GWTP on 4-5-95. The sludge was allowed to settle overnight and supernatant water was carefully removed using a Pasteur pipette attached to a vacuum aspirator. The sludge was centrifuged and supernatant water was again removed. The centrifuged sludge was rinsed twice with Milli-Q water (which was removed after re-centrifuging) and dried at 105 °C overnight. Dried sludge samples were extracted by ultrasonication in dichloromethane (2 x 10 mL) twice for 30 minutes.

Leaching of soluble organic matter from bark samples

Bark was collected from two species of indigenous Eucalypt trees, E. *rudis* and E. *camaldulensis*. Bark samples were shredded manually or crushed with a mortar and pestle, weighed, then soaked in the dark in deionised water (Barnstead Milli-Q) for seven days at ambient temperature. After completion of the aqueous leaching process, solid bark residue was separated from the supernatant by filtration through sintered glass (porosity 4). The filtered samples were freeze dried, weighed and stored in a tightly sealed vial in a dessicator prior to analysis.

The yield of freeze-dried water-soluble material obtained from the bark samples was 14.7 % by weight for E. *camaldulensis* and 4.5 % for E. *rudis*. This material is termed "tannins" in the present study.

Preparation of pine wood

A sample of wood taken from a Norfolk Island pine tree was prepared for analysis by Py-GC-MS. Sawdust was obtained by cutting the wood with a three-cornered metal file. The sawdust was extracted by ultrasonication in dichloromethane (2 x 10 mL) twice for one hour. The extracted sawdust was subject to analysis by Py-GC-MS.

3.2.2 Materials

Purified laboratory water was prepared by passing deionised or distilled water through a Barnstead Milli-Q system. This system consisted of cation and anion ion exchange resin and activated carbon columns in series. Water at the outlet of the system was filtered through a 0.2 μm membrane. Dichloromethane (AR grade) was purified by fractional distillation and stored in the dark for no longer than four weeks. Magnesium sulfate (anhydrous, Analar) was purified by Soxhlet extraction with dichloromethane for 24 hours, then dried in an oven at 120 °C overnight.

3.2.3 Analytical procedures

Analysis of DOC

Water samples were analysed for DOC at Water Corporation (by Water Corporation personnel) using a continuous flow method based on persulfate/UV oxidation similar to Method 5310 C, described by APHA, AWWA, WEF (1998). Briefly, samples were acidified and persulfate was added prior to splitting the continuous sample flow, such that one sample stream flowed through a delay coil, while the other was passed through a UV reactor. The carbon dioxide formed was separated from each sample stream

using a semi-permeable membrane that selectively allowed carbon dioxide to pass into a stream of purified water, where it was analysed by conductivity. Conductivity of the non-irradiated sample stream, representing inorganic carbon, was automatically subtracted from conductivity of the UV-irradiated sample stream, which represented total inorganic and organic carbon. The DOC was calculated as the difference between the two channels.

Analysis of sulfide and non-sulfide-reduced sulfur (NSRS)

Sulfide and NSRS were analysed as first described by Wilmot and Burkett (1992) and Wilmot and Wajon (1997). Briefly, sulfide was analysed by sparging the acidified sample with nitrogen, thereby transferring the evolved hydrogen sulfide to a zinc acetate solution, followed by colorimetric determination of the absorbed sulfide. The method for analysis of NSRS, is based on the alkaline reduction by Raney nickel of sulfur compounds (other than sulfate) to sulfide, followed by analysis of sulfide as described above. The methods are discussed in detail in Chapter 5 (Section 5.1). The fraction of sulfur termed "NSRS" includes virtually all compounds in which the oxidation state of sulfur is greater than that in sulfide (-2) and less than that in sulfate (+6) (see Section 5.1).

Analysis of NOM samples by Py-GC-MS

Py-GC-MS was carried out using a SGE Pyrojector II interfaced to a Hewlett Packard HP5890 GC and 5970 mass selective detector (MSD) operated in the EI mode at 70eV. Samples (2-5 mg) were introduced into a quartz furnace tube via a modified pelletiser. Modifications included fitting o-rings to the plunger end and the furnace nut end of the pelletiser. These modifications were made in order to minimise air leaks which caused problems when the Pyrojector was interfaced with a mass spectrometer. The modified pelletiser was constructed from a 100 mL syringe (SGE, Australia) and fitted into a Swagelock male connector (1/4") which was welded to a stainless steel piece machined to fit the existing furnace nut. Instrumental parameters and conditions for the Pyrojector and GC are listed in Table 3.1.

Table 3.1: Instrumental conditions used for Py-GC-MS analysis.

Pyrojector	
Make and model	SGE Series II
Pyrolysis temperature	600 °C
Pyrolysis pressure	35-38 psi
Standby temperature	280 °C
GC injector	
Make and type	Hewlett Packard split/splitless (used in split mode)
Carrier gas	Helium
Carrier gas pressure	23 psi
Carrier gas flow rate	1 mL/minute
Split ratio	45:1
GC oven temperature programme	
Initial temperature	-20 °C
Initial hold time	2 minutes
Rate of temperature increase (1)	4 °C/minute
Final temperature (1)	260 °C
Final hold time (1)	none
Rate of temperature increase (2)	15 °C/minute
Final temperature (2)	290 °C
Final hold time (2)	5 minutes
GC column	
Make	J&W
Phase	DB 1
Phase thickness	0.4μm
Length	40 m
Internal diameter	0.18 mm

Compounds were identified by comparison of their mass spectra with those published in the Wiley275 database.

Analysis of NOM samples by off-line thermochemolysis/methylation (TCM) using TMAH

Analysis by TCM was carried out in a manner similar to that described by McKinney et al (1995). TMAH ($(CH_3)_4N(OH).5H_2O$; Aldrich, ~97 %) or in one

case, tetraethylammonium hydroxide (TEAH) (Aldrich), were dissolved in methanol (25% w/v). An aliquot (100μL) of the methanolic solution was mixed with the isolated NOM sample (~1 mg) and heated to 250°C for one hour in a sealed Pyrex tube. After adding an internal standard (d_{12}-chrysene, 250 ng) the released compounds were extracted with dichloromethane (100 μL), and TMAH artefacts were removed as follows; the extracted compounds were applied to a column in a Pasteur pipette containing acidic alumina (0.15 g, Merck) over anhydrous magnesium sulfate and eluted with four bed volumes of dichloromethane. Depending on the anticipated concentration of analytes, the final volume of extract was reduced under a gentle stream of nitrogen. A normalisation standard (*n*-chlorohexadecane, 200 ng) was added just prior to analysis by GC-MS. Instrumental parameters used for GC-MS are listed in Table 3.2.

Table 3.2: Instrumental conditions used for GC-MS analysis.

GC and column	
Instrument make and model	Hewlett Packard 6890
Injector	HP cool on-column
Injector temperature program	Initial temp. 38 °C; oven track on
Sample volume injected	1 μL
Column make and phase	J&W DB 5 or DB1
Column phase thickness	0.4 μm
Column length	40 m
Column internal diameter	0.18 mm
Carrier gas	Helium
Carrier gas flow velocity	24.0 cm/sec
GC oven temperature program	
Initial temperature	35 °C
Initial hold time	5 minutes
Rate of temperature increase	5 °C/minute
Final temperature	300 °C
Final hold time	10 minutes
MS	
Make and model	Hewlett Packard 5973
Operating conditions	70 eV, electron impact
Operating mode	Full-scan

Compounds were identified by comparison of their mass spectra with those published in the Wiley275 database. Identification of all three trimethoxybenzene isomers was confirmed by comparing mass spectra and retention times with authentic compounds (Tokyo Kasei).

3.3 RESULTS AND DISCUSSION

3.3.1 The relationship between reduced sulfur species and dissolved organic carbon (DOC) in groundwater

An investigation of reduced sulfur compounds and DOC in selected samples of groundwater supplying Wanneroo GWTP showed that concentrations of these substances varied considerably amongst individual borewaters and appeared, to some extent, to be related to groundwater source. Concentrations of sulfide, NSRS and dissolved organic carbon (DOC) in water from thirteen bores are listed in Table 3.3. (The fraction of sulfur termed "NSRS" includes virtually all compounds in which the oxidation state of sulfur is greater than that in sulfide (-2) and less than that in sulfate (+6). NSRS is discussed in detail in Chapter 5). Twelve of the bores draw water from the sulfidic superficial aquifer and one (W305) draws from the Leederville artesian aquifer. Water from the latter aquifer is generally not sulfidic and accordingly, W305 contains the lowest concentrations of reduced sulfur compounds of all the groundwaters tested. Of the bores drawing from the superficial aquifer, those located in the south-eastern section of the borefield (W20-W60, as shown in Figure 3.1) generally contained the lowest concentrations of reduced sulfur compounds and DOC, while those to the north and to the west (W70-W320) contained the highest levels. This distribution is possibly due to the proximity of high-sulfur bores to low-lying wetland areas, which are thought to be the principal source of sulfide in these groundwaters, as discussed in the following paragraphs.

Concentrations of all of the reduced sulfur fractions analysed in groundwaters appear to correlate positively with DOC concentrations as shown in Figures 3.2 (a-c). Groundwaters with the highest concentrations of sulfide generally

also contain high concentrations of NSRS. Presumably, the NSRS components are products of sulfide oxidation and other transformations, such as incorporation of sulfur into organic matter, and it is therefore expected that the relative concentrations of these sulfur fractions in groundwater would be linked.

Table 3.3: Sulfide, NSRS and DOC in water from Wanneroo bores (sampled 13-12-94). Reduced sulfur parameters are plotted against DOC in Figures 3.2 (a-c).

	[a]Sulfide (μg/L)	[a]NSRS (μg/L)	Total reduced sulfur (sulfide + NSRS)	[a]DOC (mg/L)
Bore number				
[b]W20	700	350	1000	6.3
[b]W30	300	400	700	9.8
[b]W40	830	420	1200	9.6
[b]W50	1100	270	1400	9.0
[b]W60	560	250	810	9.1
[b]W70	1100	330	1400	13
[b]W80	1700	480	2200	25
[b]W90	1500	470	1900	23
[b]W120	1600	740	2300	49
[b]W290	1600	330	1900	19
[c]W305	<1	65	65	5.2
[b]W310	1600	370	2000	31
[b]W320	930	n.d.	930	25

Notes: [a] data sourced from Brett Jago, Water Authority of W.A. (unpublished)

[b] bores drawing groundwater from superficial (unconfined) aquifer

[c] bores drawing groundwater from Leederville (confined) aquifer

n.d. = data not available

The reasons for the apparent link between reduced sulfur compounds and DOC are less evident, but are probably associated with the dependence of sulfate reduction rates on the availability of organic carbon and sulfate. According to studies by Berner (1984), organic matter reactivity is the dominant factor controlling microbial sulfate reduction rates at high concentrations of sulfate. Sulfate becomes limiting at concentrations below about 2 mM (200 mg/L as S)(Boudreau and Westrich, 1984) and on this

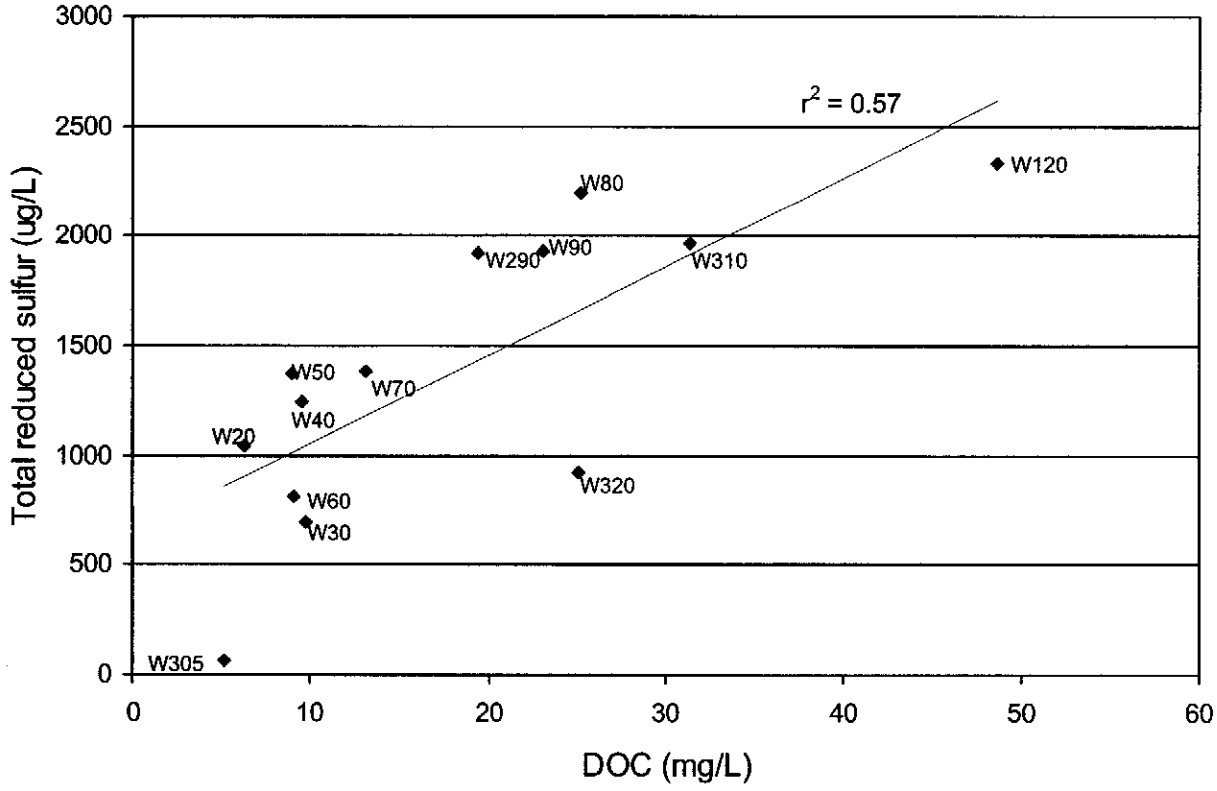


Figure 3.2 (a): Total reduced sulfur (sulfide + NSRS) *vs* DOC in thirteen groundwater samples from the superficial and Leederville aquifers (Wanneroo groundwater scheme).

Note: r^2 = correlation coefficient (r^2 and line of best fit calculated using Microsoft Powerpoint software).

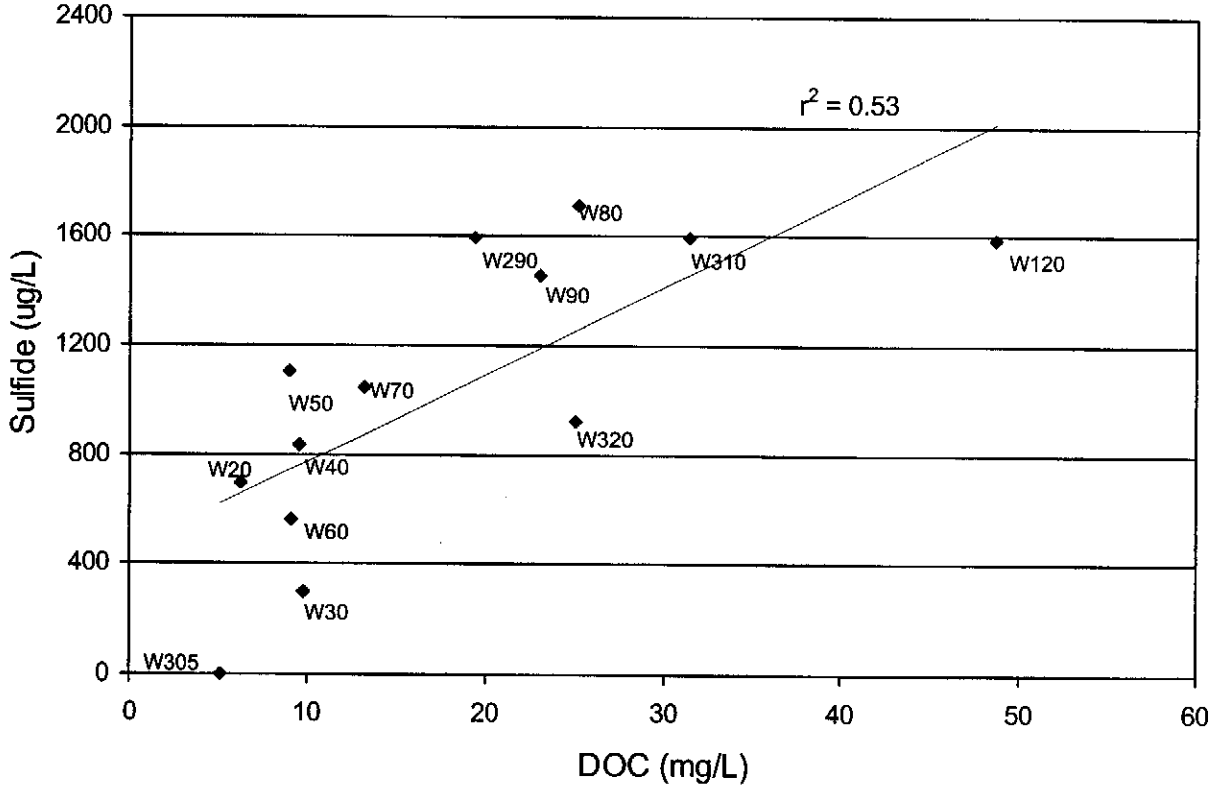


Figure 3.2 (b): Sulfide *vs* DOC in thirteen groundwater samples from the superficial and Leederville aquifers (Wanneroo groundwater scheme).

Note: r^2 = correlation coefficient (r^2 and line of best fit calculated using Microsoft Powerpoint software).

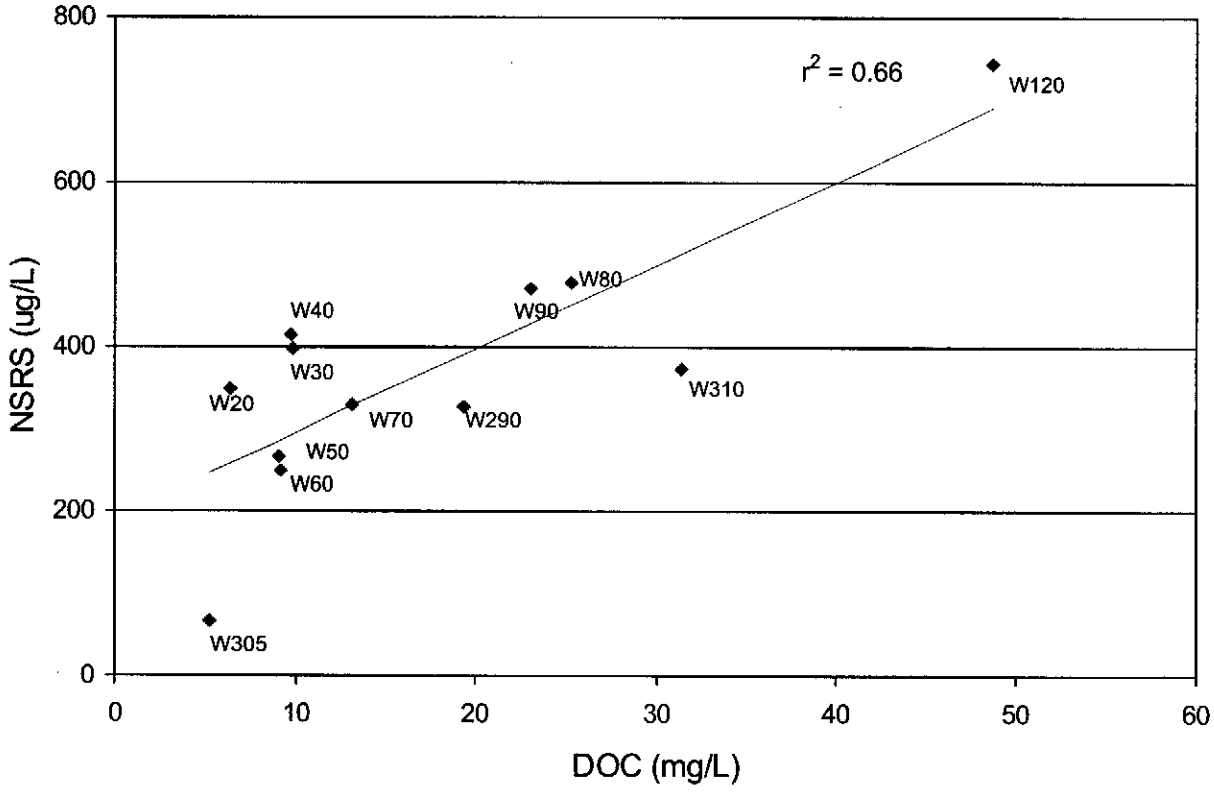


Figure 3.2 (c): NSRS *vs* DOC in twelve groundwater samples from the superficial and Leederville aquifers (Wanneroo groundwater scheme).

Note: r^2 = correlation coefficient (r^2 and line of best fit calculated using Microsoft Powerpoint software).

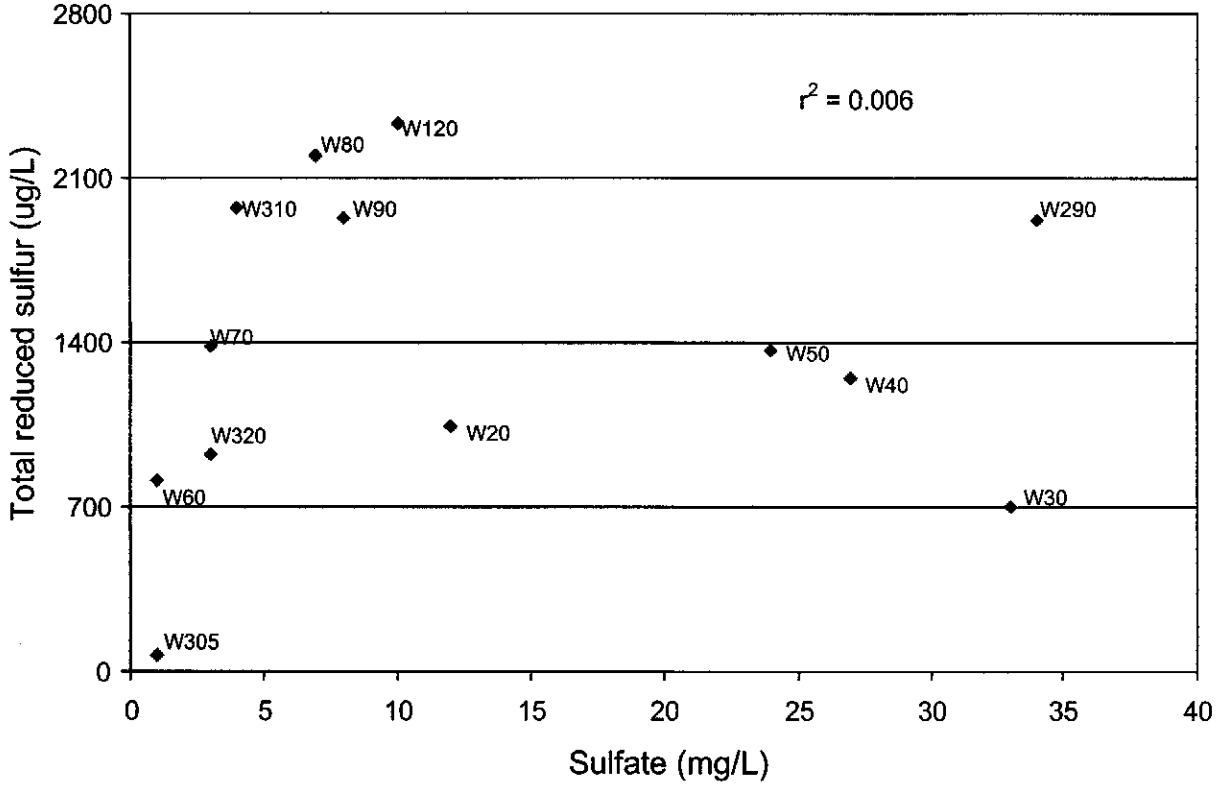


Figure 3.2 (d): Sulfate *vs* total reduced sulfur in thirteen groundwater samples from the superficial and Leederville aquifers (Wanneroo groundwater scheme).

Note: r^2 = correlation coefficient (r^2 and trendline calculated using Microsoft Powerpoint software).

basis, water originating from the superficial aquifer, including the present samples, contains sulfate in concentrations low enough to be strongly limiting (typically 10-50 mg/L (0.1 – 0.5 mM); Water Corporation, 1999). However, as shown in Figure 3.2 (d), little correlation between sulfate and sulfide concentrations exists. Sulfate reduction could therefore occur within isolated pockets of high sulfate concentration within the aquifer system, possibly in areas where dissolution of gypsum or pyrite oxidation occurs (Appelo and Postma, 1993). The origin of sulfide in these groundwaters, and its association with organic carbon is discussed briefly below.

The geochemistry and mechanisms of sulfide generation in sediments of the Swan Coastal Plain have not been studied in detail, and the processes by which sulfide arises have not been clearly established. Significant levels of sulfide are widespread throughout the superficial aquifer, as demonstrated by the strong "rotten egg" odour of hydrogen sulfide that is emitted when water is used for irrigation. Davidson (1995) limited his discussion on the presence of sulfide to: "Sulfide is present as hydrogen sulfide throughout most of the Perth Region, but less commonly within the coastal limestone belt. The gas is generated by bacterial and chemical processes associated with peaty and clayey deposits that often contain pyrite". The bottom sediments of many wetlands comprise peaty layers, which have probably arisen from accumulation of plant detritus from the surrounding vegetation. Certain local species of plants contain high levels of water-soluble organic materials such as tannins, as evidenced by the seasonal brown coloration of rivers and wetlands, and detritus from these species would be present in wetland sediments. The salinity of surface sediments associated with shallow wetlands is subject to seasonal cycling, from hypersaline to brackish (Humphries et al., 1989; Davidson, 1995). The combination of high concentrations of organic matter and seasonal high salinity, as exists in these wetland environments, is typical of the conditions required for high rates of sulfide generation by SRB (Boudreau and Westrich,1984; Lovely and Klug, 1986). Since the wetland systems are in hydraulic connection with groundwater within the superficial aquifer, these lake and swamp sediments are likely to be the most significant source of the sulfide in the present

samples. Processes of this nature would account for the close association between reduced sulfur species and organic carbon observed in Figures 3.2 (a-c).

The considerable variation existing in DOC concentrations amongst the set of groundwaters studied is probably due to a combination of surface vegetation and geomorpholgy and subsurface geology and hydrology. All of the bores investigated in the present study are located within, or near pine plantations (Pinus *pinaster*) established within the past 50 years, interspersed with remnant native vegetation and low-lying swamps and ephemeral wetlands. Since groundwater age is relatively young (groundwater age ranges from the present to 2000 years) and aquifer transmissivity is high (50-150 m/year), these relatively recent landuse changes may have had measurable effects on water quality, including DOC. It is expected that the nature and amount of DOC originating from wetland areas fringed by indigenous Eucalypt and Melaleuca species would differ considerably from that originating from pine plantations; the indigenous species are rich in highly water-soluble tannins which contribute significantly to DOC, as discussed in Section 3.3.3. Further studies of the reasons for the observed differences in the relative amounts of DOC and reduced sulfur in these samples might provide interesting insights into the geochemistry of the superfical aquifer and wetland systems, but these are beyond the scope of the present study. However, the observed link between DOC and reduced sulfur species prompted further study of the character and structure of NOM in these groundwaters, as discussed below.

3.3.2 Characterisation of NOM from surface water and groundwater using pyrolysis-GC-MS

Pyrolysis of NOM results in fragment molecules, many of which can be analysed and identified by GC-MS. Although altered to some extent during pyrolysis, these many small molecules provide a fingerprint of the original sample material and yield some information on the types of moieties present in the original structure. Chromatograms (Py-GC-MS) typical of the two water sources studied are shown in Figure 3.3. Although about 120 pyrolysis

products were identified only the 45 most abundant products are shown in Figure 3.3. These are identified by number and listed in Table 3.4.

Table 3.4: Identification of compounds in Py-GC-MS chromatograms in Figure 3.3.

Peak Number	Compound	Category (as in Figure 3.4)
1	methylcyclopentadiene isomer	hydrocarbon
2	methylcyclopentadiene isomer	hydrocarbon
3	benzene	benzene
4	thiophene	organosulfur
5	acetic acid	carbohydrate
6	methyl-1,3,5-hexatriene	hydrocarbon
7	1H-pyrrole	protein
8	toluene	toluene
9	methylthiophene isomer	organosulfur
10	methylthiophene isomer	organosulfur
11	furancarboxaldehyde isomer	carbohydrate
12	furancarboxaldehyde isomer	carbohydrate
13	methyl-1H-pyrrole isomer	protein
14	methyl-1H-pyrrole isomer	protein
15	ethylbenzene	C_2 benzene
16	o- and p-xylene	C_2 benzene
17	dimethylthiophene	organosulfur
18	styrene	protein
19	methylcyclopenten-one isomer	carbohydrate
20	m-xylene	C_2 benzene
21	methylcyclopenten-one isomer	carbohydrate
22	2-acetylfuran	carbohydrate
23	methylfurancarboxaldehyde isomer	carbohydrate
24	methylfurancarboxaldehyde isomer	carbohydrate
25	methylfurancarboxaldehyde isomer	carbohydrate
26	benzonitrile	protein
27	C_3 alkylbenzene isomer	C_3 benzene
28	phenol	tannin
29	C_3 alkylbenzene isomer	C_3 benzene
30	hydroxymethylcyclopenten-one	carbohydrate

Table 3.4 (continued)

Peak Number	Compound	Category (as in Figure 3.4)
31	1H-indene	C_3 benzene
32	cresol isomer	tannin
33	cresol isomer	tannin
34	C_2 phenol isomer	tannin
35	dimethyl indene isomer	hydrocarbons
36	naphthalene	PAH
37	benzenepropanenitrile	protein
38	acetamidofuran	aminosugar/protein
39	2,3-dihydro indene	C_3 benzene
40	1H-indole	protein
41	1-methylnaphthalene	PAH
42	2-methylnaphthalene	PAH
43	biphenyl	PAH
44	3-methyl-1H-indole	protein
45	naphthalenol	tannin

In order to simplify the results, and more effectively characterise ("fingerprint") the samples, the 120 pyrolysis products were classified into five categories, broadly based on the probable biological origin of the fragment and presented as pie charts in Figure 3.4 (raw data is listed in Appendix 1). The five categories are 'carbohydrate', 'protein', 'tannin', 'organosulfur compounds (OSC)' and 'aromatic compounds', which includes the sub-categories of benzene, toluene, xylenes, alkylbenzenes and polycyclic aromatic hydrocarbons (PAH). (No attempt was made to assign biogenic precursors to benzene, toluene and other aromatic hydrocarbons, since these may have diverse origins, and, in most cases, are probably secondary pyrolysis products (Sihombing et al., 1996)). The classifications are based on studies by Gadel and Bruchet (1987, and references therein); Sihombing et al. (1996); Bruchet et al. (1990); Bruchet et al. (1989); and van der Kaaden et al. (1983). The individual pyrolysis products obtained for each sample are listed in Table 3.4 under the category of their probable origins. The 120 selected compounds comprised almost all of the identifiable pyrolysis

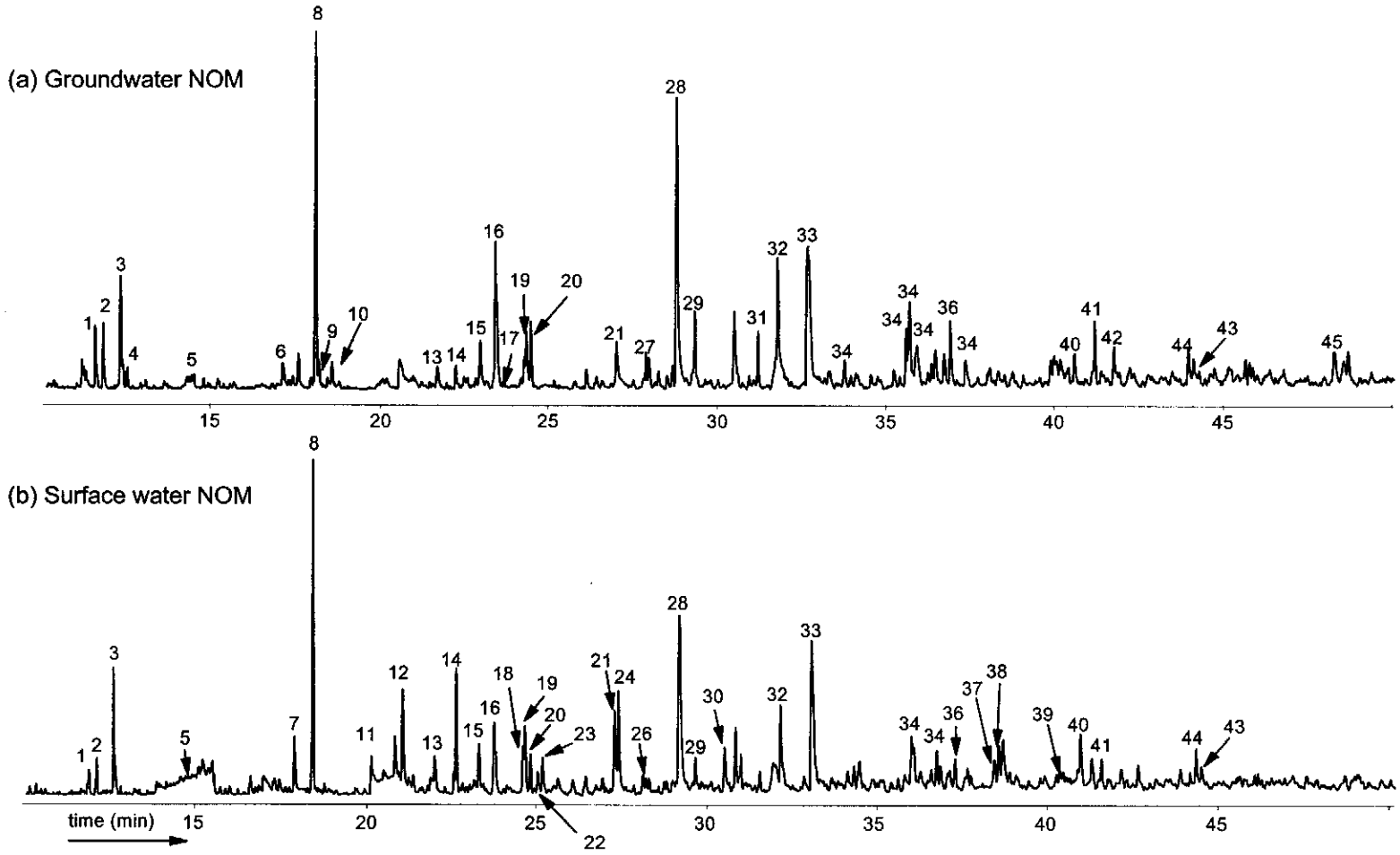


Figure 3.3: Chromatograms obtained by Py-GC-MS of NOM from (a) groundwater (Wanneroo bore W50) and (b) surface water (Mundaring Weir). Key to identification of compounds is in Table 3.4.

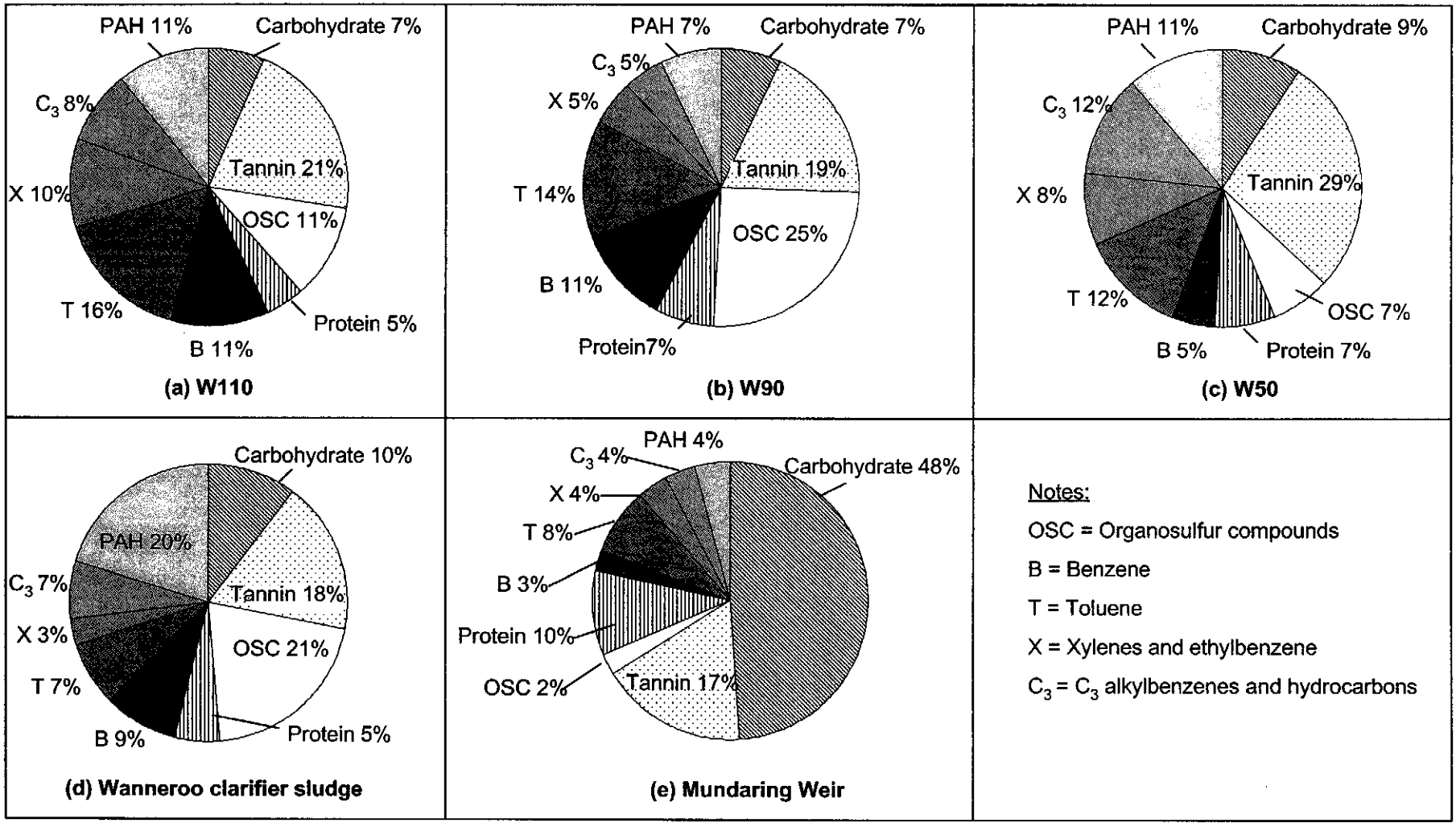


Figure 3.4: Relative yield of pyrolysis products of various (bio)polymer origin in samples of NOM from three Wanneroo groundwaters (a) bore W110; (b) bore W90; (c) bore W50, (d) Wanneroo clarifier sludge and (e) NOM from Mundaring Weir.

products in the samples studied. Peak areas of every compound in each category were determined, summed and expressed as a percentage of the sum of the peak areas of all of the 120 selected compounds. This was carried out in an identical manner for every sample, and thus provides a convenient method of comparison of individual samples, and an estimation of the relative contribution of each biopolymer precursor category to the sample.

Overall, results in Figure 3.4 show distinct differences between groundwater and surface water sources. Groundwater NOM produced pyrograms higher in phenol and alkylphenols (the 'tannin' category), aromatic hydrocarbons and thiophenes (OSC), while the major input to surface water NOM appears to be carbohydrate-related, as shown by the higher relative abundances of carbohydrate-derived pyrolysis products. The phenolic pyrolysis products in both surface water and groundwater samples probably originate primarily from polyhydroxyaromatic moieties in tannins, and not lignin, as discussed in the next section.

Minor differences in the nature of NOM in Wanneroo groundwater from different bores were observed. NOM from W50 yielded lower amounts of OSC than that from the other two borewaters (W90 and W110). This is in agreement with the observations discussed previously (Section 3.3.1) that W50 is from the low-sulfur/low-DOC group of bores, while W90 is from the high-sulfur/high-DOC group. Clarifier sludge yielded high proportions of OSC, suggesting that material containing these moieties were largely removed in the water treatment process.

Determination of methoxyaromatic moieties in NOM samples

As discussed above, methoxyl-containing moieties were of particular interest in the present work because of their potential to contribute to DMTS formation in treated water. The technique of Py-GC-MS has been used in previous studies to determine the extent of sub-units of this type in aquatic NOM Sihombing et al. (1996). These authors found that humic acid fractions from swamp waters yielded significant amounts of lignin-derived pyrolysis

products, namely guaiacol, methylguaiacol, acetoguaiacone and vinylguaiacol. Using identical pyrolysis-GC-MS instrumentation to that employed in the present study, Sihombing et al. (1996) found that the optimum pyrolysis temperature for production of guaiacol and other methoxyaromatic compounds was 510 °C.

In order to determine whether the NOM samples in the present study contained similar methoxy-aromatic sub-units, they were subjected to Py-GC-MS under the conditions described by Sihombing et al. (1996). Samples of groundwater NOM from Wanneroo (bore W110) were pyrolysed at a range of temperatures from 400 to 660 °C. To ensure that methoxy-aromatic moieties could be detected, if they were indeed present, samples known to contain lignin (i.e. wood from Norfolk Island pine) were analysed under conditions identical to those for groundwater NOM. Sihombing et al. (1996) found that ratios of peak areas of methoxyphenols *vs* phenol decreased with increasing pyrolysis temperature, and confirming the work of these authors, this was also observed in the present study (Table 3.5).

Absolute peak areas of phenol for all of the NOM samples analysed are listed in Table 3.5. These results show that, in all cases, pyrolysis products were satisfactorily transferred from the pyrojector to the analytical column and that methoxyphenols would have been detected if present. Interestingly, the absolute amount of phenol detected decreased with decreasing pyrolysis temperature in every case, suggesting that pyrolysis was more efficient at higher temperatures, or that phenol is, in part, a secondary pyrolysis product at higher temperatures.

Numerous lignin-related methoxyaromatic compounds were present in pyrolysed samples of pine wood but none of these could be found in any of the NOM samples studied (Figure 3.5 and Table 3.5.). The methoxyaromatic compounds identified in the wood sample were guaiacol (**I**), *p*-methylguaiacol (**II**), vanillin (**III**), acetoguaiacone (**IV**). Isomers of ethyl, propyl and propenyl guaiacol (not shown in Figure 3.5) were also identified. None of these methoxyaromatic pyrolysis products could be detected in any of the samples

of NOM isolated from groundwaters, surface water or clarifier sludge. This suggests that lignin-derived material does not contribute significantly to the groundwater or surface water NOM studied. The absence of these lignin-derived moieties in all of the NOM samples suggests that methyl groups derived from lignin-methoxyl are probably not important in DMTS formation in distributed Wanneroo water.

Table 3.5: Peak areas of phenol and ratios of the response of methoxyphenols/phenol produced upon pyrolysis of pine wood and groundwater NOM at various pyrolysis temperatures. Analyses were by Py-GC-MS. (Structures are shown in Figure 3.5)

Pyrolysis temperature	Peak area phenol	Ratio of peak area of methoxyphenol/phenol			
		Guaiacol (I)	*p*-Methylguaiacol (II)	Vanillin (III)	Acetoguaiacone (IV)
Wood from Norfolk Island Pine					
600 °C	35727	0.74	0.71	0.17	0.21
550 °C	72324	1.24	1.48	0.27	0.27
510 °C	20477	1.45	1.41	0.30	0.40
480 °C	79056	1.34	1.66	0.22	0.33
400 °C	4646	1.83	2.34	0.33	0.51
NOM from Wanneroo groundwater (bore W110)					
660 °C	127568	n.d.	n.d.	n.d.	n.d.
600 °C	65874	n.d.	n.d.	n.d.	n.d.
570 °C	15175	n.d.	n.d.	n.d.	n.d.
540 °C	35623	n.d.	n.d.	n.d.	n.d.
510 °C	23672	n.d.	n.d.	n.d.	n.d.
480 °C	14802	n.d.	n.d.	n.d.	n.d.
400 °C	5156	n.d.	n.d.	n.d.	n.d.
NOM from Mundaring Weir					
600 °C	219268	n.d.	n.d.	n.d.	n.d.
540 °C	82019	n.d.	n.d.	n.d.	n.d.
500 °C	39463	n.d.	n.d.	n.d.	n.d.
Wanneroo Clarifier Sludge					
600 °C	128684	n.d.	n.d.	n.d.	n.d.
510 °C	25436	n.d.	n.d.	n.d.	n.d.

Note: n.d. = not detected

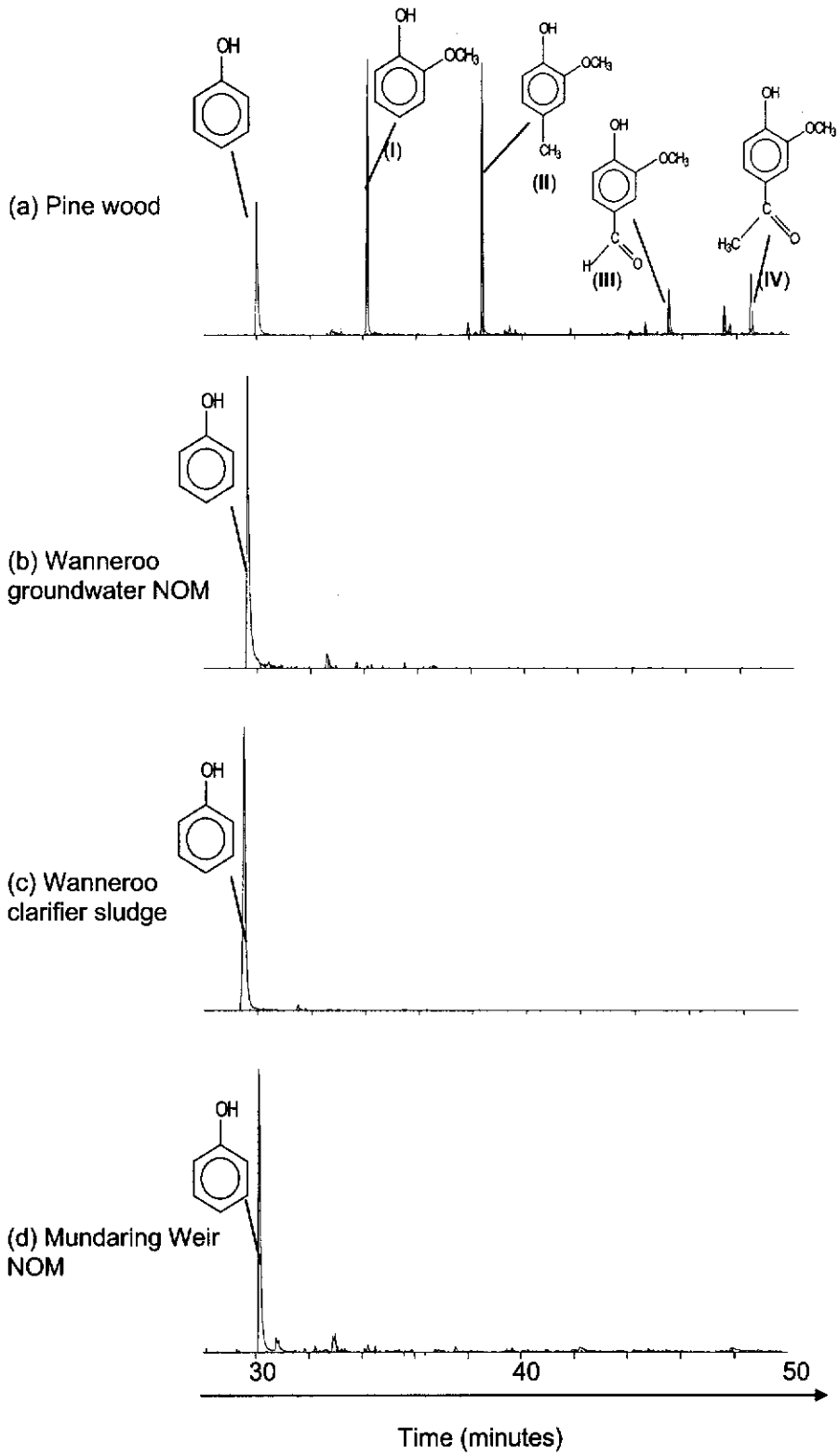


Figure 3.5: Partial ion chromatograms (m/z 94, 123, 124) showing phenol and methoxyaromatic compounds obtained from pyrolysis (Py-GC-MS) of (a) pine wood; NOM samples from (b) Wanneroo groundwater (W110); (c) Wanneroo clarifier sludge; (d) and Mundaring Weir. Methoxy aromatic compounds are guaiacol (**I**); *p*-methylguaiacol (**II**); vanillin (**III**); and guaiacone (**IV**).

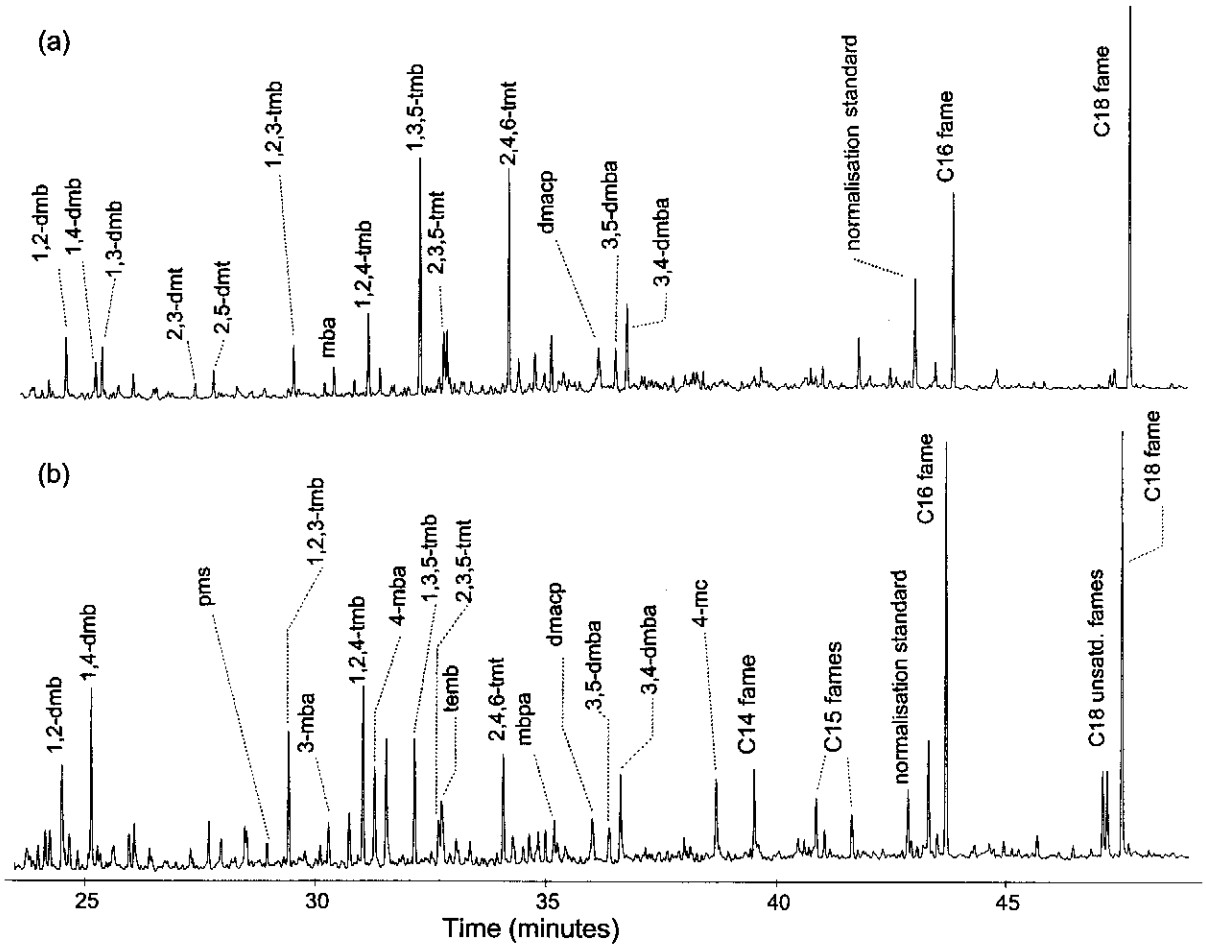
3.3.3 Analysis of NOM from surface water and groundwater using thermochemolysis-methylation (TCM) with TMAH

TCM is a useful tool to complement information obtained by Py-GC-MS in studies of functional groups contained within NOM. TCM is thought to cleave carbon-oxygen bonds in ether and ester moieties, with simultaneous, or subsequent methylation of the free oxygen atom (Challinor, 1991; McKinney et al. 1995). Carboxylate moieties such as benzenecarboxylic acids and fatty acids are subject to decarboxylation reactions during Py-GC-MS, and therefore cannot be detected by this technique. Analysis by TCM overcomes some of these problems of flash pyrolysis: during TCM, decarboxylation does not occur to the same extent as in flash pyrolysis, and the polar carboxylic and phenolic molecules produced are methylated, allowing for better chromatographic resolution (Del Rio et al., 1998; Saiz-Jimenez et al., 1993; Kralert et al., 1995). Further, methylated sulfur compounds such as DMDS, DMTS and DMTeS have previously been detected in product mixtures derived from treatment of humic substances with TCM (Martin et al., 1994) and in the present study it was thought that these might be useful indicators of the presence of polysulfide moieties in NOM.

In the present study, two NOM samples (surface water NOM from Mundaring Weir and groundwater NOM from W50) were compared using the technique of TCM. Briefly, TMAH (100 μL of a 25 % w/v solution in methanol) was mixed with the NOM sample (~1 mg) in a Pyrex tube. After removal of the methanol under a stream of nitrogen, the vial was sealed under vacuum and heated to 250 °C for 1 hour. After cooling, the contents of the vial were extracted with dichloromethane (~ 500 μL) which was analysed by GC-MS after an alumina clean-up to remove TMAH artefacts. Chromatograms of product mixtures obtained by treatment of the two NOM samples with TMAH are shown in Figures 3.6 (a) and (b).

Indicators of carbohydrate character

Analysis by Py-GC-MS showed that surface water NOM appeared to have



Key to compound identification	
pms	permethylated monosaccharide
dmb	dimethoxybenzene
dmt	dimethoxytoluene
tmb	trimethoxybenzene
tmt	trimethoxytoluene
temb	tetramethoxybenzene
mba	methoxybenzoic acid methyl- ester
dmba	dimethoxybenzoic acid, methylester
dmacp	dimethoxyacetophenone
mbpa	methoxybenzene propanoic acid, methyl ester
mc	methoxycinnamic acid, methyl-ester
fame	fatty acid, methyl ester

Figure 3.6: Total ion chromatograms of NOM isolated from (a) groundwater (W 50) and (b) surface water (Mundaring Weir) after treatment with TCM.

more carbohydrate character than NOM from groundwater samples (Figure 3.4). Evidence of polysaccharide input to surface water NOM was further supported by analysis using the alternative technique of TCM. In the present study, TCM-derived extracts of surface water NOM revealed the presence of a series of compounds tentatively identified as permethylated mono-saccharide units. Mass spectra of this group of compounds all yielded characteristic fragments at *m/z* 75, 88 and 101, and these are potentially useful as markers for polysaccharide input to NOM. The most abundant of these compounds was identified by library matching of mass spectra as methyl-6-deoxy-tri-O-methyl-D-mannopyranoside (labelled "pms" in Figure 3.6 (b)), but initial studies on model compounds (rutin and quercitin) show that it is more likely to be an isomer of this compound. These carbohydrate markers were absent from groundwater NOM, confirming results obtained by Py-GC-MS, which showed that surface water NOM has greater carbohydrate character than groundwater NOM.

Carboxylate moieties

The degree of oxidation undergone by humic substances is, to some extent, reflected by the abundance of carboxylic acid moieties within the material (del Rio et al. 1994). These moieties are difficult to detect by Py-GC-MS because of their polar nature and their tendency to undergo decarboxylation reactions during pyrolysis. As discussed above, the technique of TCM partially overcomes this deficiency by converting carboxyl moieties into their methyl esters. Total ion chromatograms in Figures 3.6 (a) and (b) show that TCM analysis of NOM samples from both surface and ground-water sources yielded methyl esters of aliphatic and aromatic mono- and di-carboxylic acids. The most prominent of these are methyl esters of hexadecanoic, hexadecenoic, octadecanoic and octadecenoic acids (labelled "fame" in Figure 3.6); methoxybenzoic acid isomers (mba); 3,4- and 3,5-dimethoxybenzoic acid isomers (dmba); methoxybenzene propanoic acid (mbpa); cinnamic acid and p-methoxycinnamic acid (mc). When abundances of carboxylic acid derivatives are compared with internal standards in each of the two samples in Figures 3.6 (a) and (b), it is apparent that surface water

NOM has greater carboxylate character. This indicates that NOM from this source may have been subjected to more highly oxidising conditions than NOM in groundwater, entirely consistent with the environments from which the two water types are sourced; surface water NOM is more likely to undergo photolytic and chemical oxidation through exposure of the water to atmospheric oxygen and sunlight (dissolved oxygen (D.O.) >90% saturation); groundwaters are almost totally anoxic (D.O. <5% saturation), and NOM from this source is probably largely derived from bottom sediments in coloured lakes and wetlands which are in hydraulic contact with groundwaters. Fatty acid methyl esters might also be derived from bacteria and algae, the latter of which would be more abundant in surface waters than in the highly coloured lakes and swamps from which much of the groundwater NOM originates.

Sulfur-containing moieties

Anoxic and sulfidic aquifer conditions are reflected by the high abundance of thiophenes in pyrolysates of groundwater NOM, relative to the corresponding surface water sample, where these compounds were virtually undetectable (Figure 3.3). TCM analysis of groundwater NOM also yielded compounds which are probably specific to certain sulfidic environments, and do not appeat to have not been previously reported as TCM products. These were identified as methyl 3-methylthiopropanoate (**V**) and methyl 3-methylthiobutanoate (**VI**) (Figure 3.7 (a)) by matching of their mass spectra with those contained in the Wiley275 database. Their mass spectra are characterised by ions at *m/z* 61 and *m/z* 75, diagnostic for terminal and sub-terminal methyl-sulfur groups. Partial ion chromatograms (*m/z* 61 and 75) for two samples of groundwater NOM and a sample of surface water NOM (Figure 3.7 (a-c)) show that the compounds were not detected in TCM-derived extracts of surface water NOM. In experiments using tetraethyl-ammonium hydroxide (TEAH) the ethylthio analogues of these compounds were formed, suggesting that they originate from ester-bound hydro-sulfide moieties, rather than methyl-sulfide moieties. A possible origin in sediments is 3-mercaptopropionic acid (3-MPA), a degradation product of dimethylsulfoniopropionate (DMSP) which is produced by marine organisms

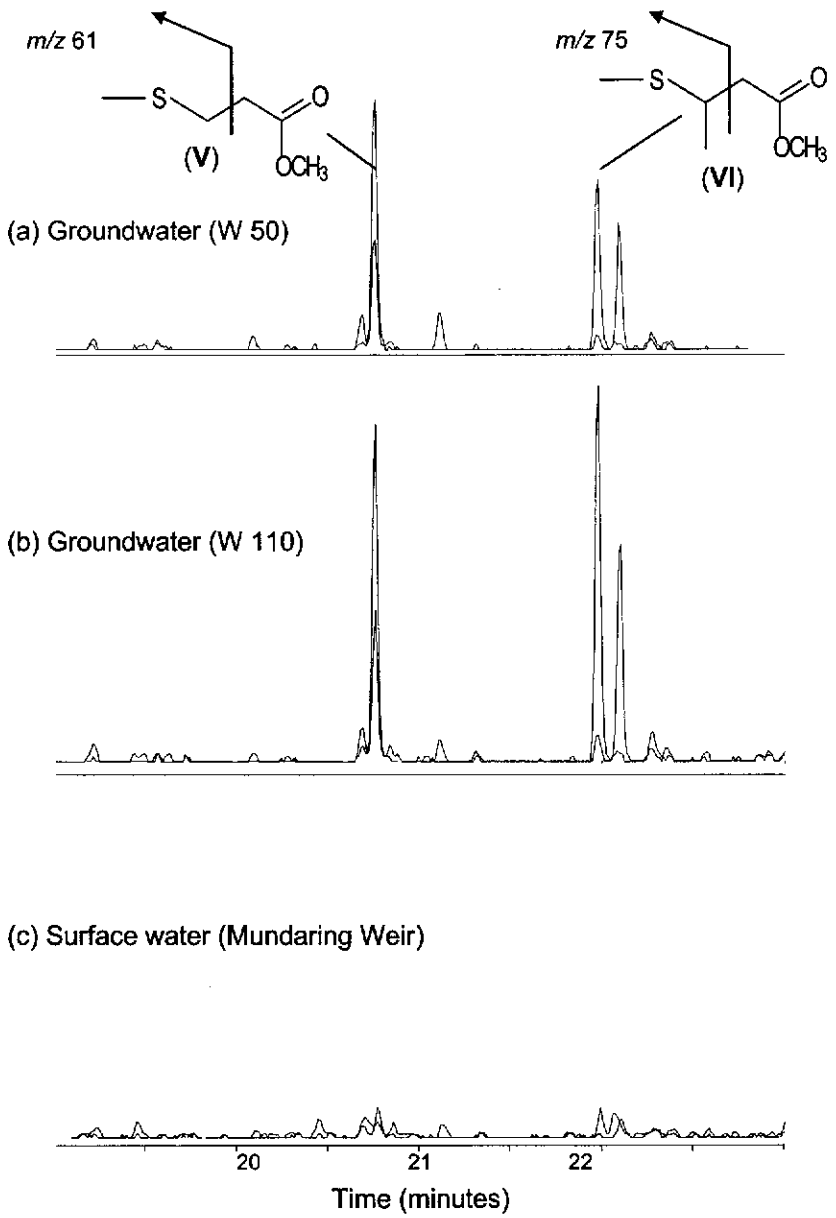


Figure 3.7: Partial ion chromatogram (*m/z* 61, 75) from TCM analysis of NOM from (a) Wanneroo groundwater (bore W50); (b) Wanneroo groundwater (bore W110); and (c) Mundaring Weir surface water. Sulfur-containing moieties, methyl-3-methylthiopropanoate (**V**) (*m/z* 61) and methyl-3-methylthiobutanoate (**VI**) (*m/z* 75) are present in groundwater NOM and absent in surface water NOM.

to regulate salinity changes (MacCrehan and Shea 1995, and references therein). 3-MPA is probably produced, for similar reasons, in sediments associated with wetlands of the Swan Coastal Plain, since these environments are subject to substantial annual salinity changes. These sulfur compounds, released after treatment with TMAH, therefore have the potential to be used as marker compounds to identify NOM sourced from this type of environment. The compounds are also of interest because they might be associated with processes that form DMSP. The presence of DMSP and related methyl sulfur compounds in groundwater (discussed in Chapter 2) could be indirectly associated with formation of DMTS in distributed water. As mentioned above, in previous studies (Martin et al., 1994) methylated sulfur compounds such as DMTS have been found in TCM-product mixtures of humic substances, but these compounds were not detected in TCM-product mixtures from any of the present NOM samples.

Trimethoxybenzenes (TMBs) and trimethoxytoluenes (TMTs) as indicators of tannin input

The high abundances of di-and tri-methoxybenzene (TMB) isomers and 2,4,6-trimethoxytoluene (TMT) are notable in TCM-derived extracts of NOM from both surface water and groundwater (Figures 3.6 (a) and (b)). The origin of TMBs has been attributed to lignin, tannin and carbohydrate precursors by Pulchan et al. (1997), and to cutan by McKinney et al. (1996). The distribution of these and other polymethoxyaromatic compounds in TCM analysis of cutan (McKinney et al., 1996) is not dissimilar to those observed here; however, analysis of these cutans by Py-GC-MS yielded mainly a series of aliphatic compounds ranging from C_5 to C_{32}, and samples in the present study did not yield correspondingly high amounts of these compounds, suggesting that cutan does not contribute significantly to NOM in our samples. Tannins obtained from the deciduous bark of indigenous eucalypt trees (E. *rudis* and E. *camaldulensis*) by aqueous leaching, also yielded TMBs and 2,4,6-trimethoxytoluene as the most abundant products when subjected to TCM analysis. This is shown by comparison of the total ion chromatogram of a TCM-derived extract of tannin from E. *camaldulensis*

(Figure 3.8) with those from the NOM samples studied (Figures 3.6 (a) and (b)). Relative distributions of TMB isomers in NOM from groundwater and surface water, and *E.* rudis tannin treated with TMAH are shown Figures 3.9 (a-c). Eucalypt tannins yielded large amounts of the *meta-* substituted compounds 1,3,5-TMB and 2,4,6-TMT, together with smaller amounts of 1,2,3-TMB, and can thus be considered to be potential precursors to these products in NOM. The origin of 1,2,4-TMB is less clear, and being the most abundant isomer in surface water NOM extracts, its source is of some interest. Cellulosic material has been suggested as a potential source by Pulchan et al. (1997).

The present studies led to further work in our laboratory to determine some of the mechanisms that occur in the TCM process (Huynh, 1999; Huynh et al., 2000). These recent studies showed that TMTs and TMBs can arise from TCM of moieties present in tannins, largely through decarboxylation of aromatic rings. Results of model compound studies showed that aromatic carboxylic acids with hydroxyl groups at the *o-* or *p-* position undergo some decarboxylation to form methoxybenzenes. Gallic acid (3,4,5-trihydroxy-benzoic acid), a common component of hydrolyzable tannins (De Leeuw and Largeau, 1993), produced a high abundance of 1,2,3-trimethoxybenzene, and in some cases methoxytoluenes may also be produced from benzenecarboxylic acids.

The tannins from the eucalypts in the present study are condensed tannins (i.e. proanthocyanidin polymers), with structures composed of chains of polyhydroxy flavan-3-ol units linked via carbon-carbon bonds, as shown in Figure 3.10 (De Leeuw and Largeau, 1993). Upon treatment with TMAH, the flavanoids, quercitin and pelargonidin, components of many of these condensed tannins (Walker, 1975), produced significant concentrations of 1,3,5-trimethoxybenzene and 2,4,6-trimethoxytoluene. These polymethoxyaromatics are among the most abundant compounds in TCM product mixtures from NOM and tannins (Figures 3.6 and 3.8). These results show how tannins could give rise to methoxybenzenes and methoxytoluenes upon TCM treatment. The findings support the hypothesis that our NOM

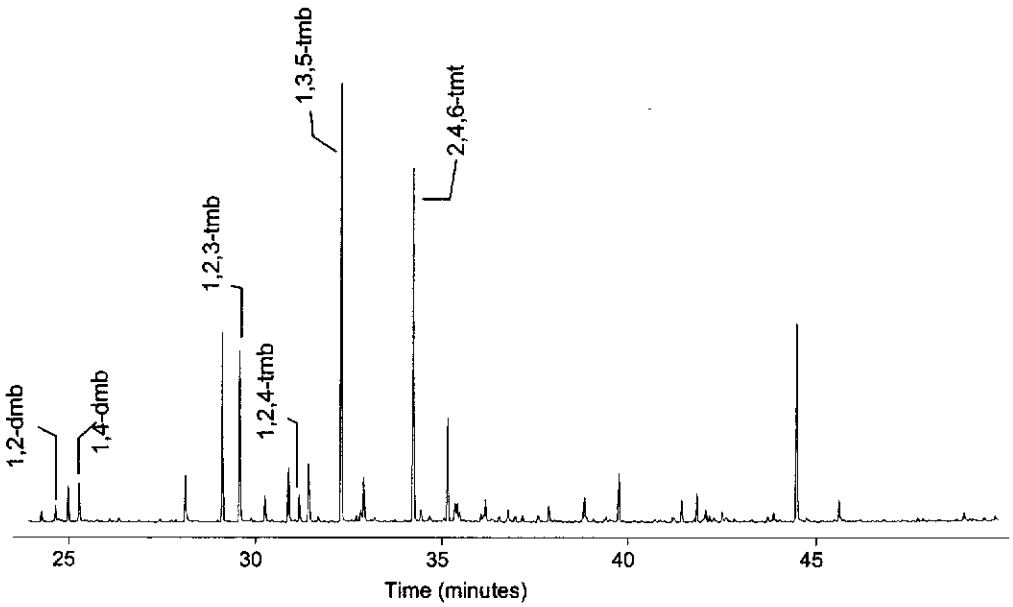


Figure 3.8: Total ion chromatograms of tannins leached from bark of E. *camaldulensis* after treatment with TCM. Key to compound identification is given in Figure 3.6.

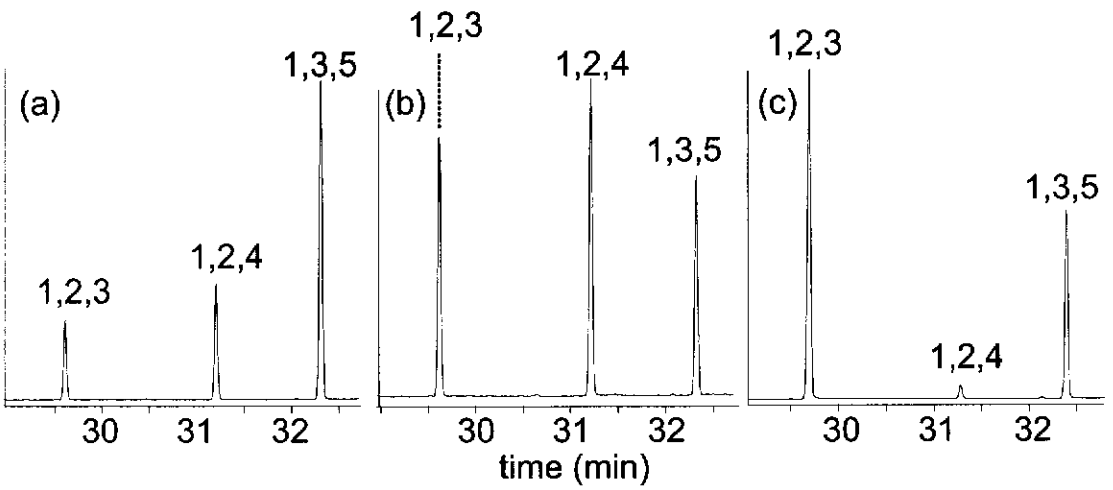


Figure 3.9: Partial ion chromatograms (m/z 168) of samples treated with TMAH, showing relative abundances of trimethoxy benzene isomers (a) groundwater NOM, (b) surface water NOM (c) tannin extracted from E. *rudis*.

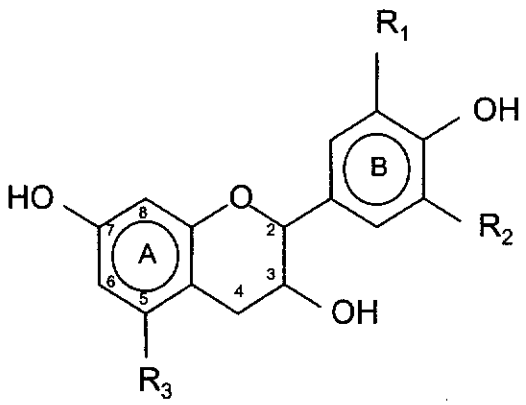


Figure 3.10: Structures of polyhydroxy flavan-3-ol repeating units that constitute condensed tannins (proanthocyanidin polymers). In proanthocyanidin units, $R_1 = R_2 = OH$; in prodelphinidin units, $R_1 = R_2 = H$; in phloroglucinol-type A ring, $R_3 = OH$; in resorcinol-type A ring, $R_3 = H$. Units may be joined via $C_4 \rightarrow C_8$ interflavan bonds, forming linear chains or $C_4 \rightarrow C_6$ interflavan bonds, forming branched chains (De Leeuw and Largeau, 1993)

samples, which yielded high amounts of these compounds, have had significant tannin input. Further, the conclusion that NOM in the present samples originates largely from tannins is in agreement with the conclusions from Py-GC-MS studies of these samples (Section 3.3.2), where it was shown that lignin-derived moieties did not contribute significantly to the NOM (i.e. the NOM probably arises largely from tannins rather than lignin).

3.4 CONCLUSIONS

Groundwater from the unconfined aquifer contained significant concentrations of sulfide and other reduced sulfur compounds (NSRS), most of which are likely to be derived from sulfide. The concentrations of these reduced sulfur fractions appeared to be linked to the DOC content of groundwater. This observation can be rationalised on the basis of microbial sulfate reduction occurring within the aquifer and wetland systems, since sulfate reduction rates are known to be dependent on the bioavailability of organic carbon.

In the present work several groups of marker compounds (obtained after pyrolysis or treatment of the sample with TMAH) have been identified which show that significant differences exist between the surface-and ground-water NOM samples studied. Groundwater NOM is characterised by high yields of organosulfur compounds as pyrolysis products, and by compounds thought to be derived from 3-MPA, produced upon treatment with TMAH. Surface water NOM has greater carbohydrate character, shown by the production of carbohydrate-related pyrolysis products, and by the presence of per-methylated monosaccharide units in NOM treated with TMAH. TCM-derived extracts of surface water NOM also contained a higher abundance carboxylic moieties, suggestive of a more highly oxidising environment. It was shown that tannins are the most likely source of TMB and TMT isomers and that tannin input is probably significant in NOM from both groundwater and surface water sources. Lignin-derived moieties were not detected by Py-GC-MS, despite careful method development and scrutiny of NOM samples for these sub-groups. The absence of methoxyphenols in all of the samples

studied suggests that lignin-derived material does not contribute strongly to aquatic NOM in these environments. Further, the results of the study suggest that DMTS formation in the Wanneroo distribution system is not influenced by lignin-methoxyl in NOM. However, the presence of compounds related to 3-MPA suggests that methylated sulfur compounds such as DMSP might be produced in sediments associated with groundwater, and these could conceivably affect DMTS formation.

In the present study no conclusive evidence was found to show that groundwater NOM contains DMTS precursors and that surface water does not; however, the existence of distinct differences in the character of NOM from the two water sources studied supports the hypothesis that a component of NOM might be a key factor in whether or not DMTS is formed in distributed water.

CHAPTER FOUR

NOVEL TECHNIQUES FOR THE ANALYSIS OF INORGANIC POLYSULFIDES AND DIMETHYLPOLYSULFIDES IN AQUEOUS SAMPLES

ABSTRACT

Methyl iodide reacts readily with di-anionic inorganic polysulfides to form the methylated analogues, dimethylpolysulfides (DMPSs) (Korchevin et al., 1989). This reaction has been used previously as a qualitative tool to detect the presence of polysulfides in aqueous and biological samples (Wajon and Heitz, 1995; Ginzburg et al., 1999). To date, it has not been demonstrated that the method can be used quantitatively, nor that it is specific for inorganic polysulfides. In the present study, the method was further investigated to determine whether quantitative recovery of polysulfides could be achieved, and to investigate the potential for interference from other sulfur compounds. The reaction between methyl iodide and polysulfides was tested in 15 polysulfide standard solutions ranging in concentration from 0.15 to 370 μg/L. The concentration of polysulfide in samples was calculated from the concentration of polysulfide recovered as DMPSs. Deuterated analogues of the DMPS analytes (DMPS-d_6) were synthesized and used as internal (surrogate) standards. It was shown that in solutions of polysulfide spiked into purified water (pH 10), the reaction between methyl iodide and polysulfides is quantitative and linear over the range of concentrations tested. The average recovery of polysulfide sulfur (n=15) was 98%, with a relative standard deviation (RSD) of 10.4%. The method was tested for interferences from organic sulfur compounds and the inorganic sulfur nucleophiles, thiosulfate and sulfite. None of the organic compounds nor thiosulfate interfered measurably. Sulfite interfered but only at concentrations that are unlikely to co-exist with polysulfides in natural systems (i.e. sulfite in 1000-fold excess over polysulfide).

A modified procedure based on purge and trap for the analysis of DMPSs is

described. Utilising a commercially available purge and trap instrument, modified so that "Grob" activated carbon traps could be used, it was possible to partially automate and streamline the procedures used by previous authors (Wajon et al. 1988).

4.1 INTRODUCTION

4.1.1 Analysis of inorganic polysulfides

At present there exists no analytical method to determine individual homologues of inorganic polysulfides at trace levels. This is demonstrated by discussions in the recent literature on the requirement for new analytical tools to quantify these species in environmental samples (Miller et al.,1998; Wang et al., 1998; Ginzburg et al., 1999). Various methods have been employed to date, but none of these can measure concentrations of individual homologues at sub-milligram-per-litre levels.

Techniques used previously to quantitatively measure polysulfides include UV absorbance, liquid chromatography-based techniques and electrochemical methods. Direct measurement by UV spectrophotometry is suitable only for solutions containing millimolar concentrations, or greater (Schwarzenbach and Fisher, 1960; Giggenbach, 1972; Danielsson et al., 1996), and lacks the sensitivity required for many applications. Quantification is complicated by the co-existence of multiple species with overlapping absorption spectra. Attempts to overcome this by separation of individual homologues using liquid chromatography techniques, with detection by UV absorbance have not been successful to date. Steudel et al. (1989) and Clarke et al. (1994) analysed millimolar concentrations of polysulfides in a mixture of reduced sulfur compounds by ion pair chromatography and UV detection. The methods were semiquantitative for polysulfides because it was not possible to resolve individual homologues. Di-alkyl polysulfides (R_2S_2 to R_2S_{10}) and polysulfanes (H_2S_2 to H_2S_{13}) have been separated on reverse phase columns (Clark et al., 1984; Möckel, 1984 a,b) but it has not been possible to effect similar separations of dianionic polysulfide homologues.

Electrochemical techniques are sensitive and generally more precise than chromatographic methods, and can distinguish between many anionic sulfur species. Polarography has been successfully used to speciate reduced sulfur in sediment porewaters, enabling quantitative analysis of thiosulfate, sulfite, bisulfide and polysulfide sulfur (S^0) (Luther et al., 1985). By this method, all of the zero-valent sulfur (polysulfides and orthorhombic sulfur) was analysed indirectly as thiosulfate, after reaction with sulfite. Individual polysulfide homologues could not be distinguished, and their concentrations were theoretically derived using equilibrium constants. Similarly, Wang et al. (1998) used square-wave cathodic stripping voltammetry to determine total S^0 in sediment porewaters, from which hypothetical values for the various forms of zero-valent sulfur were calculated. Calculations were based on the sample pH, total concentrations of elemental sulfur and sulfide and equilibrium constants for the formation of polysulfides. In both cases, the calculated values of total S^0 deviated from the measured values, partly because some of the samples were supersaturated with respect to orthorhombic sulfur.

Rozan et al. (2000) and Luther et al. (2001) used linear sweep voltammetry to simultaneously identify and quantify total polysulfides, elemental sulfur and sulfide. These authors employed solid-state gold amalgam microelectrodes for in-situ speciation of reduced sulfur compounds in microbial mats and hydrothermal vents. Sulfide was rapidly oxidised to form polysulfides upon disruption of sediments during sampling, illustrating the advantages inherent in in-situ determination of these species (i.e. minimal disruption to the study environment is critical for accurate speciation of sulfur compounds since transformations such as oxidation can occur rapidly). However, as with the previous methods described above, it was not possible to distinguish between the individual polysulfide homologues.

Inorganic polysulfides react rapidly and quantitatively with alkyl halides in aqueous solution to form dialkylpolysulfides (Korchevin et al., 1989) which can be analysed by GC techniques. Previous workers (Wajon and Heitz, 1995; Ginzburg et al., 1999) used a technique based on this reaction, for

semi-quantitative assessment of polysulfide sulfur in groundwater and biological samples. In the present chapter the applicability of the technique to the quantitative determination of microgram-per-litre (nanomolar) concentrations of polysulfide sulfur in anoxic groundwaters and in biofilm samples is demonstrated. The potential for interference from organosulfur compounds, elemental sulfur, sulfite, thiosulfate and insoluble metal sulfides is investigated, and it is shown how interferences can be mitigated.

4.1.2 Trace analysis of volatile organic compounds

Sensitivity limitations of most chromatography detectors require a pre-concentration step for determination of nanogram-per-litre concentrations of volatile organic compounds in aqueous samples. A large number of different techniques have been developed for this purpose, the majority of which are based on purge and trap procedures (Bellar and Lichtenberg, 1974; Borén et al, 1985; Gerbersman et al, 1995; Gibson et al., 1994), or closed loop stripping analysis (CLSA) (Schwarzenbach et al, 1985, 1977; Khiari et al., 1997; Grob and Grob, 1974; Jüttner, 1988).

The essential elements of purge and trap procedures are (a) liberation of volatile, non-polar compounds by purging the water sample with a stream of inert gas; (b) trapping of these analytes on an adsorbent material, and (c) desorption of analytes. In many applications analytes are thermally desorbed from the trap and transferred directly onto a gas chromatography (GC) column. Automated purge and trap systems designed to interface with gas chromatographs have been commercially available for more than two decades. The principles of purge and trap-thermal desorption-GC are graphically represented in Figure 4.1

Selection of appropriate parameters such as type and amount of adsorbent material, purge gas type, flow rate and volume, sample and trap temperatures, sample flask configuration, bubble size and control of water vapour in the trap are crucial in achieving optimum method performance for a particular set of analytes. A principal consideration is the breakthrough

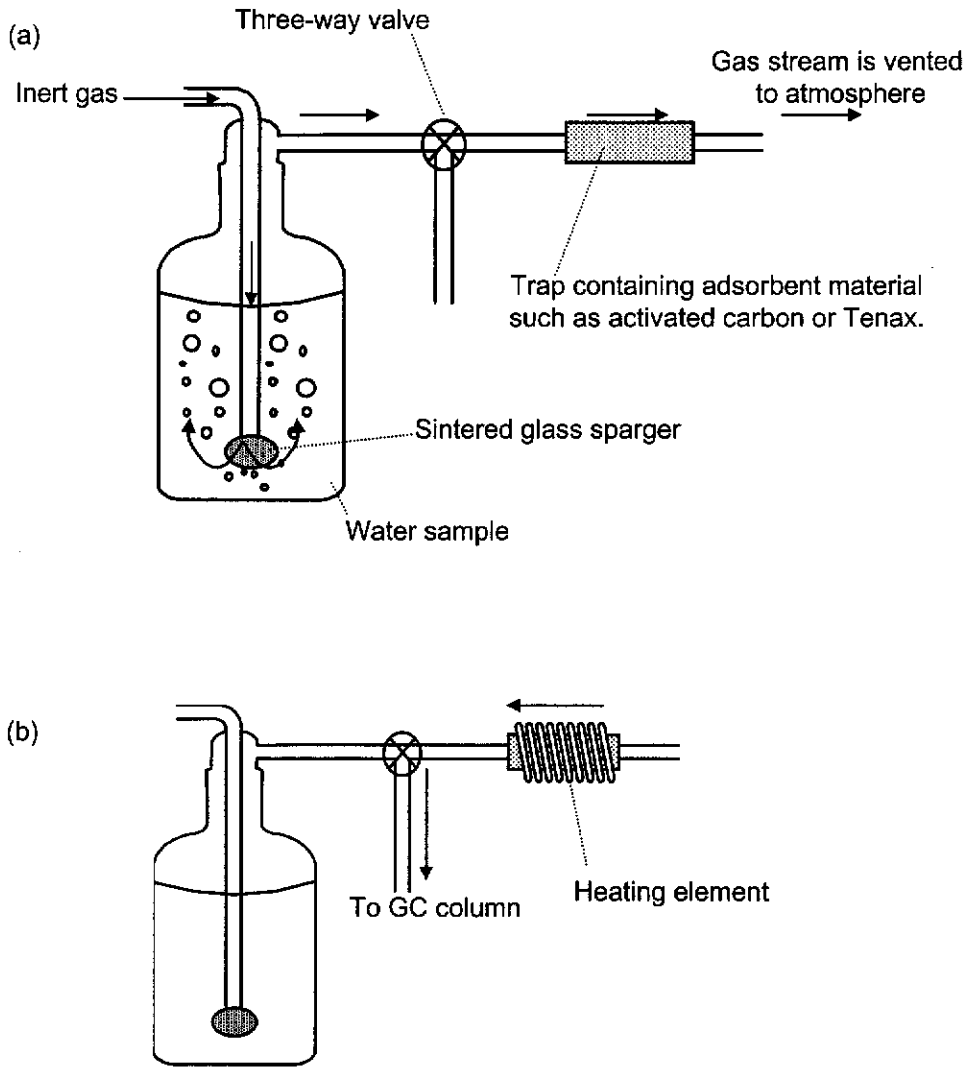


Figure 4.1: Principles of purge and trap-thermal desorption:

(a) Step 1: Purge and trap. Inert gas is passed through water sample to purge analytes which are trapped on adsorbent material. Gas stream passes through trap to atmosphere.

(b) Step 2: Thermal desorption. Flow of gas is reversed and is changed to GC carrier gas. Trap is heated to desorb analytes which are swept directly onto GC column, where they can be re-focussed at sub-ambient temperature.

volume (V_b) for a given analyte (Comes et al., 1996, 1993). This is defined as the maximum volume of purge gas which can pass through the trap with no detectable loss of analyte from the trap. When the analyte begins to elute from the trap the breakthrough volume is said to have been exceeded.

Depending on the concentration of the analyte and the type of adsorbent used, the behaviour of analyte on adsorbent can be described in linear or in Langmurian terms. Comes et al. (1996, 1993) discussed the Langmurian behaviour of BTEX and several industrial odorants on Tenax, Carboxen-569 and HayeSep Q adsorbents. They described the relationship between V_b and the concentration of analyte in the (purge) gas phase (c) by eq 4.1;

$$V_b = bm_{max}/(1+bc) \qquad ...[4.1]$$

where b is the distribution coefficient between the solid (adsorbent) and gas phases, m_{max} is the maximum mass of analyte that can be retained in a monolayer on the adsorbent surface. V_b for a particular analyte is dependent on the type and amount of adsorbent material (b and m_{max}), and concentration of analyte. The type of adsorbent material has a particularly marked effect on V_b. For low concentrations, as in trace analysis, a linear relationship is usually appropriate.

The problems of breakthrough volume are largely overcome by closed loop stripping analysis (CLSA), a technique first described by Grob and Zürcher (1976), and essentially a variation of purge and trap. The apparatus, shown in Figure 4.2, consists of a thermostatted purge vessel containing the water sample, a sintered glass sparger, a heated activated carbon adsorbent trap and metal bellows pump connected in a closed circuit. The ambient air trapped in the system when all openings are sealed is recirculated through the sample via the carbon trap and the pump. After completion of the purge cycle, typically 90-120 minutes, the trap is removed from the holder and analytes are eluted from the carbon using a small quantity of solvent, which is then analysed by gas chromatograpy.

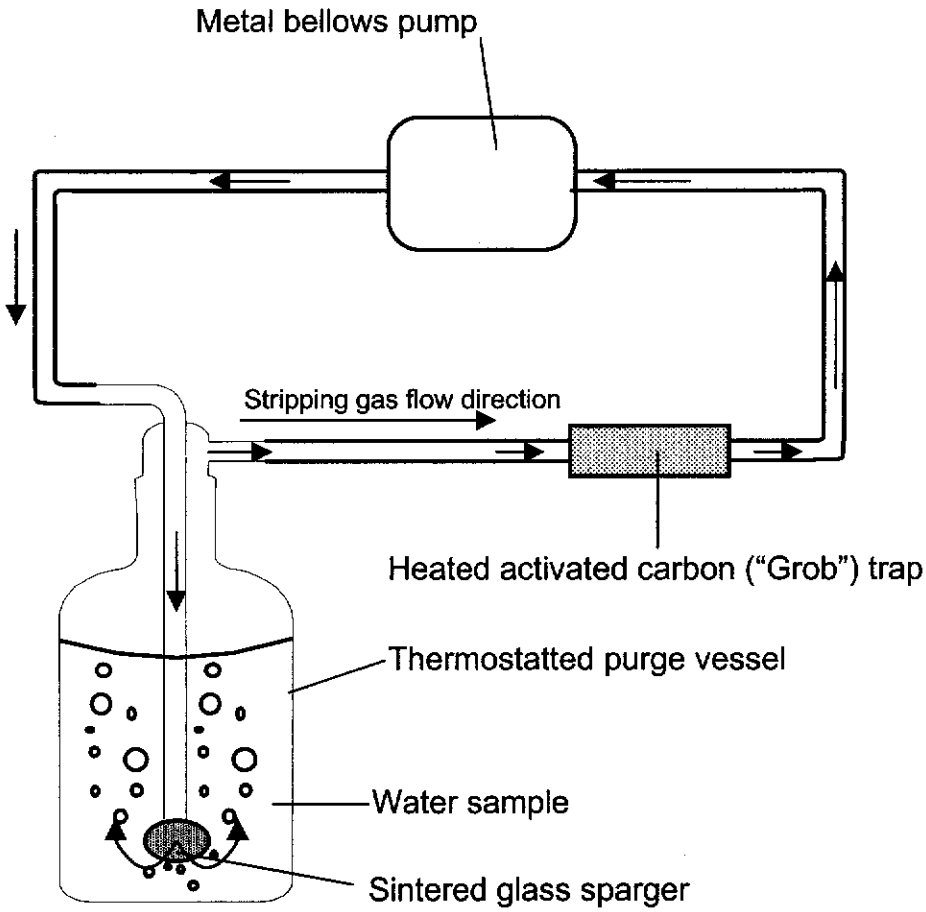


Figure 4.2: Closed loop stripping apparatus (CLSA).

According to Grob Zürcher (1976) the problems of inefficient recovery of analytes because the breakthrough volume is exceeded (as occurs with purge and trap procedures) is overcome with CLSA. Use of the closed loop system ensures that analytes are readsorbed onto the trap during the analysis, even if they have a very low V_b. This means that the limitations of sample volume and trap size can be ignored, and the range of compounds which can be efficiently recovered in a single analysis is much greater than with purge and trap methods. Acceptable recoveries of analytes ranging in molecular weight from benzene to octadecane can be achieved with sample volumes in the order of 1L, and purge flow rates of 1-2 L/minute, with a trap containing only 1.5 or 5 mg of activated carbon adsorbent (Khiari et al, 1997; Schwarzenbach et al., 1978). The small trap size is of great advantage because only micro-litre amounts of solvent are required to desorb the analytes (typically a total of 20-40 μL), resulting in a concentration factor of up to 50 000 fold. This is of considerable advantage in ultratrace analysis. In cases where the analyte is thermally labile and sufficiently non-volatile to be effectively separated from the solvent during gas chromatographic analysis (e.g. as in the case of DMPSs), elution of analytes from the trap using a solvent is preferred over thermal desorption.

Considerable overlap exists in the applicability of the two methods, and both are suitable for the analysis of species that can be purged from water. In CLSA, the use of a solvent to extract analytes from the adsorbent precludes analysis of very volatile compounds that co-elute with the solvent during GC analysis, but purge and trap-thermal desorption-GC is an ideal method for analysis of these types of compounds. Generally, purge and trap is used for compounds of higher volatility, but the range of compounds that can be determined in a single analysis is greater using CLSA. Purge and trap is usually directly interfaced with GC and therefore lends itself readily to automation. This technique is therefore more commonly used for routine, high-throughput analyses than CLSA, which is inherently more cumbersome and labour-intensive. CLSA is, however, exceptionally useful in environmental and taste-and-odour problem-solving applications (i.e. analysis of 'non-target' analytes) where it is desirable to recover as wide a

range of analytes as possible in a single analysis.

Borén et al. (1985) noted that, while the development of the CLSA technique was "a great accomplishment in the field of ultra-trace analysis" (and remains so, almost twenty later), the design of the system caused several problems, which limited its applications. According to these authors, use of the closed system limited the stripping temperature (i.e. purge temperature) to 30 °C, while the optimum stripping temperature for many compounds of interest was 60-90 °C. A more serious problem associated with CLSA was contamination of samples during analysis, either by leakage of air into the system or by reversible adsorption of contaminants from 'dirty' samples onto surfaces within the system. In order to overcome these problems, these authors developed a modified version of CLSA, which they termed 'open loop stripping'. This system was similar to purge and trap, except that the adsorbent used was the same as that used for CLSA (a small filter tube containing 1.5-5 mg of activated carbon, manufactured by Bender and Hobein, and termed a 'Grob tube'). These authors showed that breakthrough was not a problem for most analytes, even with purge gas flow rates of up to 2 L/minute for 1 hour. It was found that the activated carbon filter tube (Grob tube) was superior to other adsorbents tested, although it was not specified what these were. In the study by Borén et al. (1985), the analytes of interest were of lower volatility than many compounds typically analysed by purge and trap-thermal desorption, and this might partly explain why breakthrough was not a serious problem in their study. For example, these authors analysed compounds ranging in volatility from naphthalene (b.p. 218 °C) to 2,3,6-trichloroanisole (b.p. 240 °C), while purge and trap-thermal desorption procedures are typically used for more volatile compounds such as benzene (b.p. 78 °C), xylenes and halogenated solvents, such as perchloroethylene (b.p. 121 °C) (Chang and Klee, 1997).

In the present study, a purge and trap procedure similar to that described by Borén et al. (1985) was used. A commercially available purge and trap instrument (Tekmar LSC 2000) was modified so that an activated carbon filter of the type developed by Grob (Grob tube), as discussed above, could

be used. One major advantage of this technique was that method parameters, including the purge gas flow rate, purge time, sample temperature and trap temperature could be stored in the microprocessor memory of the Tekmar instrument. The stored method parameters were automatically controlled and a particular method could be activated at the touch of a button. Together with other modifications discussed in Sections 4.2.3 and 4.3.2, this resulted in a procedure that was simple, rapid ,accurate and robust, in comparison to the previously used CLSA procedure described by Wajon et al. (1988).

Analysis of dimethylpolysulfides (DMPSs)

(a) Extraction and pre-concentration

Procedures for the extraction and pre-concentration of nanogram-per-litre concentrations of volatile organosulfur compounds such as hydrogen sulfide, methanethiol (MT), dimethylsulfide (DMS) and DMDS are typically based on purge and trap-thermal desorption systems. Henatsch and Jüttner (1988) used a Tenax cryotrap cooled with solid carbon dioxide to extract and pre-concentrate sulfur compounds in the volatility range from hydrogen sulfide to DMDS. Leck and Bagander (1988) analysed a similar range of compounds using a glass U-tube trap filled with glass beads and cooled with liquid nitrogen. Gibson et al. (1994) and Caron and Kramer (1989) also used a liquid nitrogen trapping scheme, the latter authors using a Hall electrolytic conductivity detector, to obtain limits of detection in the sub-nanogram-per-litre range for sulfur gases up to DMDS. A relatively simple procedure using Molecular Sieve 5A which did not require sub-ambient trapping, was described by Deprez et al. (1986). Gold-sputtered glass wool was also found to be a useful adsorbent at ambient temperatures (Gibson et al., 1994; Kittler et al., 1992).

Dimethylpolysulfides are susceptible to disproportionation, particularly at high temperatures, and procedures based on thermal desorption are therefore unsuitable for the analysis of these compounds. Both DMTS and DMTeS are

unstable at temperatures lower than those commonly used for thermal desorption (>200 °C). According to Kende et al. (1965), DMTeS has a half-life of 0.5 seconds at 170 °C and even at 80 °C it formed DMTS (8%) and DMPeS (10%) after 30 minutes. Due to their thermal instability, dimethylpolysulfide homologues higher than DMDS cannot be analysed using thermal desorption techniques and consequently CLSA has been used in previous studies of these compounds (Wajon et al., 1988; Ginzburg et al., 1998).

(b) GC-MS analysis of DMPSs

Wajon et al. (1985a) have described in detail gas chromatographic procedures for the analysis of DMPSs, and discussed the difficulties arising from disproportionation of the homologues due to their thermal instability. The authors found that column heating rates and the presence of active sites within the injector liner were crucial factors in the analysis of these compounds. DMPeS disproportionated to DMDS, DMTS, DMTeS and elemental sulfur at a heating rate of 4°C/minute, but eluted as a pure Gaussian peak at lower heating rates. Injector liners which were even slightly active caused losses of analyte (up to 30% at an injection temperature of 260°C). Significant losses were observed at injector temperatures as low as 140°C when using poorly maintained liners. Disproportionation of the higher homologues occurred at injection temperatures as low as 140°C. These problems were overcome by using direct cool on-column injection, and by programming the GC oven so that dimethylpentasulfide eluted from the column at a temperature lower than 115°C.

4.2 EXPERIMENTAL

4.2.1 Solvents, reagents and glassware

Blank water

Purified laboratory water was prepared by passing deionised or distilled water through a Barnstead Milli-Q system. This system consisted of cation

and anion ion exchange resin columns and activated carbon columns in series. Water at the outlet of the system was filtered through a 0.2 μm membrane. Water prepared in this way was used for all operations requiring water throughout the present study. This included dilution of reagents and analysis of reagent "blanks" or procedural "blanks". Occasional analyses of the water by the methods described in Section 4.2.3 and 5.2.2 (i.e. CLSA) revealed that it contained traces of plastic additives such as phthalate esters and fatty acids, but that it was always free of contaminants that could interfere with the present studies.

Solvents and glassware

Analytical grade hexane (ChromAR HPLC, Mallinckrodt) was used without further purification. Laboratory reagent grade pentane, methanol and dichloromethane (DCM) were purified by fractional distillation. All glassware was thoroughly washed with purified solvents then heated to 650 °C overnight.

Reagents

Iodomethane (Unilab, Ajax) was purified by fractional distillation and stored at 4 °C. Sodium sulfate (Univar, Ajax) was heated to 600 °C overnight and stored in a sealed container. All reagents and compounds used as standards were analytical reagent grade, except where specified. Hydrazine hydrate ($NH_2NH_2.H_2O$; 24-26% in water) was purchased from Fluka and used as received.

Buffer solutions

Buffer solutions were prepared as follows:

Buffer at pH 9.80 was prepared by mixing sodium bicarbonate (0.05 M; 50 mL) and sodium hydroxide (0.1 M; 7.6 mL) and diluting to 100 mL in Milli-Q water.

Buffer at pH 8.60 was prepared by mixing sodium tetraborate, dried to constant weight at 105 °C (0.025 M; 50 mL), with hydrochloric acid (0.1 M; 13.5 mL) and diluting to 100 mL in Milli-Q water.

Buffer at pH 6.86 (0.4 M) was prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4; 6.804 g; BDH, Analar) and disodium hydrogen phosphate (Na_2HPO_4; 7.050 g; BDH, Analar) in Milli-Q water (250 mL). Both phosphate salts were dried to constant weight at 105 °C before use.

Buffer at pH 4.80 was prepared by mixing potassium hydrogen phthalate, dried to constant weight at 105 °C (0.1 M; 50 mL; BDH, Analar), with hydrochloric acid (16.5 mL; 0.1 M) and diluting to 100 mL in Milli-Q water.

Dimethyldisulfide (≥98%) was purchased from Aldrich and stored in flame-sealed glass ampoules after the supplied ampoule had been opened.

4.2.2 Synthetic methods

Preparation of inorganic polysulfide solutions

Solutions of inorganic polysulfide were prepared according to the method of Korchevin et al (1989). Milli-Q water (800 mL) was de-aerated by boiling while purging with high-purity nitrogen. After cooling, sodium hydroxide (3.2 g) was added and the solution was re-heated to about 70 °C, while stirring and purging with nitrogen. After complete dissolution of the sodium hydroxide, hydrazine hydrate ($N_2H_4.H_2O$; 2.0 g) and elemental sulfur (2.56 g) were added. The mixture was stirred at 70-90 °C until the sulfur had dissolved completely. As the sulfur dissolved, the solution turned from clear to brown to deep green, indicative of polysulfide formation. The solution was cooled under nitrogen and a yellow colour developed.

This stock polysulfide solution (0.1 M) was stored under nitrogen and used within twenty-four hours to prepare dilute intermediate and working solutions at the concentrations listed in Table 4.3.1. The diluted solutions were

prepared in ascorbic acid (0.05% w/v) and sodium hydroxide (0.01 M). Water used for dilutions was comprehensively de-aerated prior to use and all operations were carried out under strict exclusion of oxygen. Precautions to prevent oxidation of inorganic polysulfides during preparation and dilution of standard solutions are discussed further in Section 4.3.1.

Synthesis of dimethyltrisulfide

Dimethyltrisulfide was prepared according to the method of Milligan et al., 1963. Iodomethane (7.1g, Unilab, Ajax) was added to a solution of sodium thiosulfate pentahydrate (10.9 g, AR, BDH) in phosphate buffer (20 mL; 0.2M). The phosphate buffer (pH 8) had been previously prepared by dissolving potassium dihydrogen phosphate (6.8 g) and sodium hydroxide (0.93 g) in water (250 mL). *S*-Methylthiosulfate was formed by vigorous stirring of the mixture at about 30 °C for about 1 hour. This was then extracted with pentane (3 x 5 mL) to remove any dimethyldisulfide. The aqueous *S*-methylthiosulfate solution was saturated with sodium chloride (Ajax, Univar), and 35% w/v formaldehyde (BDH, Analar) was added (10 mL). A solution of sodium sulfide (1M, 25 mL) was added over a period of 30 minutes while stirring. During this step the pH was maintained at 7-8 by addition of 1:1 hydrochloric acid, and the temperature was kept between 20 °C and 30°C. The solution was stirred for a further 90 minutes and then extracted with dichloromethane. After distilling under vacuum, analysis by GC-MS showed the product to be >99% DMTS. Neat DMTS was stored in flame-sealed ampoules in the dark.

Synthesis of a mixture of perdeuterated DMDS and DMTS

Preparation of a mixture of deuterated DMDS and DMTS was carried out in a similar manner to the procedure for preparation of DMTS described above, except that iodomethane-d_3 (Aldrich, 99.5% purity, 1 g) was used in place of native iodomethane. The pentane extraction step to remove DMDS-d_6 was omitted, as was the addition of formaldehyde. Products were not purified by distillation, but instead were extracted with dichloromethane (3 x 2 mL). The

combined extracts were washed once with Milli-Q water (3mL) and dried over anhydrous magnesium sulfate before evaporation of the solvent at 55°C. Analysis by GC-MS showed that deuterated DMDS and DMTS were obtained in the ratio of approximately 25:75. The yield of dimethylpolysulfides was 0.29 g (~76% based on iodomethane). The product mixture was divided into aliquots of about 70 μL and stored in the dark in flame-sealed ampoules.

Synthesis of DMPSs and deuterated DMPSs (n=2-5)

A mixture of DMPSs that contained dimethylpolysulfide homologues from DMDS to DMPeS was synthesized according to the method of Korchevin et al. (1989). Using a molar ratio of sulfur:hydrazine hydrate:sodium hydroxide of 0.2:0.1:0.1, these authors obtained a mixture of DMPSs containing DMDS (3.4%), DMTS (71%) and DMTeS (15%). They did not report the presence of DMS or DMPeS, but probably did not detect the latter compound because of its thermal instability and attendant difficulties in analysis by GC techniques, as discussed in Section 4.1.2. In the present study, a polysulfide solution was prepared as described above under "Preparation of inorganic polysulfide solutions", except that the proportions of sulfur, hydrazine hydrate and sodium hydroxide were 2:1:1 (i.e. 5.12 g sulfur; 4.00 g hydrazine hydrate; and 3.2 g sodium hydroxide were added to de-aerated water (800 mL)), to maximise yield of the higher homologues.

After preparation of the solution of inorganic polysulfides, it was cooled under nitrogen. Iodomethane (18.3 g; 8.0 mL) was added while stirring. After stirring for 2 hours, the mixture was extracted with pentane (2 x 30 mL) and dried over anhydrous magnesium sulfate. Pentane was removed by evaporation at 55-65 °C. Analysis of the headspace of the product mixture by GC-MS showed that it was free of pentane and iodomethane. Analysis of the product mixture by GC-FID showed that it contained the DMPS homologues in the proportions shown in Table 4.6 and that the percentage purity was 89.3%. Analysis of the mixture by GC-MS (Figures 4.11 (a) and (b)) showed that a small amount of elemental sulfur was present as the only contaminant.

A mixture of deuterated analogues of DMPSs was prepared in an identical manner, except that iodomethane-d_3 was used instead of isotopically unmodified iodomethane. The preparation was scaled down five-fold from that described above.

4.2.3 Sampling and analysis procedures

Methylation of inorganic polysulfides

Typically, analysis of samples for inorganic polysulfides involved addition of methyl iodide (0.05% v/v) directly into the water sample. The samples were allowed to react in darkness for 24 hours before analysis for DMPSs. The concentration of inorganic polysulfide sulfur was calculated by subtracting DMPSs originally present in the samples from the concentration of DMPSs resulting from reaction with methyl iodide.

In the case of hypoxic groundwater, where it was necessary to avoid contact with air, samples were taken by attaching a glass syringe (10-100 mL) to the sampling point. The syringe was flushed with sample, then filled to exclude headspace, before adding methyl iodide (0.05% v/v) via a microsyringe. The syringe containing the sample was then sealed using a custom made Luer-lock cap and transported to the laboratory on ice. Samples were stored at 4 °C and analysed twenty-four hours after the addition of methyl iodide. Prior to isolation of DMPSs by purge and trap, a mixture of internal standards (DMPS-d_6, typically 155 ng (2 μL) in methanol) was added to the sample via a microsyringe.

Sampling for analysis of DMDS and DMTS

Samples of distribution system water to be analysed for DMDS and DMTS, were taken in pre-annealed glass Winchesters (2.5 L) fitted with Teflon-lined caps. Sample containers were filled to exclude headspace, stored at 4 °C whenever possible, and analysed within twenty-four hours of sampling.

Isolation of DMPSs from water samples by purge and trap

An aliquot of water sample (typically 25 mL) was placed in the sample container of the Tekmar LSC 2000 purge and trap and internal standard was added (typically 29 ng, or 5 μL, of a mixture of DMDS-d_6 and DMTS-d_6 in methanol). The Tekmar instrument was fitted with a trap assembly that had been custom-made such that Grob tubes (1.5 or 5 mg; Drs Bender and Hobein, Switzerland or Brechbuehler, Switzerland) could be used in place of the manufacturer's traps (Figure 4.3). Instrument parameters used for the present procedure are listed in Table 4.1. The Grob tube (i.e. trap) was heated to 55 °C, using the Tekmar's auxiliary heater, to prevent condensation of water in the tube.

Table 4.1: Instrument parameters for Tekmar LSC 2000 purge and trap used in the isolation of DMPSs from water samples.

Sample size	5 - 25 mL
Sample temperature	ambient
Preheat time (trap)	3 minutes
Purge time	30 minutes
Purge gas	nitrogen @ 45 mL/min
Dry purge	2 minutes
Trap type	Grob tube containing activated carbon (1.5 mg)
Trap temperature	55° C

Analytes were eluted from the Grob tube with dichloromethane (DCM; 30-40 μL). The Grob tube was attached to a 5 mL syringe using Teflon heat-shrink tubing. An aliquot of DCM (10 μL) was placed onto the carbon filter, with the syringe and tube assembly in the vertical position, as shown in Figure 4.4. The assembly was rotated 90°, to the horizontal position, and the syringe barrel was moved backwards and forwards such that the solvent plug passed through the carbon filter forty times. The syringe/Grob tube assembly was swung back to the horizontal position and the plug of solvent was removed using a blunt-tipped 10 μL syringe. The solvent extract was placed into a microvial (100 μL), containing an aliquot of normalisation standard (*n*-$C_{16}Cl$;

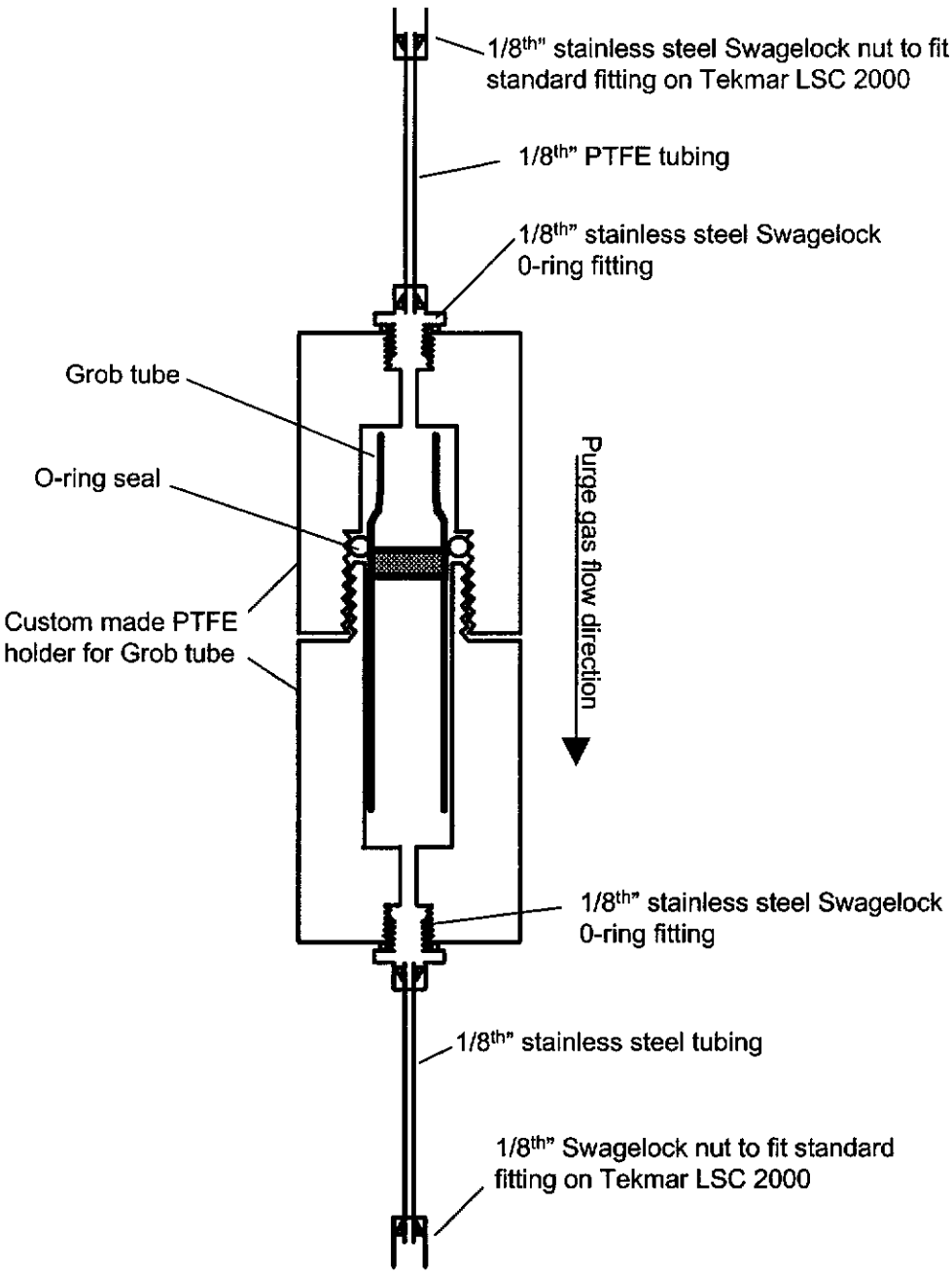


Figure 4.3: Custom made assembly to integrate Grob tube with Tekmar LSC 2000 purge and trap. The assembly is designed to replace existing Tekmar traps.

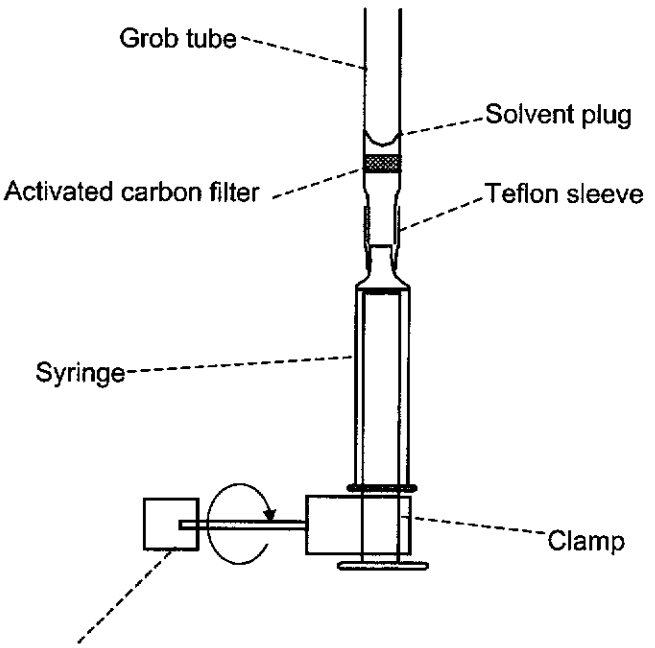


Figure 4.4: Syringe assembly used for extraction of Grob tubes.

10uL, or 20 ng, in hexane). The procedure was repeated three times, using 3 x 8 μL aliquots of DCM and all the extracts were combined in the microvial. The microvial was capped using a custom-made Teflon cap to prevent evaporation of the solvent (as occurred when conventional crimp-top caps were used) and consequent loss of analytes. The Teflon caps were replaced with crimp-top caps just prior to GC-MS analysis.

Grob tubes were pre-cleaned immediately after use by allowing a volume of DCM (about 0.5mL) to flow through the tube, then placed into a sealed vial containing a solvent mixture (DCM/methanol approximately 1:1). The tubes were stored in the solvent mixture ready for cleaning, just prior to analysis of the next batch of samples. Tubes were cleaned by passing about four tube volumes of each of methanol, Milli-Q water, methanol and DCM sequentially through each tube under gravity flow. Residual solvent was removed by placing the tubes in a vacuum chamber for five minutes.

Analysis of DMPSs by gas chromatography-flame ionisation detection (GC-FID)

DMPS mixtures were analysed using GC-FID under the conditions listed in Table 4.2. Note that different oven temperature programmes were used, depending on whether or not the mixtures contained only DMDS and DMTS, or all of the dimethylpolysulfide homologues (DMDS to DMPeS).

Table 4.2: Conditions used for analysis of DMPS mixtures by GC-FID.

Instrument	
Make and model	Hewlett-Packard 5890
Detector	Flame ionisation
Column	
Make and phase	J&W DB 1
Internal diameter	0.32 mm
Length	30 m
Phase thickness	3 μm

Table 4.2 (continued)

Oven temperature program (for mixtures containing DMDS and DMTS only)	
Initial temperature	35 °C
Initial hold time	5 minutes
Temperature increase (rate)	5 °C/minute
Final temperature	300 °C
Final hold time	10 minutes
Oven temperature program (for mixtures containing homologues from DMDS to DMPeS)	
Initial temperature	35 °C
Initial hold time	5 minutes
Temperature increase #1 (rate)	4 °C/minute
Final temperature #1	90 °C
Hold time #1	0 minutes
Temperature increase #2 (rate)	1 °C/minute
Final temperature #2	136 °C
Hold time #2	0 minutes
Temperature increase #3 (rate)	20 °C/minute
Final temperature #3	300 °C
Hold time #3	10 minutes

Analysis of DMPSs by GC-MS

Mixtures of DMPSs were analysed using GC-MS under the conditions listed in Table 4.3. Note that different oven temperature programmes were used, depending on whether or not the mixtures contained only DMDS and DMTS, or all of the dimethylpolysulfide homologues (DMDS to DMPeS).

Table 4.3: Conditions used for analysis of DMPS mixtures by GC-MS.

GC and column	
Instrument make and model	Hewlett Packard 6890
Injector	HP cool on-column
Injector temperature program	Initial temp. 38 °C; temperature programmed for oven track
Sample volume injected	1 µL
Column make and phase	J&W DB 5 or DB1
Column phase thickness	0.4 µm

Table 4.3 (continued)

Column length	40 m
Column internal diameter	0.18 mm
Carrier gas	Helium
Carrier gas flow velocity	24.0 cm/sec
GC oven temperature program (for mixtures containing DMDS and DMTS only)	
Initial temperature	35 °C
Initial hold time	5 minutes
Rate of temperature increase	5 °C/minute
Final temperature	300 °C
Final hold time	10 minutes
Oven temperature program (for mixtures containing homologues from DMDS to DMPeS)	
Initial temperature	35 °C
Initial hold time	5 minutes
Temperature increase #1 (rate)	4 °C/minute
Final temperature #1	90 °C
Hold time #1	0 minutes
Temperature increase #2 (rate)	1 °C/minute
Final temperature #2	136 °C
Hold time #2	0 minutes
Temperature increase #3 (rate)	20 °C/minute
Final temperature #3	300 °C
Hold time #3	10 minutes
MS	
Make and model	Hewlett Packard 5973
Operating conditions	70 eV, electron impact
Operating mode	Full-scan and selected ion monitoring (SIM)
Ions monitored in SIM mode	*m/z* 64; 79; 82; 91; 94; 97; 100; 126; 129; 132;158; 164; 190; 196

Statistical calculations

Statistical parameters and "lines of best fit" were calculated using Microsoft Powerpoint software.

4.3 RESULTS AND DISCUSSION

The present section is divided into two parts; in the first part (4.3.1) a new procedure for the analysis of inorganic polysulfides by derivatisation with methyl iodide is described. The discussions focus specifically on issues related to methylation of inorganic polysulfides, the first part of the analytical procedure. Topics discussed include the potential for interferences from other sulfur nucleophiles and organic polysulfides; effect of pH change on polysulfide speciation; hydrolysis of methyl iodide; and the precision and accuracy of analyses of "blank" water samples spiked with inorganic polysulfides.

The second part (4.3.2) deals with analysis of DMPSs, either as DMPSs formed as a result of adding methyl iodide to a sample, or as DMPSs already present in a sample. The methodology for isolation and preconcentration of DMPSs from water samples and subsequent analysis of these compounds by GC-MS is described. Method validation, including an analysis of the precision and accuracy of the method, and new modifications and improvements to a previously used method are described.

4.3.1 In-situ methylation of inorganic polysulfides

Preparation of polysulfide standard solutions

Quantitative preparation of very dilute polysulfide solutions requires strict exclusion of oxygen because of rapid oxidation of these compounds, even by traces of residual dissolved oxygen in the dilution water. In the present work, dilution water was de-aerated comprehensively by boiling and sparging with ultra high purity inert gas (nitrogen or argon) containing the lowest possible concentration of oxygen (<1 ppm O_2). All operations involving transfer of polysulfide solutions were conducted under oxygen-free conditions in an argon-filled glovebag. Ascorbic acid (0.5% w/v) was added as a redox buffer. Polysulfide solution (3.7 g/L), prepared according to Korchevin et al. (1989), was diluted using the de-aerated dilution water to prepare intermediate

standards and a series of working standards at the concentrations listed in Table 4.4. Methyl iodide (0.05% v/v) was added to the final working standards prior to adding the appropriate aliquot of polysulfide solution. When these very dilute solutions were prepared by reversing the order of addition of methyl iodide and polysulfide, the recovery of polysulfide was decreased considerably, presumably because underivatised inorganic polysulfide reacted rapidly with the very small residual amounts of dissolved oxygen present in the dilution water. Presumably when methyl iodide was already present in the water to which polysulfide was added, it reacted rapidly to form the methylated polysulfides, which are oxidised much more slowly than inorganic polysulfides (the stability of DMDS and DMTS in aqueous solutions is discussed in Section 4.3.2). The higher the polysulfide concentration in the solution being prepared, the smaller the effect from residual dissolved oxygen.

Under the conditions described above it was possible to quantitatively recover inorganic polysulfide, at the nanomolar level as DMDS and DMTS. Quantitative recovery of polysulfides in the concentration range from 0.15 μg/L to 370 μg/L is demonstrated in Figure 4.5, where the total polysulfide recovered as DMDS and DMTS is plotted against the concentration of polysulfide spiked into the solution under test. The calibration was linear over the concentration range tested, as shown by the graph in Figure 4.5. (Figure 4.5 is presented in log/log form to illustrate the linearity of results over the wide concentration range tested). The raw data are listed in Table 4.4. Only concentrations of DMDS and DMTS are listed, since DMTeS and DMPeS were not produced at detectable concentrations in these solutions. The molar ratio of DMDS/DMTS produced in each solution is also listed in Table 4.4.

Standard polysulfide solutions were prepared according to the method of Korchevin et al. (1989), since this offers several advantages over alternative methods. Other methods previously used include reaction of sulfide with elemental sulfur (Borchardt and Easty (1984), or dissolution of single polysulfide salts (e.g. Schwarzenbach and Fischer, 1960). The use of sulfides (e.g. sodium sulfide) presents some practical difficulties because of

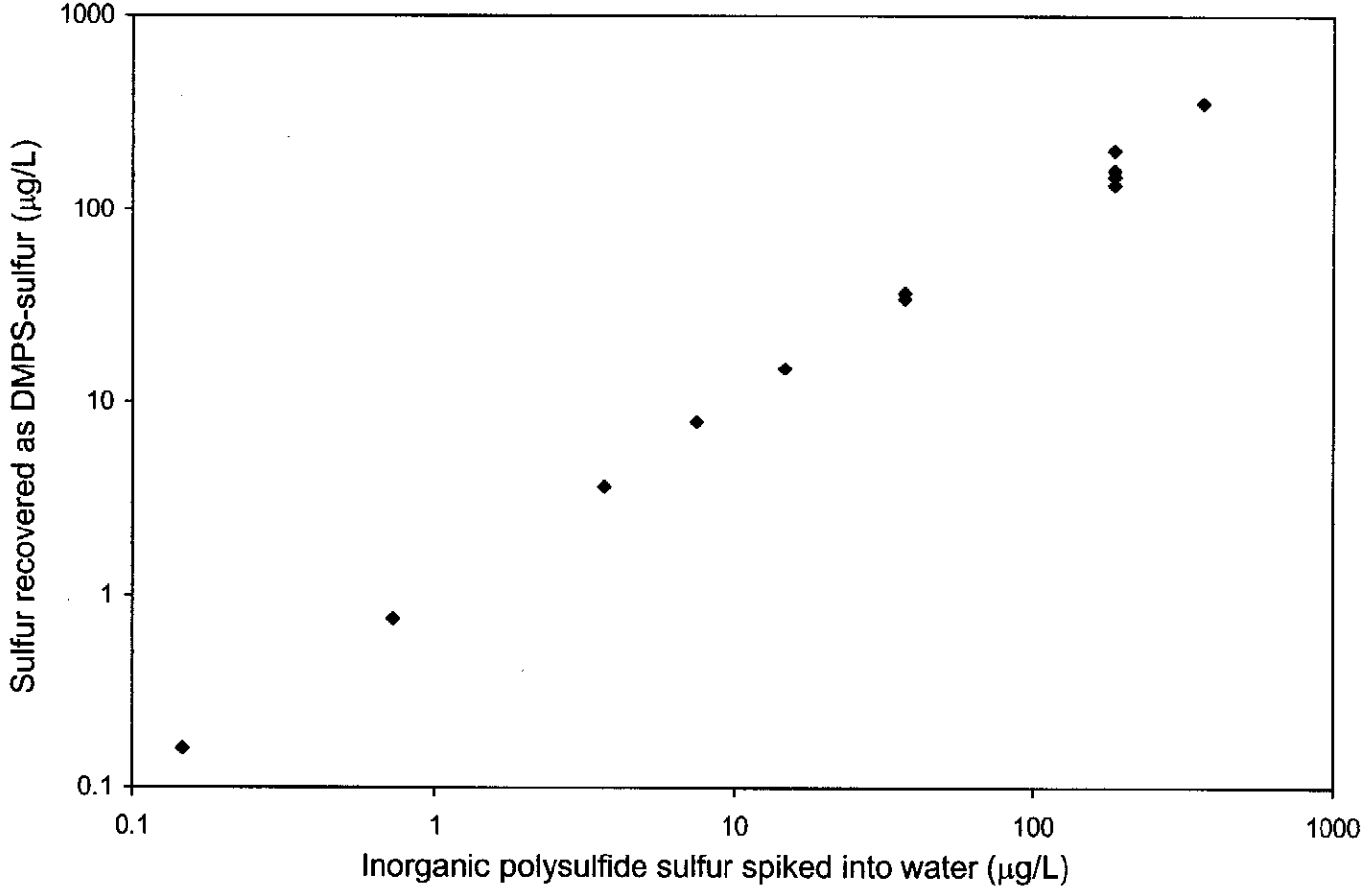


Figure 4.5: Recovery of inorganic polysulfides as dimethylpolysulfides (DMPS) after derivatisation with methyl iodide. (Concentration range = 0.15 to 370 µg/L, n = 15, RSD = 10.4%, r^2 = 0.97. Note log/log scale).

their reactivity with oxygen and their propensity to absorb moisture. Preparation of single salts of polysulfides is difficult and time consuming and the salts must be stored and handled under oxygen-free conditions. In the method of Korchevin et al., elemental sulfur is heated in a solution of sodium hydroxide in the presence of hydrazine hydrate. These reagents are relatively cheap and easily purchased and do not require special handling procedures. Elemental sulfur is extremely stable in the presence of oxygen and does not absorb water. It can therefore be accurately weighed with no requirement for protection from air or moisture and is ideal for preparation of standards of known sulfur concentration. Oxygen-free conditions are required only as the sulfur starts to react with hydroxide to form the polysulfide solution.

Table 4.4: Recovery of polysulfide sulfur as DMDS and DMTS after reaction with methyl iodide.

Polysulfide spike (µg/L - S)	DMDS recovered (µg/L)	DMTS recovered (µg/L)	Molar ratio DMDS/DMTS	*Recovery of polysulfide sulfur (µg/L - S)	*Recovery of polysulfide sulfur (%)
0	0.021	0.008		0.021	n/a
0.146	0.102	0.111	1.2	0.154	105
0.146	0.108	0.112	1.3	0.159	109
0.731	0.376	0.630	0.80	0.736	101
0.731	0.561	0.488	1.5	0.753	103
3.66	4.11	1.04	5.3	3.59	98
7.40	3.46	7.24	0.64	7.87	106
14.8	11.7	9.73	1.6	15.3	104
14.8	6.93	13.6	0.68	15.1	102
37.0	12.7	37.7	0.45	37.4	101
37.0	38.0	11.1	4.6	34.3	92
185	75.2	107	0.94	133	72
185	114	160	0.95	199	108
185	76	124	0.82	146	79
185	110	112	1.3	160	87
370	195	290	0.90	354	96
Average			1.5		98
Standard deviation (%)					10.4

Notes: n/a = not applicable; *Recovery of polysulfide sulfur measured as DMDS and DMTS.

Precision and accuracy of the method

According to Swartz and Krull (1997) the accuracy of a method is established by collecting data from a minimum of nine determinations over a minimum of three concentration levels covering the specified range. The accuracy of the present method is demonstrated by the data presented in Table 4.4. A mean recovery of 98% was achieved over the concentration range 0.15 to 370 μg/L. Repeatability, or precision as determined by the relative standard deviation was calculated at 10.4 % for the synthetic solutions in Table 4.4. Precision in the case of analysis of "real" samples will be discussed in Section 4.3.2.

Effect of pH

Polysulfide stability and speciation is extremely sensitive to pH changes as discussed in Chapter 2 (Giggenbach 1972; Pasiuk-Bronikowska et al., 1992, p. 64). According to Giggenbach (1972), at pH values around 14, disulfide is expected to be the major species, and as pH decreases, the average polysulfide chain length increases. Chen and Morris (1972) contradicted this, predicting that polysulfides of chain length 4-5 predominate at pH > 7.3. Ginzburg et al. (1999) observed that as pH increased, the relative proportions of trisulfide and tetrasulfide increased, while the fraction of disulfide decreased. While there appears to be some confusion regarding the effect of pH on polysulfide chain length, it has been clearly established that as pH decreases to values < 7, polysulfides disproportionate to give elemental sulfur and sulfide, and the absolute concentration of polysulfide decreases (Steudel, 1996; Giggenbach, 1974a; Schwarzenbach and Fischer, 1960).

In the present study, to investigate the effect of pH on polysulfide recovery, a polysulfide solution was prepared, such that the major homologues expected (according to Korchevin et al, 1989) were DMDS and DMTS in a ratio of about 1:3 at 2.4 g/L total sulfur. This was then diluted to give working solutions of 12 μg/L-S buffered (0.01 M buffer) at pH values between 4.4 and 10, before adding methyl iodide (0.05% v/v) and analysing for

dimethylpolysulfides. One sample was prepared by spiking polysulfide directly into de-aerated purified water without the addition of buffer. Recoveries of total polysulfide, and relative ratios of DMTS to DMDS between pH 4.4 and 9.8 are shown in Figure 4.6. The recovery of total polysulfides (as DMPSs) from a synthetic solution of 12 μg/L polysulfide-S decreased from 98% at pH 9.8 to 6.9% at pH 4.8. This decrease in recovery at low pH is indicative of the formation of elemental sulfur due to polysulfide disproportionation, as discussed above.

Contrary to some literature reports stating that the polysulfide chain length should increase with decreasing pH, the proportion of DMDS relative to DMTS in the above experiment increased with decreasing pH. The reason for this discrepancy is unclear, but may be related to the method of preparation of the polysulfide solution. Giggenbach (1974) reported that the average polysulfide chain length increased if solutions were prepared at high pH (i.e. average chain length increased with increasing starting pH). Korchevin et al. (1989) found that it was possible to control chain length to some extent by varying the proportions of sulfur and sodium hydroxide or potassium hydroxide. The latter authors were able to prepare polysulfide solutions with average chain lengths from 1.9 to 3.4 in solutions of pH 13-14.

Clearly, factors other than pH influence polysulfide chain length, and it appears that these are not yet fully understood. Gun et al. (2000) predicted that the average polysulfide chain length should decrease with decreasing concentrations of total reduced sulfur, such that at very low concentrations disulfide would be the only species in solution. As shown in Table 4.4 there did not appear to be any clear trend with respect to concentration and polysulfide chain length in the present work, although the trisulfide was the highest homologue detected, agreeing to some extent with the work of Gun et al. (2000) (O. Lev, personal communication, 2001). In Table 4.4 the molar ratio of DMDS/DMTS for each of the standard solutions analysed (pH >13, 0.2 M sodium hydroxide) varied considerably from 0.45 to 5.3, with an average value of 1.5 and an average chain length between 2 and 3. The reason for the wide variation in DMDS/DMTS production is unclear. As stated

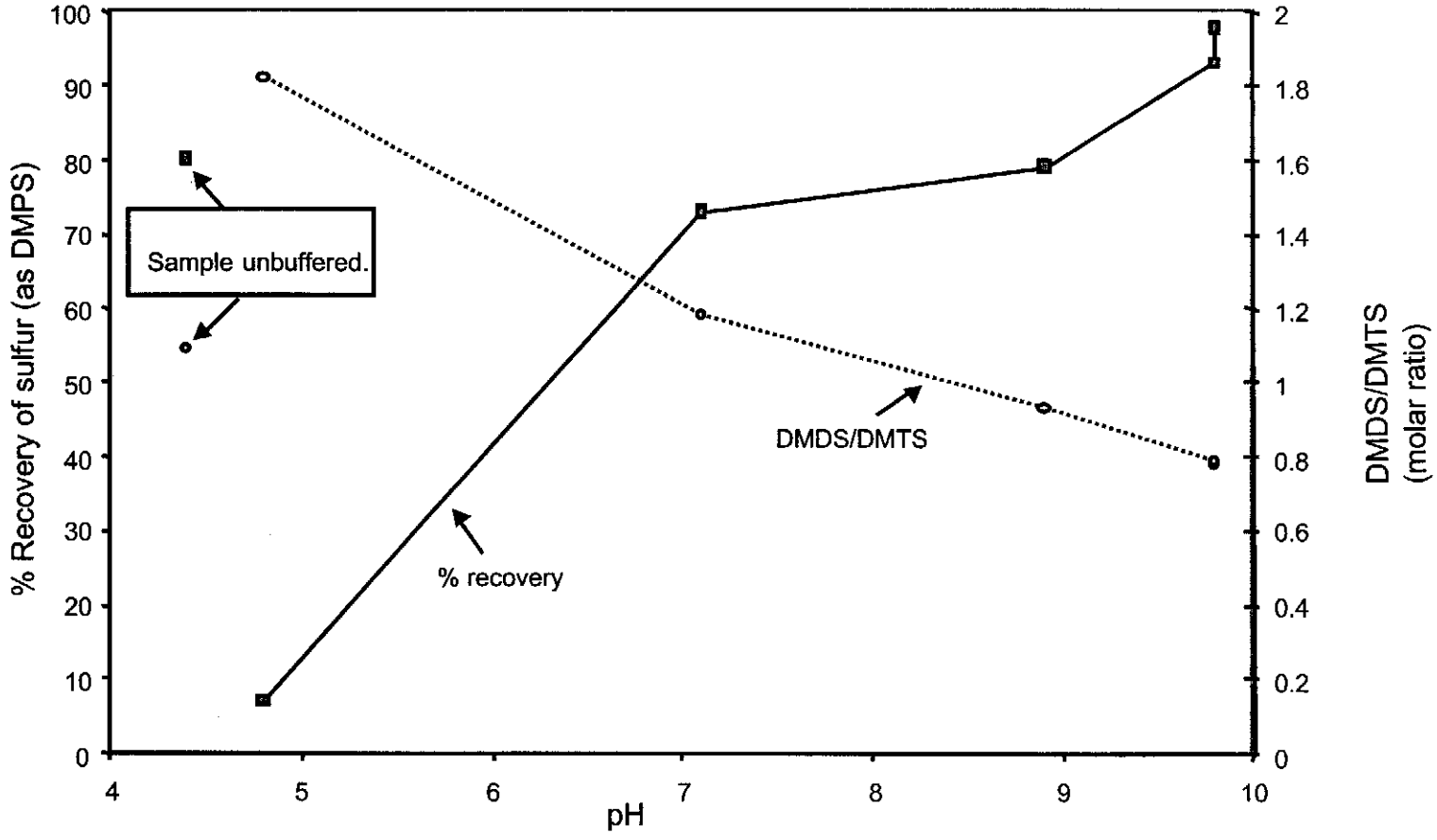


Figure 4.6: Effect of pH on recovery of polysulfides and on distribution of disulfide *vs* trisulfide. The sample at pH 4.2 was not buffered and the pH recorded is the final pH, after reaction with methyl iodide.

previously, factors affecting polysulfide distribution are not fully understood and may include determinants other than pH and concentration, which have not yet been identified.

Effect of methyl iodide on sample pH

Addition of methyl iodide to poorly buffered samples can result in a decrease in pH, due to the liberation of a proton from the hydrolysis of methyl iodide (eq 4.2).

$$CH_3I + H_2O \rightarrow CH_3OH + I^- + H^+ \quad \ldots[4.2]$$

The effect of hydrolysis of methyl iodide ($t_{1/2}$ = 110 days at pH 7 and 25 °C, Montgomery, 1996) on the pH of a sample is negligible in environmental waters that have adequate buffering capacity, but this is not always the case in very dilute solutions. This is shown by pH values in various natural water samples before and after addition of methyl iodide as listed in Table 4.5. The pH decreased considerably in groundwaters W60, W80 and W90, since these have a lower buffer capacity than Wanneroo raw water and W110, as indicated by the alkalinity values listed in Table 4.5.

Table 4.5: The effect of the addition of methyl iodide (0.05 %v/v, 8 mM) on pH in some aqueous environmental samples.

Sample	pH at t, where t = time (hours after the addition of methyl iodide (0.05% v/v))					*Alkalinity as CaCO3 (mg/L)
	t = 0	t = 1	t = 18	t = 48	t ≥ 72	
Wanneroo Raw water	7.40	7.40	7.39	7.41	7.40	60
Groundwater W60	5.34	5.14	n.d.	5.09	2.82	16
Groundwater W80	5.30	5.22	n.d.	5.18	2.81	20
Groundwater W90	5.25	5.15	n.d.	5.11	2.78	22
Groundwater W110	5.79	5.78	n.d.	5.77	5.78	90
Milli-Q water	6.90	n.d.	4.41	n.d.	2.59	<1

Notes: n.d. = not determined; * Reference: Water Corporation (1999)

Although a decrease in pH occurs in some samples upon the addition of methyl iodide, polysulfide recovery should not be affected if polysulfide is methylated rapidly, before significant hydrolysis of methyl iodide occurs. This is shown by the sample in Figure 4.6, which had been prepared by spiking polysulfide (12 μg/L) into unbuffered deaerated deionised water. The nominal pH of deionised water was 6.9 prior to addition of polysulfide, but decreased to 4.4 twenty-four hours after the addition of methyl iodide. However, unlike the sample buffered at pH 4.8 (phthalate; 0.01M) before the addition of polysulfide, the recovery of polysulfide in the unbuffered sample was relatively high (80 % recovery for unbuffered sample *vs* 7% recovery for sample buffered at pH 4.8). This probably occurred because methylation of polysulfides took place at pH 6.9 or greater, and the pH of the solution decreased at a rate slower than the rate of polysulfide methylation.

These observations illustrate the importance of maintaining the natural sample pH until methylation of polysulfides is complete, and for this reason (and to minimise oxidation), methyl iodide should be added to the sample at the time of sampling. Further, while recovery of polysulfides may be slightly decreased in very poorly buffered samples (where $CaCO_3$ alkalinity <1 mg/L), preliminary results showed that 80% recovery was achievable in unbuffered deionised water. Since almost all natural water samples contain at least some residual buffering capacity, methyl iodide hydrolysis should have minimal effect in most cases.

Potential for interference from sulfoxyanions

In sulfidic environments, or at the interface between sulfidic environments and the oxic zone, polysulfides are likely to co-occur with other metastable intermediates of sulfide oxidation such as thiosulfate and sulfite (Luther and Church, 1992; Luther et al., 2001). Reaction of sulfite with DMTS according to eq 4.3 (Milligan et al., 1963) would result in a net removal of sulfur from the DMPS pool, and could thereby interfere with the measurement of polysulfides. Similarly, reaction of polysulfides with thiosulfate would result in a redistribution of S^0, as shown in eq 4.4.

$$SO_3^{2-} + CH_3SSSCH_3 \Leftrightarrow CH_3S^- + CH_3SSSO_3^- \Leftrightarrow CH_3SSCH_3 + S_2O_3^{2-} \quad ...[4.3]$$

(Milligan et al. 1963)

$$^-SSO_3^- + CH_3SSSCH_3 \Leftrightarrow CH_3SSSO_3^- + CH_3SS^- \Leftrightarrow CH_3SSSSCH_3 + SO_3^{2-} \quad ...[4.4]$$

The effects of these reactions are demonstrated in Table 4.6 where it is shown that DMDS and DMTS were not quantitatively recovered from water containing a large excess of thiosulfate and of sulfite. The effect is particularly marked in the case of sulfite which is more nucleophilic, and more reactive towards sulfur-sulfur bonds than thiosulfate (Milligan et al 1963, Oae 1991, Barbash and Reinhard 1989a).

Table 4.6 Recovery of DMDS and DMTS standards in sulfoxyanion solutions. (DMDS and DMTS were spiked at 29 ng/L-S, approximately 1nM)

Sulfoxyanion (31 mM, 1000 mg/L Sulfur)	% recovery	
	DMDS	DMTS
None	93[a]	92[a]
Thiosulfate	97[b]	40[b]
Sulfite	42[b]	6[b]

Note: [a] average of 10 determinations

[b] one determination only

Thiosulfate and sulfite react with methyl iodide to form the S-methyl derivatives, S-methylthiosulfate and methylsulfonate respectively, and methylation essentially quenches their reactivity with polysulfides. In order to determine the extent of interference from sulfite and thiosulfate in the present analytical technique, polysulfide/methyl iodide reaction mixtures were spiked with an excess of these sulfoxyanions. The sulfoxyanions were added after the methylation reaction of polysulfide was complete (after 24 hours of reaction). A further aliquot of methyl iodide (0.05% v/v) was added to a

duplicate set of experiments just prior to spiking with the sulfoxyanions. This was done to determine whether methylation of sulfoxyanions would prevent their reaction with dimethylpolysulfides. These solutions were allowed to react for 16 hours, and then analysed for dimethylpolysulfides. Results are shown in Table 4.7.

Table 4.7: Effect of a large excess of sulfite and thiosulfate on recovery of polysulfide sulfur (2.00 μg/L-S), when analysed by *in-situ* methylation.

Sulfur nucleophile spiked (2 mg/L sulfur, 65 μM)	Methyl iodide added (% v/v)	Recovery of dimethylpolysulfide (μg/L)		Recovery of polysulfide sulfur (μg/L)	% recovery of polysulfide sulfur
		DMDS	DMTS		
none	*0.05	1.65	1.24	2.07	104
SO_3^{2-}	*0.05	1.40	0.30	1.18	57
SO_3^{2-}	*0.05+#0.05	1.59	0.71	1.62	78
SSO_3^{2-}	*0.05	1.69	1.30	2.14	107
SSO_3^{2-}	*0.05+#0.05	1.69	1.23	2.08	104

Notes: *Methyl iodide added once only, as described in Section 4.2.3.
#Additional methyl iodide added just prior to spiking with thiosulfate or sulfite (after 24 hours of reaction between polysulfide and methyl iodide)

Results in Tables 4.6 and 4.7 confirm that both sulfite and thiosulfate interfere with analysis of polysulfide/dimethylpolysulfide, when present in high molar excess. DMDS is depleted to a lesser extent than the trisulfide, and overall, the results suggest that the process in eq 4.3 is the dominant process, and that the process involving attack by thiosulfate (eq 4.4) is less significant. Results in both Tables show clearly that sulfite is far more reactive as an interferant than thiosulfate. Thiosulfate does not affect DMPSs if present at one thousand-fold molar excess or less. The effects of sulfite at these levels can be minimised by adding an excess of methylating reagent.

It is unlikely that sulfite would occur in great excess of polysulfide, especially in environments where S^0 is high. Yao and Millero (1995) found that sulfite was an initial product in the oxidation of sulfide to produce elemental sulfur,

thiosulfate and sulfate in the presence of iron and manganese oxides. Sulfite was shortlived and transient in the reaction, and was probably involved in the oxidation of elemental sulfur to form thiosulfate. Luther et al. (1985) found that sulfite was present at concentrations one to two orders of magnitude less than the total S^0 concentration, and significantly less than polysulfide concentrations in salt marsh porewater samples.

Limitations of methylation of polysulfide due to hydrolysis of methyl iodide

The competing nucleophilic substitution (S_N2) reaction between methyl iodide and hydroxide ion (or water) places a practical limit on the minimum concentration of polysulfide which can be detected with the present method. Although significant reaction occurs between strong nucleophiles (e.g. polysulfides) and alkyl halides, as the concentration of the nucleophilic species decreases, the reaction rate eventually becomes so slow that the competing reaction between water and the alkyl halide becomes dominant. At very low concentrations of sulfur nucleophile, alkyl halide is hydrolysed to the alcohol prior to any significant reaction occurring.

A comprehensive discussion of the competitive effects of water on the extent of reaction between various sulfur nucleophiles and alkyl halides is given by Barbash and Reinhard (1989a). According to these authors, the following expression can be used to determine whether significant reaction will take place between a nucleophilic species and an alkyl halide in aqueous medium:

$$\log\left\{\frac{k_{Nu}}{k_{H2O}}\right\} > \log\left\{\frac{[H_2O]}{[Nu]}\right\} \quad \text{(Barbash and Reinhard 1989a)} \qquad ...[4.4]$$

where

k_{Nu} is the second order rate constant for the nucleophilic substitution reaction between the sulfur nucleophile and methyl iodide,

k_{H2O} is the hydrolysis rate constant for the alkyl halide substrate at a given pH, and

[Nu] is the concentration of the sulfur nucleophile.

Rate constants for the reaction of polysulfides with methyl iodide have not been determined, but can be estimated from comparison with kinetic parameters reported for other sulfur nucleophiles and alkylhalides. Using eq 4.4, together with data compiled by Barbash and Reinhard (1989a), it was calculated that the lowest concentration of thiosulfate ($S_2O_3^{2-}$) that will react with methyl iodide in aqueous solution is 0.2 to 2 μM; for sulfite ion (SO_3^{2-}) it is 4.3 μM; and for bisulfide ion (HS^-) it is 5.5 μM. The approximate order of reactivity of sulfur species in S_N2 reactions with alkyl halides in aqueous solution is given as

$S_5^{2-} \approx S_4^{2-} \approx$ n-hexylsulfide > n-butylsulfide > $HS^- \approx SO_3^{2-} \geq S_2O_3^{2-}$ > RSR >> H_2O
(Barbash and Reinhard, 1989a)

showing that polysulfides are substantially more reactive than other species tested. For the reaction with *n*-hexylbromide, the values of log ($\{k_{Nu}\}/\{k_{H2O}\}$) for tetrasulfide and pentasulfide were 1.5-2.0 units higher than those for bisulfide, thiosulfate and sulfite anion. Hence, in the reaction with alkyl halides, these polysulfide species are in the order of 100 times more reactive than the other sulfur nucleophiles listed above. Accordingly, it is estimated that significant reaction should occur between polysulfide and methyl iodide in water, even when polysulfide is present at the low nanomolar level (i.e. several hundred ng/L). This is in agreement with results in Table 4.4 which show that polysulfide sulfur can be quantitatively recovered as dimethylpolysulfide at concentrations as low as 4.6 nM (150 ng/L).

Potential for reaction of methyl iodide with organic sulfides, organic polysulfides and elemental sulfur

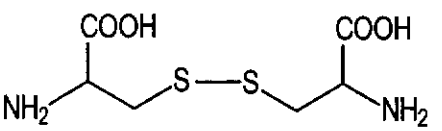
Under some conditions, thioethers are sufficiently nucleophilic to displace

halide ion from haloaliphatic substrates to form the corresponding alkylthioethers (Barbash and Reinhard 1989a). Dialkylsulfides, dialkyldisulfides and dialkylpolysulfides react with methyl iodide in polar solvents, such as dimethylformamide (DMF) at 70-75 °C to form methylated sulfur compounds (Schouten et al. 1993, Shaw 1989, Corey and Jautelat 1968) according to eq 4.6.

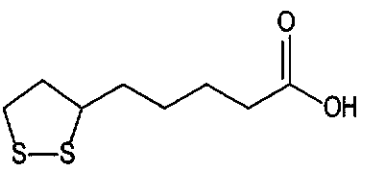
$$RS_nR' + CH_3I \xrightarrow[70\ ^\circ C]{DMF} R-\overset{CH_3}{\overset{|}{S_n^+}}-R'\ I^- \xrightarrow[70\ ^\circ C]{DMF} RS_nCH_3 + R'I \qquad \ldots[4.6]$$

The nucleophilic displacement reaction proceeds via the formation of a sulfonium ion intermediate, followed by cleavage of the C-S bond. The initial product of reaction with the organic polysulfide is RS_nCH_3 and, presumably after further reaction with methyl iodide, the dimethyl compounds, $CH_3S_nCH_3$ (n=1-3).

According to eq 4.6, organic polysulfides could conceivably contribute to the fraction of polysulfide measured by the present method, although the conditions required (70 °C) were far more severe than those used in the present procedure. In order to determine the potential for organosulfur contribution using the present method, aliquots of "blank" water were spiked with various organic disulfides and trisulfides and then subjected to the analytical procedure. The organic polysulfides tested included deuterated DMDS and DMTS, cystine or lipoic acid. The latter two substrates are representative of cyclic and linear functionalised organosulfur compounds commonly found in natural systems. Their structures are shown below:



Cystine



Lipoic acid

The experiments using the deuterated DMDS and DMTS were conducted to test the capacity of simple dialkylpolysulfides to undergo the reaction in eq 4.6 under mild conditions. Results of these experiments, listed in Table 4.8, show that cleavage of carbon-sulfur bonds by methyl iodide, to yield dimethylpolysulfides is insignificant (<0.01-0.2%) for the three organosulfur substrates investigated. Under the relatively mild conditions (dilute solution at 25°C), less than 1% reaction occurred in all cases. It is therefore unlikely that organic sulfur compounds contribute to the fraction of polysulfide that is detected by the present method.

Table 4.8: Concentrations of DMDS and DMTS formed from reaction of selected organic polysulfides with methyl iodide.

Compound(s) tested	pH	Concentration (μg/L – S)	DMDS (μg/L)	DMTS (μg/L)	Total polysulfide (μg/L -S)	Recovery polysulfide (%)
Lipoic acid	7.0	140	0.005	0.001	0.004	<0.01
Lipoic acid	(a)	140	0.024	0.021	0.032	0.02
Cystine	7.0	320	0.006	0.004	0.007	<0.01
Cystine	(a)	320	0.005	0.002	0.005	<0.01
DMDS/DMTS-d_6	(a)	5.8	0.015	0.003	0.012	0.22

Notes: (a) unbuffered, pH changed from neutral to acidic during the course of the reaction.

In light of the above results it was not expected that methyl iodide would react with elemental sulfur to form DMPSs. Two experiments were nevertheless conducted so that this could be confirmed. Samples of purified water and sulfidic groundwater were spiked with elemental sulfur (5 μg/L and 300 μg/L, respectively, as S_8 dissolved in a minimum volume of dichloromethane/methanol) before adding methyl iodide (0.05% v/v). DMPSs were not detected in the spiked sample of purified water and the amounts of DMPSs in the spiked sample of sulfidic groundwater were not significantly different to those in other samples of the same groundwater which had not been spiked. The results show that elemental sulfur does not interfere, even in the presence of sulfur nucleophiles such as sulfide.

Are the chain lengths of individual inorganic polysulfide homologues preserved upon reaction with methyl iodide?

While analytical methods that can quantitatively determine the amounts of individual inorganic polysulfide homologues in dilute aqueous samples have not been previously available, with the present new method, polysulfide homologues can be readily quantified after derivatisation with methyl iodide. In aqueous systems, inorganic polysulfides exist as equilibrium mixtures of di-anionic chains of sulfur atoms, with the number of sulfur atoms in the chains varying from 2-5 (see Section 2.2.2). As discussed in the present Chapter and in Section 2.2.2, several factors, including pH, temperature and the concentration of total sulfur species in solution affect the composition of these polysulfide mixtures (i.e. the relative concentrations of individual homologues). Literature reports on the composition of a polysulfide solution under a given set of conditions are not always in agreement, and consequently there is some uncertainty regarding the exact composition of these mixtures, even under well-controlled conditions. Therefore, it has not been possible to show conclusively that addition of methyl iodide to a solution of inorganic polysulfides does not alter the composition of these mixtures, and that the chain lengths of the individual homologues are preserved upon methylation.

However, many of the experiments in the present Chapter show that DMPSs are quite stable relative to inorganic polysulfides, and that once the polysulfides are methylated, these species no longer interact or redistribute. For example, no reactions have been observed between the perdeuterated DMPSs that are routinely added to water samples as internal standards (described in Section 4.3.2) and the isotopically unmodified DMPSs, showing that these species do not interact under typical sample conditions. In the present Section it was shown that sulfur nucleophiles such as sulfite can result in the redistribution of S^0, converting DMTS into DMDS (eq 4.3). The reaction mechanism that occurs in this process is similar to that involving the redistribution of inorganic polysulfides in equilibrium mixtures. The reactivity of sulfite with DMPSs was considerably inhibited by adding an excess of

methyl iodide, presumably because the nucleophilic sulfur atom of sulfite was methylated. Methyl iodide probably also quenches the reactivity of aqueous nucleophilic polysulfide anions in a similar manner, that is, via methylation of the nucleophilic terminal sulfur atom. Since the DMPSs formed are relatively stable, it is assumed that methyl iodide quenches redistribution between inorganic polysulfides and freezes the equilibrium, providing a snapshot of the mixture of inorganic polysulfide homologues that was present in the sample prior to methylation.

4.3.2 Analysis of dimethylpolysulfides (DMPSs)

Improvements in the isolation and preconcentration of DMPSs

In previous work Wajon et al. (1988) used closed loop stripping analysis (CLSA) to isolate and concentrate dimethylpolysulfides from water samples, but in the present study it was found that CLSA presented several practical difficulties. In the method described by Wajon et al. (1988) large sample sizes, of around one litre were required, making sampling and refrigerated sample storage cumbersome. The method was lengthy, requiring stripping times of ninety minutes. The technique for extracting analytes from Grob tubes was awkward since it relied on alternately placing a microtube containing solvent into the warm hands of the operator and then an ice-bath to draw the solvent through the Grob trap. Further, the CLSA method suffered from serious memory effects, especially when samples containing high concentrations of analyte were analysed prior to much "cleaner" ones, so it was often very difficult to obtain acceptable results for procedural "blank" analyses. Considerable efforts were therefore made to streamline and simplify the procedure, to enable faster sample throughput and to redress problems with "blanks" and memory effects.

As discussed in Section 4.1.2, problems with dirty blanks and memory effects using CLSA have been discussed by Boren et al. (1985), who remedied the problem by using an open stripping system, similar to purge and trap, instead of the closed loop system. These authors suggested that the problems were

caused, in part, by activated carbon particles becoming dislodged from the Grob trap and adsorbing to the inside surfaces of the equipment and partly by air leaking into the CLSA system. They reasoned that these problems would not exist in the case of an open system because any carbon particles would be swept downstream of the trap to vent to the atmosphere. Since the open system is under constant positive pressure, air is less likely to leak into the system than with the CLSA system, where areas of negative and positive pressure exist. In the present study an open system based on that described by Boren et al (1985) was used in preference to CLSA.

A disadvantage of the open stripping system over CLSA is that the range of compounds of different volatility that may be recovered in one analysis is lower, especially in the case of very volatile analytes, such as DMDS (b.p. 109 °C). Breakthrough of analytes, which can occur in an open system, is not a serious problem in CLSA because compounds which are desorbed from the carbon trap due to breakthrough are subsequently re-adsorbed. In the case of the open system, however, method parameters such as purge gas (i.e. stripping gas) flow-rate and purge time must be more carefully optimised to suit the required analytes. Purge gas volume must be sufficient to liberate analytes from the water, but should not exceed the breakthrough volume for the given analytes. Where it is necessary to analyse a number of compounds of different volatilities (i.e. different breakthrough volumes), such as DMPS homologues, these parameters may not be optimum for any one compound. In this case a low water sample volume (<100 mL) is advantageous because lower sample volumes require lower purge gas volumes to efficiently purge any given compound, but the breakthrough volume remains constant. Therefore, the lower the sample volume, the greater the range of volatility of analytes that can be efficiently recovered using purge and trap systems. With the availability of increasingly sensitive GC-MS systems it has been possible to decrease sample volume to 25 mL, while achieving analytical limits of detection well below the odour threshold concentration of DMTS (10 ng/L). The decrease in sample volume also made it possible to shorten the purge time from 90 minutes to 30 minutes, achieving a considerable increase in sample throughput.

The analytical system used to isolate and pre-concentrate DMPS homologues from aqueous samples is based on a commercially available Tekmar LSC 2000 purge and trap which was modified to accommodate a Grob tube containing activated carbon adsorbent. The modification, shown in Figure 4.3, allowed the configuration of the instrument to be switched easily and rapidly from the conventional purge-and-trap thermal desorption mode to the new "Grob-tube solvent desorption" mode, and vice-versa. The advantage of using the Tekmar instrument is that experimental parameters such as temperatures of heated zones, purge time and heating times are all controlled by a microprocessor. The entire purge and trap procedure proceeds automatically after push-button activation of the appropriate method by the operator. The Tekmar instrument gives precise, automated control over heating zones, purge gas flow rates and times, and allows method parameters to be stored in memory. Partial automation of the extraction procedure thus adds convenience, and reduces the potential for operator error.

Details of experimental conditions (listed in Table 4.1) are described briefly below. In a typical extraction procedure, the water sample (5-25 mL) was spiked with an internal standard mixture (DMPS-d_6 in methanol), preheated for 3 minutes at 50°C, then purged with nitrogen at a flow rate of 100 mL/min for 30 minutes. After a 2 minute dry-purge step, where the purge gas by-passed the sample, the Grob tube was removed from the apparatus and the analytes were eluted from the carbon using 4 x 8 μL aliquots of dichloromethane. A normalisation standard (*n*-chlorohexadecane, 20 ng) was added to the combined extract, which was then analysed by GC-MS.

Analysis of DMPSs by gas chromatography-mass spectrometry (GC-MS)

As discussed in Section 4.1.2, dialkylpolysulfides, especially the higher homologues, undergo disproportionation at relatively low temperatures. Wajon et al. (1985) showed that to avoid disproportionation of DMTS and higher dimethylpolysulfides, it was necessary to use slow column heating rates and to avoid contact between the sample and reactive heated zones in

the chromatographic system. Disproportionation of DMPeS to elemental sulfur and the lower dimethylpolysulfide homologues occurred if this compound eluted from the column at a temperature of 127 °C or higher. The GC conditions used by Wajon et al. (1985) were designed to minimise chromatographic run-time, while ensuring that DMPeS eluted at 115°C, and were as follows: Initial temperature of 30 °C (1 minute), followed by a temperature increase of 4 °C/minute to 90 °C, then to 130 °C at 1 °C/minute and then to 280 °C at 10 °C/minute. In the present study sample extracts were introduced onto the GC column via a cool on-column injector, as described by these latter authors. For the analysis of mixtures containing DMTeS and DMPeS, the oven temperature program was similar to that described above, except that the initial temperature used was 35 °C and the temperature program rate of 1 °C/minute was extended from 130 °C to 136 °C. In the case of drinking water samples, where DMTeS and DMPeS were never detected, it was possible to use a uniform oven temperature program rate of 5 °C/minute from 35 °C to 300 °C, thereby decreasing analysis time. Full details of chromatographic conditions used for these analyses are listed in Table 4.3.

Quantification of analytes using deuterated internal standards: Precision and accuracy of analytical procedure

Perdeuterated dimethylpolysulfide homologues were used as internal standards to assist in quantification of compounds. In the case of drinking water samples, where the higher DMPSs were always absent, a mixture containing only perdeuterated DMDS and DMTS was used. In cases where samples contained DMTeS and DMPeS, such as in groundwater samples, a mixture of all perdeuterated homologues from DMDS to DMPeS was used. Using the oven temperature programmes detailed in Tables 4.3 it was possible to obtain baseline resolution between DMPS homologues and their deuterated analogues, as shown in Figures 4.7 (a) and (b).

The use of internal standards and a normalisation standard served as a useful guide to ensure that the extraction system and chromatographic

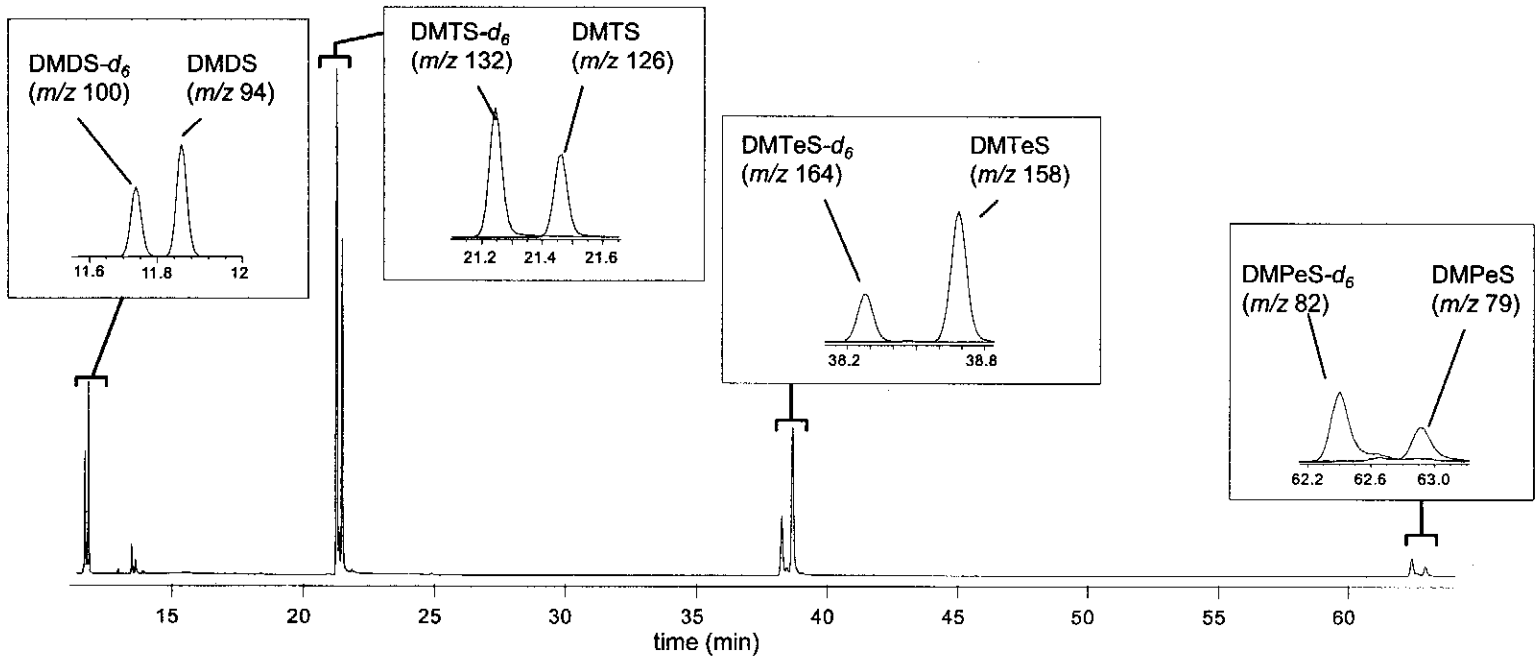


Figure 4.7 (a): Chromatogram (GC-MS in SIM mode) of extract of sulfidic groundwater (bore W60) after treatment with methyl iodide. Insets show analyte peaks and their corresponding deuterated analogues, added as internal standards.

GC conditions: Initial temperature 35 °C; 5 minute hold time; temperature rate 4 °C/minute to 90 °C, then 1 °C/minute to 136 °C, then 20 °C/minute; final temperature 300 °C; final hold time 10 minutes.

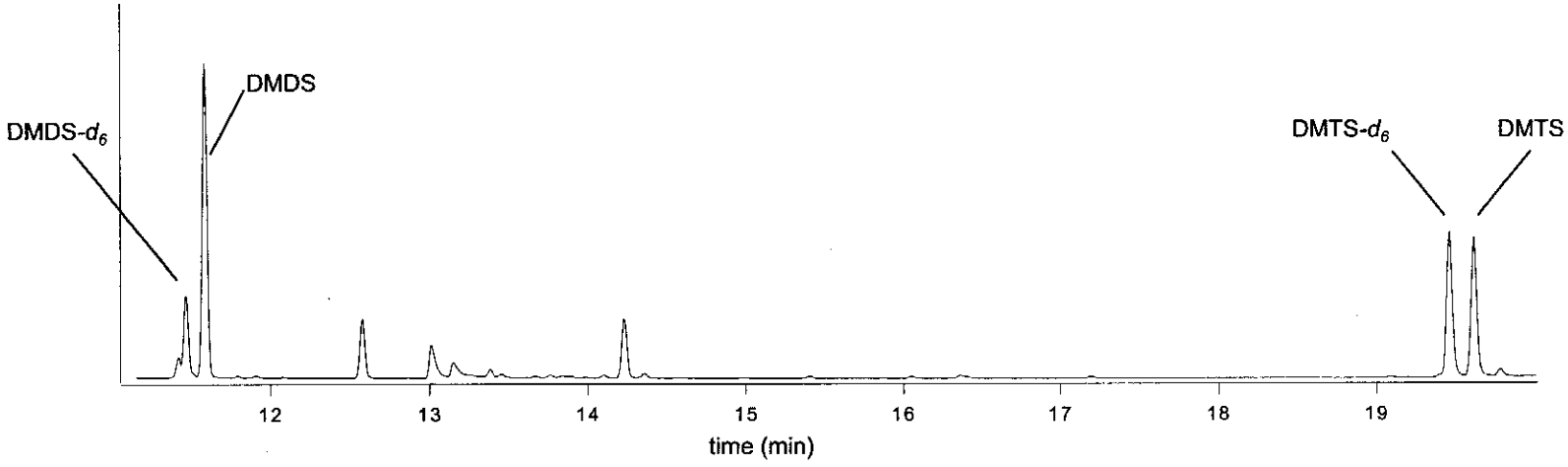


Figure 4.7 (b): Chromatogram (GC-MS in SIM mode) of DMDS and DMTS and the corresponding deuterated analogues, added as internal standards.

GC conditions : Initial temperature 35 °C; 5 minute hold time; temperature rate 5 °C/minute; final temperature 300 °C; final hold time 10 minutes.

systems continued to perform efficiently and reproducibly. The appropriate mixture of internal standards was added to every sample prior to extraction, and the concentration of analytes was calculated on the basis of their recovery, as described in eq 4.7. The absolute recovery of each internal standard was determined by adding a normalisation standard, *n*-chlorohexadecane after extraction of the sample and prior to analysis by GC-MS. Control charts plotting the recovery of internal standards were constructed and used routinely to monitor the performance of the analytical procedure. Examples of recoveries of deuterated DMDS and DMTS over a period of eleven months, representing 98 analyses are shown in Figures 4.8 (a) and (b). Recoveries of DMDS-d_6 were slightly higher and more reproducible than those for DMTS-d_6 (average recovery = 87.7 and 79.2%; standard deviation = 14.5 and 15.1% for DMDS and DMTS respectively).

Equation 4.7: Calculation of analyte concentration using internal standards.

Amount of analyte in unknown sample (ng) =

$$\frac{\left(\frac{\text{peak area analyte}}{\text{peak area deuterated standard}}\right)_{\text{unknown}} \times \text{[concentration of analyte in standard (S)]}}{\left(\frac{\text{peak area analyte}}{\text{peak area deuterated standard}}\right)_{\text{Standard of known concentration (S)}}}$$

Note: (S) = standard containing a known amount of DMDS and DMTS

It should be noted that quantitative determination of thermally labile and reactive compounds such as dimethylpolysulfide homologues at nanogram-per-litre concentrations presents a considerable analytical challenge. Scrupulous maintenance of GC-MS instrumentation, especially injector systems and capillary columns was required to obtain consistently acceptable resolution and maximum sensitivity. Peak tailing and poor

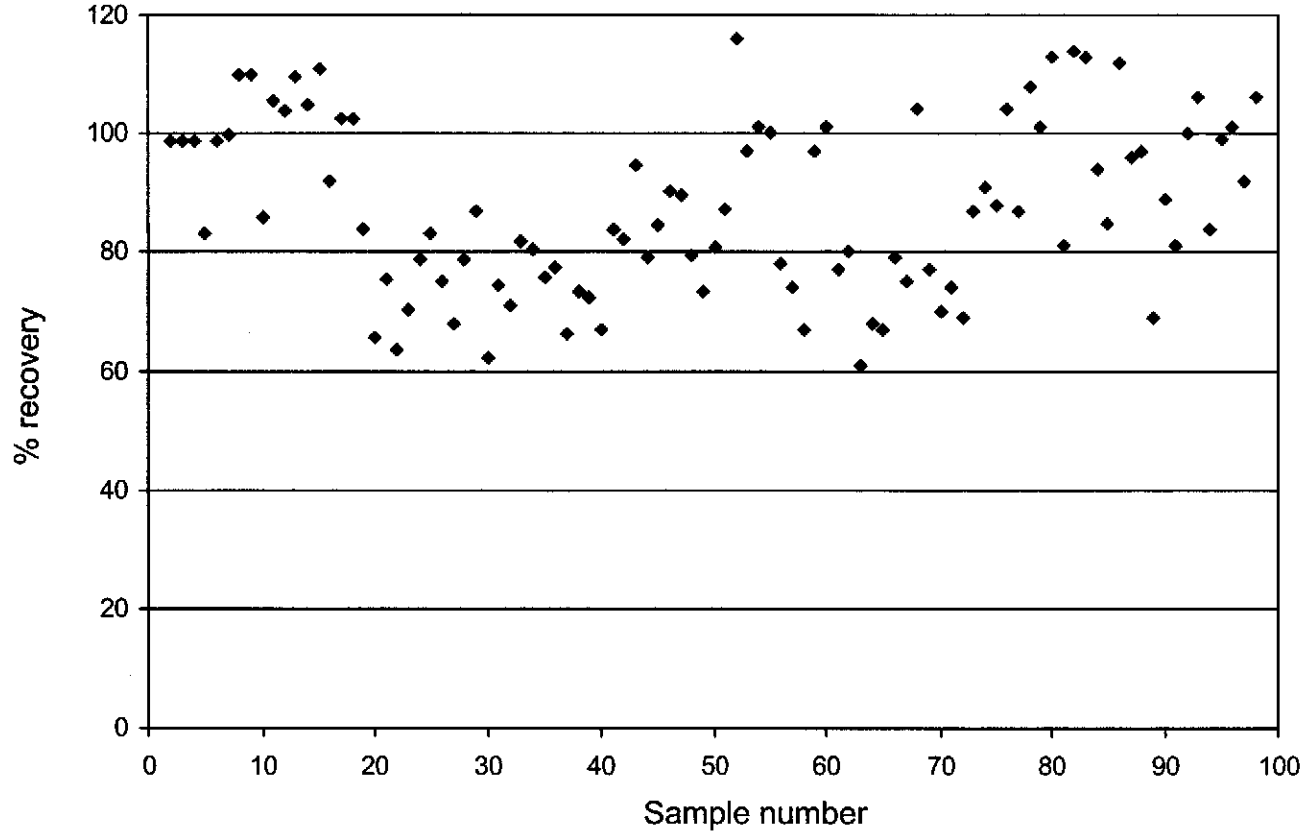


Figure 4.8 (a): Percent recovery DMDS-d_6 for 98 samples analysed over a period of 11 months. Average = 87.7, standard deviation = 14.5.

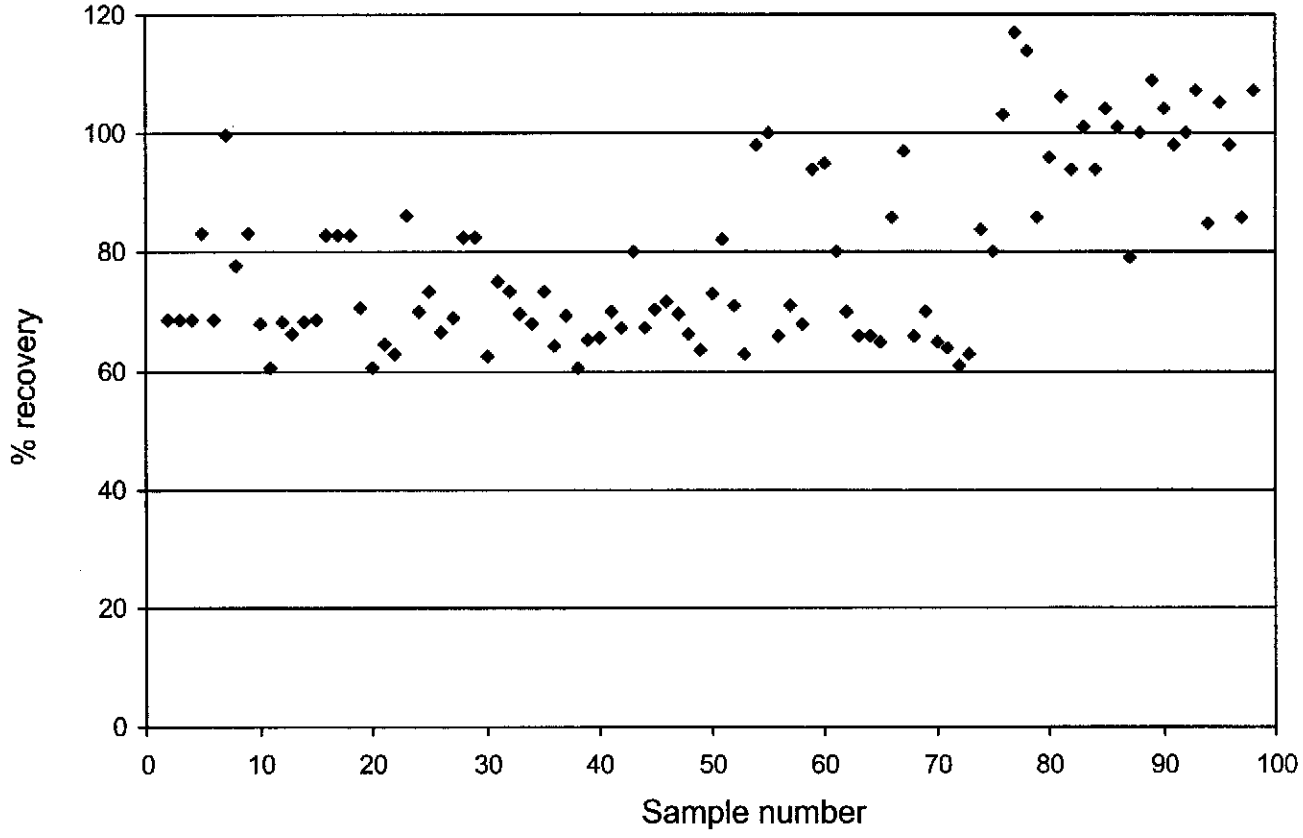


Figure 4.8 (b): Percent recovery DMTS-d_6 for 98 samples analysed over a period of 11 months. Average = 79.2, standard deviation = 15.1.

resolution most commonly occurred when chromatographic performance was compromised by a dirty, or active injector system (Figure 4.9 (a) and 4.10 (a)). In the author's experience problems were most often due to cored septa and/or pieces of septum lodged in the injector and/or pre-column (retention gap).

A particular advantage of using deuterated analogues of analytes as quantification standards is that potential effects of poor chromatography on quantification are usually not as serious as when the internal standards used are chemically and physically different to analytes. Although every effort was made to maintain excellent chromatographic performance, changes occurring after only a few samples can affect quantification when analytes are chemically different to quantification standards. When using deuterated analogues of analytes, both the quantification standard and the analyte are affected in identical ways and poor chromatography does not affect quantification as seriously. This is demonstrated by the chromatograms in Figures 4.9 (a) and (b) and 4.10 (a) and (b) where results using deuterated internal standards are compared with those obtained using chlorooctane as an internal standard. Mixtures of chloroalkanes were used as internal standards in previous studies (Wajon et al., 1986 and 1988). In the present study, mixtures of DMTS and an internal standard were analysed when chromatographic performance was poor as shown in Figures 4.9 (a) and 4.10 (a). The same mixtures were re-analysed after remediation of the problem, as shown in chromatograms in Figures 4.9 (b) and 4.10 (b). The solutions analysed in Figure 4.9 (a) and (b) contained chlorooctane as the internal standard, while in those in Figures 4.10 (a) and (b), DMTS-d_6 was used as the internal standard. The results represented in Figures 4.9 (a) and 4.10 (a) were treated as the "known" standard and concentrations of DMTS in samples in Figures 4.9 (b) and 4.10 (b) were calculated as described in eq 4.7. This resulted in a discrepancy of 19% in the case of chlorooctane, compared with only 4 % where DMTS-d_6 was used. In the case where DMTS-d_6 was used, chromatographic performance was considerably worse than where chlorooctane was used, but surprisingly, the effect on the final analytical result (i.e. DMTS concentration) was far less severe. This is

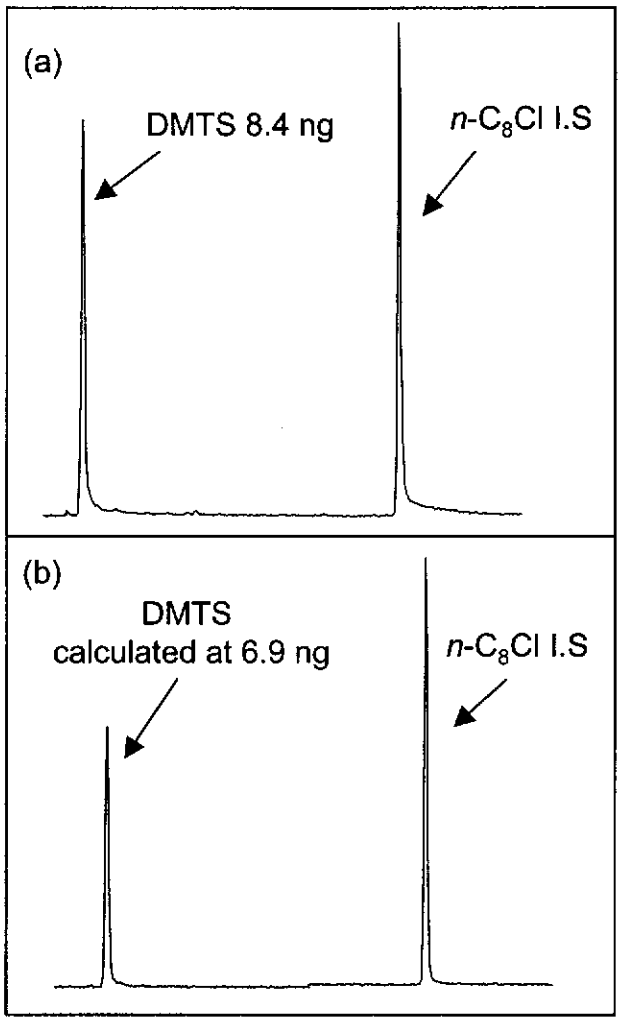


Figure 4.9: (a) DMTS (8.4 ng) and *n*-chlorooctane internal standard (I.S) analysed using poorly maintained GC;

(b) the same sample re-analysed after correcting the chromatography problems. Calculation of the concentration of DMTS in (b), based on (a) resulted in a 19% error.

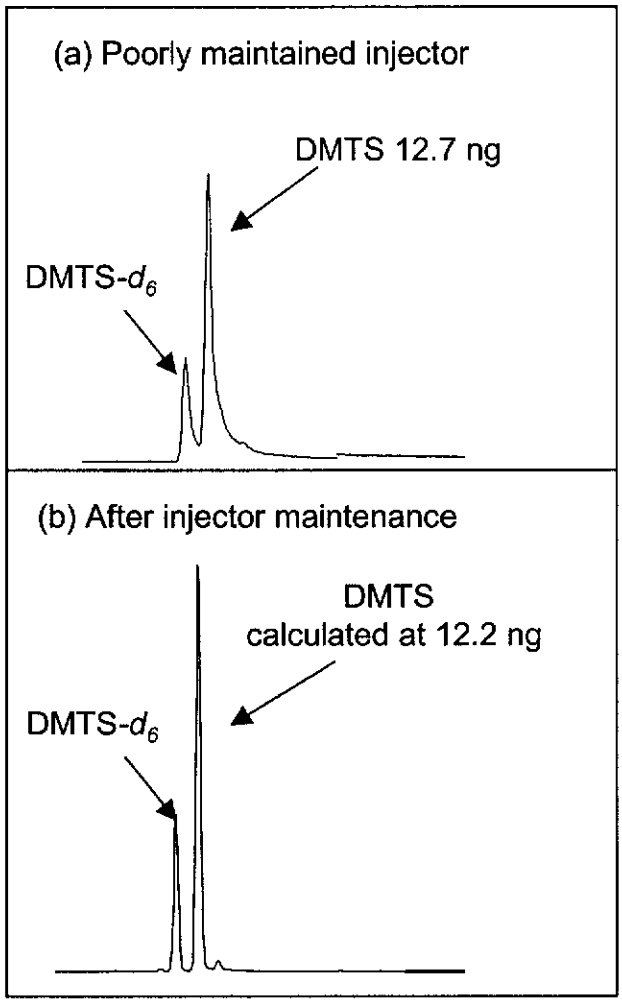


Figure 4.10: (a) DMTS (12.7 ng) and DMTS-d_6 internal standard run using poorly maintained GC;

(b) the same sample re-analysed after correcting the chromatography problems. Calculation of the concentration of DMTS in (b), based on (a) resulted in a 3.9% error.

Note that quantification was carried out using a different ion for each compound: for DMTS the ion used was *m/z* 126, and for DMTS-d_6 the ion was *m/z* 132.

because the perdeuterated standards have very similar physicochemical properties to the analytes, and the chromatographic behaviour of the standards is therefore very similar to that of the analytes. In contrast, chloroalkanes have quite different physicochemical properties to DMPSs, and their chromatographic behaviour would therefore be quite different to that of the analytes, thus accounting for the relatively large errors in quantification. It should be noted that similar results were obtained in the case of DMDS (i.e. that quantification using DMDS-d_6 as an internal standard provided more accurate results than when chlorooctane was used), and that similar observations were made on several different occasions on different sets of samples (i.e. the examples shown in Figures 4.9 and 4.10 were not unrepresentative). These observations show that the use of quantification standards that have similar physicochemical properties to the analyte is particularly important in the case of functionalised compounds such as these sulfur species, which are sensitive to active sites in the chromatographic system and changes in chromatographic conditions.

As shown in Figures 4.8 (a) and (b), recoveries of both DMDS and DMTS were often less than 65% or greater than 105%. In many cases this was due to poor chromatographic performance, as discussed above, rather than to problems with the purge and trap extraction procedure. Since the chemical structure and nature of DMDS-d_6 and DMTS-d_6 (internal standards) and *n*-chlorohexadecane (normalisation standard) are quite different, the chromatographic behaviour of these two compound types also differs considerably. It should be noted that since only the internal standards, and not the normalisation standard were used to calculate analyte concentration, poor recoveries as shown in Figures 4.8 (a) and (b) were not necessarily an indication of poor analytical outcomes.

Quantification of dimethyltetrasulfide (DMTeS) and dimethylpentasulfide (DMPeS)

In almost all of the treated drinking water and pipewall biofilm samples analysed in the present study, DMDS and DMTS were the major homologues

and DMTeS and DMPeS were either not detected, or did not appear to contribute significantly to total dimethylpolysulfides. However, methylation of inorganic polysulfides in pipewall biofilm, reservoir sediment and sulfidic groundwater samples almost always produced what appeared to be small quantities DMTeS, and occasionally some DMPeS was detected. DMTeS and DMPeS were not available as quantification standards in pure form, although mixtures of DMPSs containing homologues with 2-4 sulfur atoms are readily prepared (Korchevin 1989 et al.; Milligan et al., 1963). Separation of the higher homologues from these mixtures is time-consuming and requires specialised vacuum distillation equipment because of their thermal instability. Since these higher homologues appeared to be absent in most of the samples of interest, it was considered beyond the scope of the present study to prepare them in pure form. However in order to ensure that their concentrations in water samples had not been grossly underestimated, their recoveries and GC-MS response factors were investigated.

Molar response ratios for dimethylpolysulfide homologues were determined by analysing mixtures containing known concentrations of DMDS, DMTS and a normalisation standard (1,2,4,5-tetramethylbenzene (TeMB))) using a flame ionisation detector (FID). The FID response is determined by the combustion of carbon in air, and is proportional to the amount of hydrocarbon material passing through it. Since all DMPS homologues differ only by the number of sulfur atoms and all contain the same number of methyl groups, the FID molar response should be very similar for each homologue. This principle was used to estimate molar response ratios in a mixture of DMPS homologues prepared as described by Korchevin et al. (1989). These authors used various ratios of reactants to obtain mixtures containing dimethylpolysulfide homologues with average chain lengths from 1.9 to 3.4 sulfur atoms. The latter mixture reportedly contained 15% DMTeS, and therefore should also contain an appreciable quantity of DMPeS, although this was not stated by the authors. A mixture using the prescribed quantities of reactants to achieve the maximum average sulfur chain length was prepared and analysed by GC-FID and GC-MS after addition of a normalisation standard and appropriate dilution. Separate solutions

containing known concentrations of DMDS and DMTS and TeMB were prepared from pure standards and these were similarly analysed. Analyses were carried out in duplicate or triplicate. Molar response ratios of DMDS and DMTS *vs* TeMB were then calculated as described in eq 4.8.

$$\text{Molar response ratio} = \frac{\text{response (i.e. peak area) per mole of analyte}}{\text{response per mole of normalisation standard (TeMB)}} \quad \ldots[4.8]$$

Molar response ratios for DMDS and DMTS obtained using GC-FID are listed in Table 4.9. As predicted, the values are very similar for both homologues and it is therefore assumed that their average (0.21) would be a valid approximation for the response ratios of DMTeS and DMPeS. This value was used to calculate the concentrations of all four homologues in a mixture prepared as described above. The calculated concentrations of DMPS homologues and percentage recoveries of total DMPSs are shown in Table 4.10.

Table 4.9: Molar response ratios for DMDS and DMTS *vs* TeMB analysed in triplicate.

Triplicate #	Spiked concentration (ng/μL)			Molar response ratio	
	DMDS	DMTS	TeMB	DMDS	DMTS
1	274	332	208	0.223	0.224
2	274	332	208	0.209	0.197
3	274	332	208	0.217	0.210
Average				0.216	0.212

Calculation of the percentage recovery of total DMPSs showed that around 10% of the material was not accounted for. Analysis by GC-MS of the mixture of DMPSs, at a slow GC temperature program rate (1°C/minute from 35 to 300 °C) revealed that it contained a small amount of residual elemental sulfur (Figure 4.11(a)). This was probably an impurity from the synthetic procedure and would contribute to the mass of mixture analysed as "DMPS

homologues", partly accounting for the discrepancy of 10% in Table 4.10.

Table 4.10: Calculated concentrations of DMPS homologues in a mixture containing DMDS, DMTS, DMTeS and DMPeS. Only the total mass of the mixture is known. Response ratios in Table 4.9 were used to calculate concentrations of each homologue and the sum of these was then used to calculate the total percent recovery.

Concentration mixture analysed (ng/μL)	Calculated concentration (ng/μL)				Sum of DMPS homologues (ng/μL)	Recovery of total DMPSs (%)
	DMDS	DMTS	DMTeS	DMPeS		
1160	88	422	323	171	1004	86.5
1160	92	433	327	179	1031	88.9
922	73	364	272	147	857	93.0
922	74	347	259	139	819	88.8
Average						89.3

Close examination of the chromatogram of the DMPS mixture (Figure 4.11(b)) also showed that DMPeS did not elute as a Gaussian peak, as it should under the conditions employed (Wajon et al., 1985). The unusual peak shape is probably caused by partial disproportionation of DMPeS, as discussed by Wajon et al. (1985). When the sample was analysed using a slow heating rate (1°C/minute from 35 to 300 °C) the peak shape improved, becoming almost Gaussian, as shown in Figure 4.11(a). The slow temperature program was 260 minutes in duration, making analysis cumbersome, and this was considered impractical for routine analyses. Although partial disproportionation of DMPeS occurred, resulting in underestimation of concentrations of this compound, the faster temperature program rate in Table 4.3 was chosen as a compromise. Given the small number of samples found to contain DMPeS in the present study, this was not considered a serious problem. Thus, disproportionation of DMPeS and the elemental sulfur impurity in the standard mixture accounted for a major part of the 10 % discrepancy of DMPS homologues revealed in Table 4.10.

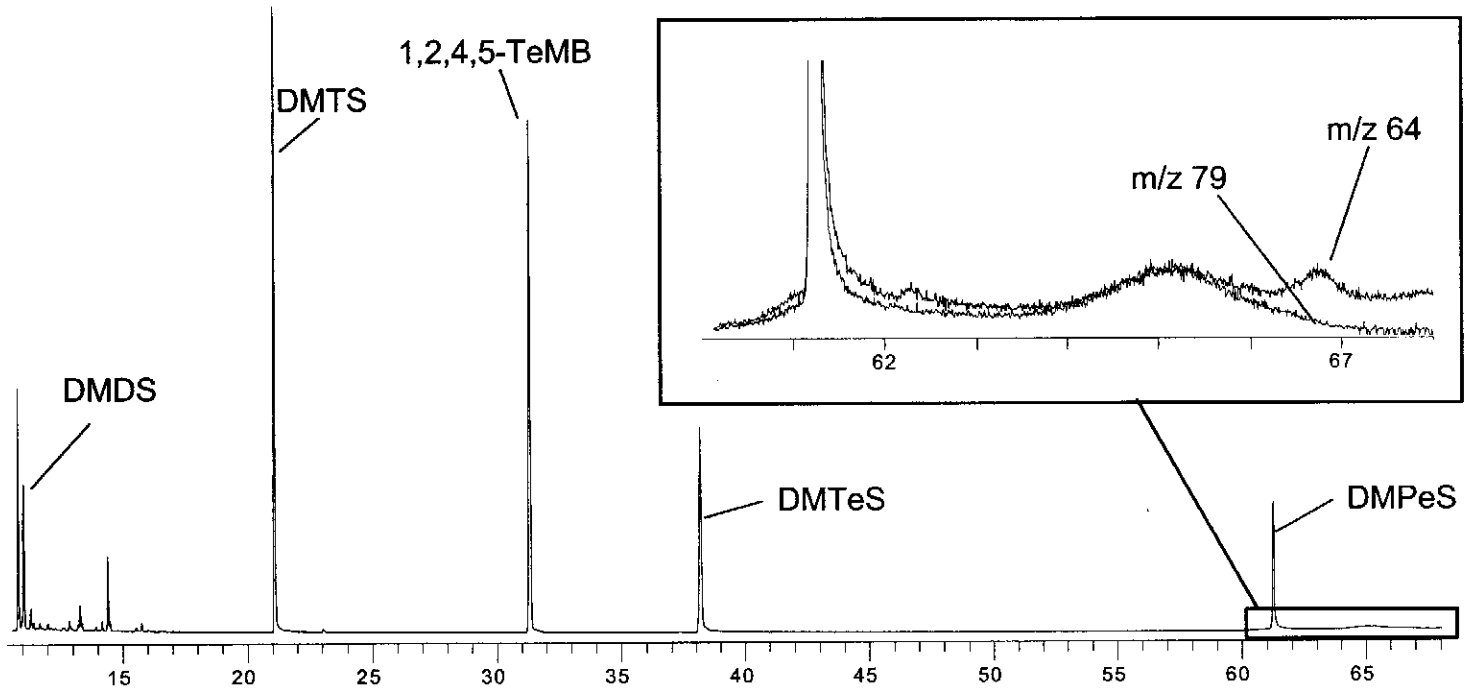


Figure 4.11 (a): Chromatogram (GC-MS, full scan) of mixture of DMPS standards and 1,2,4,5-TeMB using slow temperature programme. Inset shows extracted ions (*m/z* 79, diagnostic for DMPeS, and *m/z* 64, diagnostic for elemental sulfur) eluting after DMPeS. The "humps" are probably due to partial disproportionation of DMPeS.

GC oven temperature program: Initial temperature 35 °C; 5 minute hold time; temperature rate 4 °C/minute to 90 °C, then 1 °C/minute to 136 °C, then 20 °C/minute; final temperature 300 °C; final hold time 10 minutes.

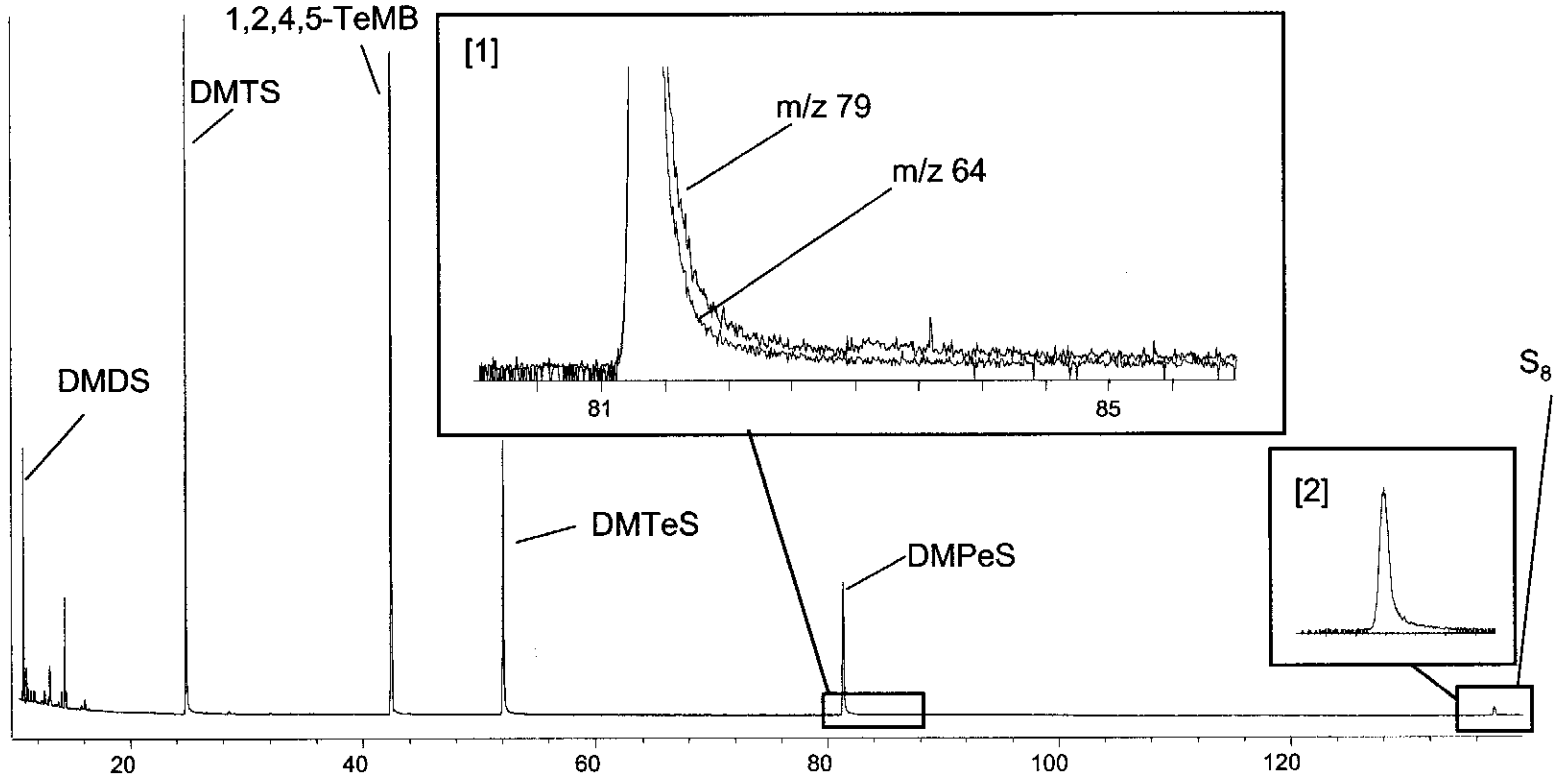


Figure 4.11(b): Chromatogram (GC-MS, full scan) of mixture of DMPS standards and 1,2,4,5-TeMB using fast temperature programme. Inset [1] shows extracted ions (*m/z* 79, diagnostic for DMPeS, and *m/z* 64, diagnostic for elemental sulfur) eluting after DMPeS. Inset [2] shows extracted ion (m/z 64) for elemental sulfur peak.

GC oven temperature program: Initial temperature 35 °C; 5 minute hold time; temperature rate 1 °C/minute; final temperature 300 °C; final hold time 10 minutes.

Recovery of DMTeS and DMPeS from water samples

Concentrations of DMTeS and DMPeS in water samples were calculated in the same way as those for DMDS and DMTS as described in eq 4.7. A mixture of deuterated DMPS homologues was added to the sample prior to extraction by purge and trap. The normalisation standard, *n*-chlorohexadecane was added to the sample extract just prior to analysis by GC-MS. From the ratio of deuterated standard *vs* normalisation standard it was possible to calculate the recovery of the deuterated homologue. It was assumed that the recovery of the deuterated analogue was representative of that of the native compound. Recoveries of deuterated DMPS homologues (DMDS to DMPeS) from ten multiplicate analyses of a groundwater sample (after addition of methyl iodide) are listed in Table 4.11.

Table 4.11: Recovery of DMPS-d_6 homologues (155 ng DMPS-d_6) from ten multiplicates of a groundwater sample (W60 Wanneroo borefield).

Sample #	% Recovery			
	DMDS-d_6	DMTS-d_6	DMTeS-d_6	DMPeS-d_6
1	65	102	85	27
2	83	87	108	45
3	89	82	104	51
4	88	103	103	55
5	44	74	90	46
6	60	87	80	42
7	87	93	98	66
8	100	93	94	62
9	67	78	72	38
10	101	98	104	86
Average	78	90	94	52
Rel. standard deviation (%)	19	10	12	17

Average recoveries for the three lower homologues were comparable to those of DMDS-d_6 and DMTS-d_6 in drinking water samples (Figures 4.8 (a) and (b)), but that for DMPeS was significantly lower. Measurements for DMDS and DMPeS were not as reproducible as those for DMTS and DMTeS as indicated by the higher standard deviations. In the case of DMPeS this

may have been partly due to the problem of disproportionation during analysis by GC, as mentioned in the previous section.

Linearity of DMDS and DMTS analyses

The linearity of the present analytical method for DMDS and DMTS was tested by analysing aliquots of blank water (5 mL) spiked with these compounds at concentrations from 0.9 to 267 ng. The concentration of the internal standard mixture (DMDS-d_6 and DMTS-d_6) was constant (29 ng) for each analyte concentration tested. The response (peak area analyte/peak area internal standard) for each concentration was plotted against concentration as shown in Figures 4.12 (a) and (b). Good linearity was achieved for both DMDS and DMTS within the concentration ranges tested, as demonstrated by correlation coefficients of 0.999 and 0.998 respectively.

Limits of detection (LOD) and limits of quantitation (LOQ) for DMDS and DMTS

Swartz and Krull (1997) defined the limit of detection (LOD) as the lowest concentration of an analyte in a sample which can be detected. It is usually calculated as the concentration equivalent to three times the standard deviation of the signal-to-noise ratio (LOD = 3.3 x (SD/S), where SD is the standard deviation and S is the slope of the calibration curve at low concentration. Limit of quantitation (LOQ) is defined as the lowest concentration of an analyte which can be quantified. It is calculated as the concentration equivalent to ten times the standard deviation of the signal-to-noise ratio (LOQ = 10 x (SD/S)) (Swartz and Krull (1997)).
Limits of detection and quantitation for the analysis of DMDS and DMTS using the new method were calculated according to these definitions. Five blank determinations were carried out and peak areas of ten "noise" peaks eluting where the analyte peak would normally elute were determined. Each of the 50 "noise" peak areas was then divided by the peak area of the internal standard for that sample. The standard deviation of these 50 quotients was determined (SD) and this value was then divided by the slope of the

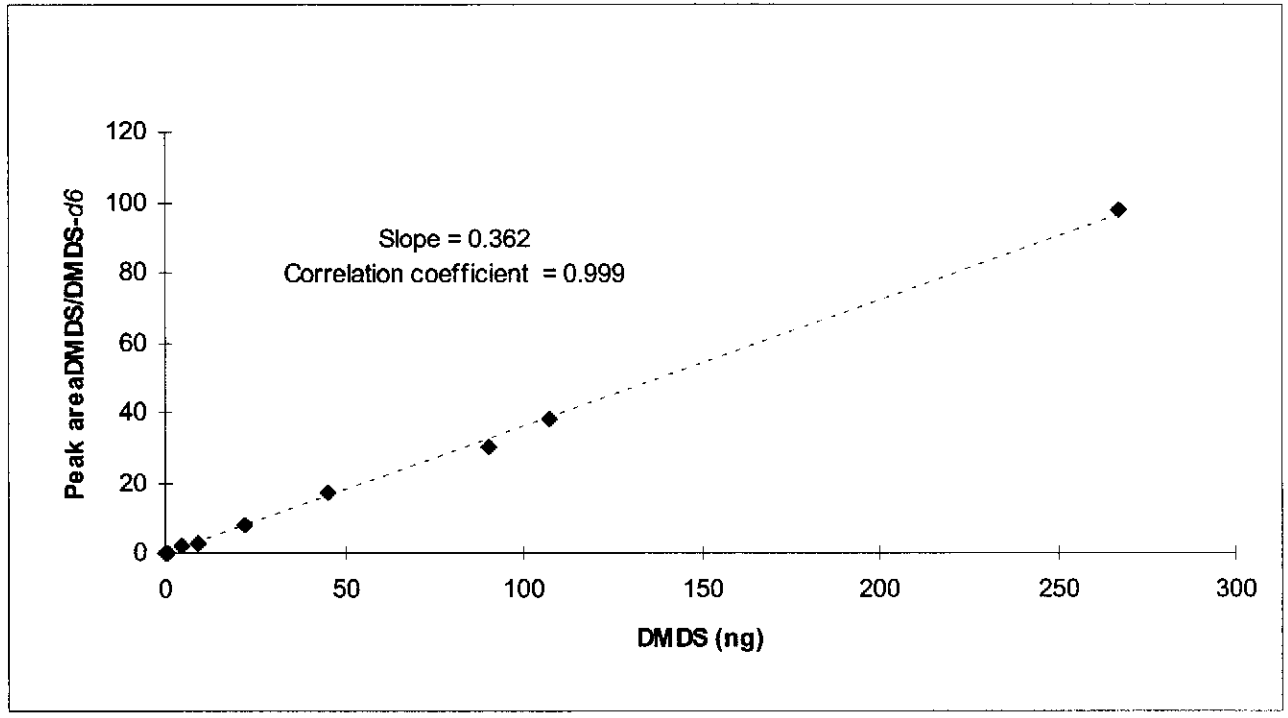


Figure 4.12(a): Calibration graph of DMDS showing peak areas of DMDS/DMDS-d_6 recovered from water versus spiked concentration of DMDS.

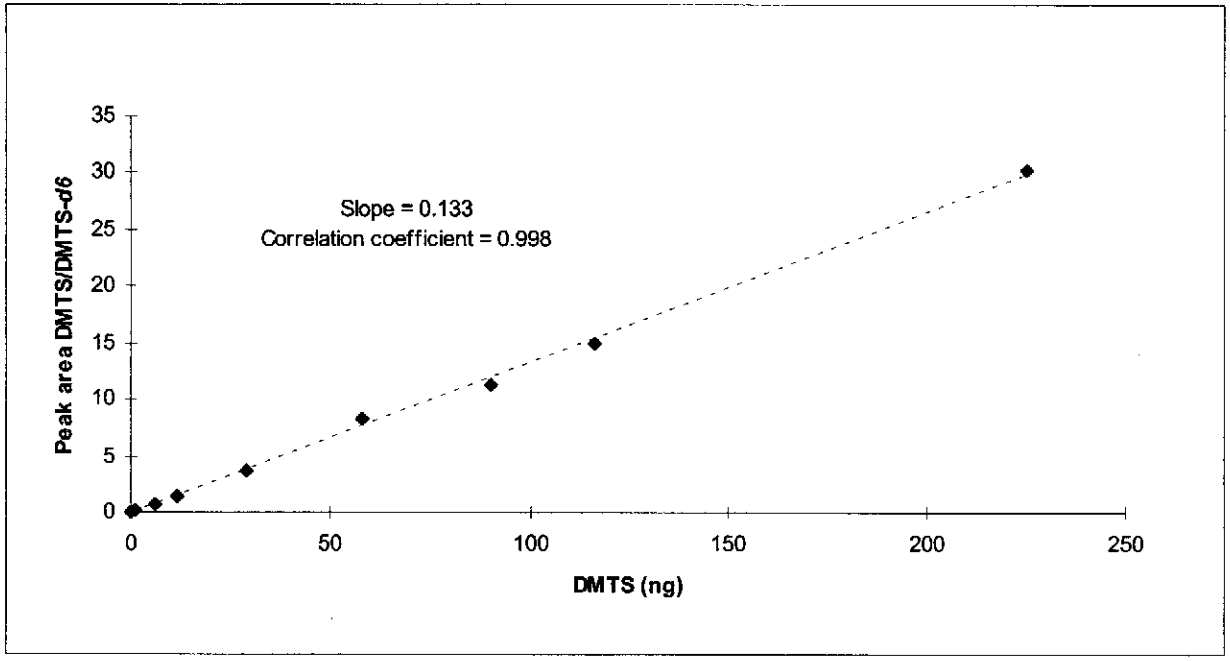


Figure 4.12(b): Calibration graph of DMTS showing peak areas of DMTS/DMTS-d_6 recovered from water versus spiked concentration of DMTS.

calibration curve, as given in Figures 4.12 (a) and (b). Values for SD and LODs and LOQs for DMDS and DMTS obtained in this way are listed in Table 4.12.

The LODs for DMDS and DMTS are 2 pg and 9 pg respectively, and these are equivalent to 0.5 ng/L and 1.8 ng/L respectively for a 5 mL water sample. LOQs are 1.4 ng/L and 5.4 ng/L respectively for a 5 mL water sample. These values are sufficiently low to detect the presence of DMTS well below its odour threshold concentration of 10 ng/L.

Table 4.12: Limits of detection (LOD) and limits of quantitation (LOQ) for DMDS and DMTS and statistical parameters used to calculate these values.

Statistical parameter	DMDS	DMTS
Standard deviation (SD) (n = 50)	0.00025	0.00036
SD/slope calib. curve	0.00070	0.0027
LOD (ng)	0.0023	0.0089
LOQ (ng)	0.0070	0.027
LOD of a 5 mL sample (ng/L)	0.5	1.8
LOQ of a 5 mL sample (ng/L)	1.4	5.4

Limits of detection and quantitation for DMTeS and DMPeS

Comparison of peak areas of DMPS homologues analysed by GC-FID and GC-MS show that the responses of these compounds relative to each other are similar for both detectors. This is shown in Figures 4.13 (a) and (b), where a chromatogram of a DMPS mixture obtained by FID is compared with the same mixture analysed by GC-MS. In the case of the mass chromatogram (Figure 4.13 (b)), only the response obtained for the quantification ions (listed in Table 4.13) is shown. In order to compare the sensitivities of the two detectors, peak areas of DMTS, DMTeS and DMPeS were divided by the peak area of DMDS (i.e. normalised against DMDS) as shown in Table 4.13.

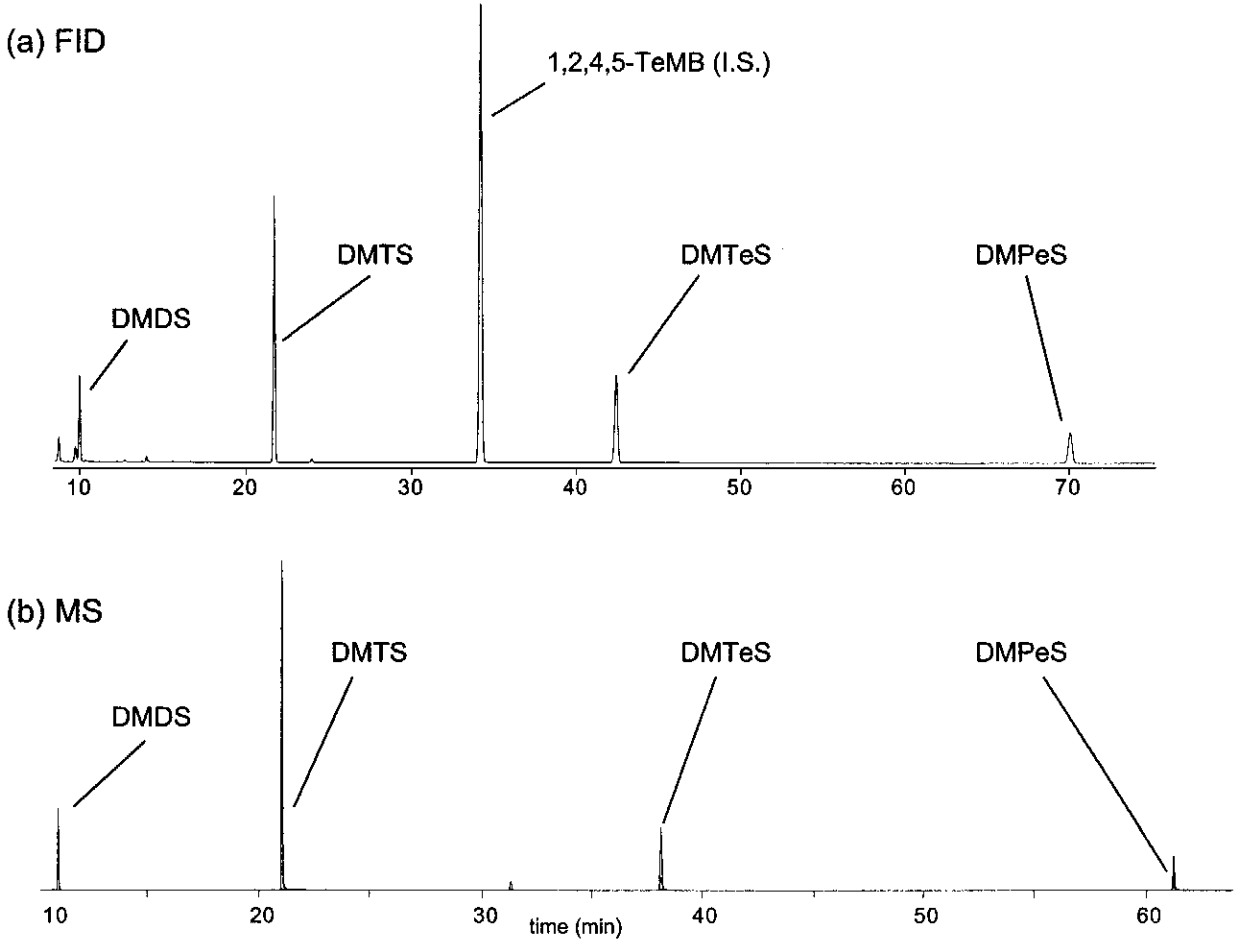


Figure 4.13: Chromatograms of DMPS mixture (~1000 ng) obtained using (a) FID and (b) MS in SIM mode (partial ion chromatogram shows the response obtained for *m/z* 79, 94 and 126. See Table 4.13).(I.S. = internal standard).

Table 4.13: Comparison of response of FID and MS for DMPS homologues. Peak areas for DMTS, DMTeS and DMPeS are divided by the peak area for DMDS (i.e. normalised against DMDS) for each detector.

	DMPS homologue (peak area homologue/peak area DMDS)			
	DMDS	DMTS	DMTeS	DMPeS
FID	1	3.6	2.2	0.96
MS	1	4.5	1.5	0.60
Diagnostic ion[(a)]	94	126	79	79

[(a)]Diagnostic ion used for quantification in mass spectrometry SIM mode

Normalised against DMDS, the response for DMTS is slightly higher when MS is used than when FID is used (4.5 and 3.6 respectively). However, the responses for DMTeS and DMPeS using MS are about two thirds of those obtained using FID. Therefore, for the present method, using GC-MS, the limits of detection and quantitation for DMTeS and DMPeS should be slightly higher than those of DMDS and DMTS (about 1.5-fold). Also, since the recovery of DMPeS from water is only about half of that obtained for the other homologues the practical LOD and LOQ for this compound would be more than twice that of DMTS. On the basis of these arguments the LODs for DMTeS and DMPeS using a 5 mL sample are estimated at 3 - 6 ng/L and the LOQ's at 10 - 20 ng/L. The lack of pure calibration standards for these compounds presented some difficulty in demonstrating that accurate quantitation is possible using the present method; however, the above studies show that good estimates of their concentrations can be made using the response factors obtained for DMDS and DMTS. The work demonstrates that their concentrations are not grossly underestimated due to low recovery from water or low analytical sensitivity.

Sampling of sulfidic groundwaters

Sampling of anoxic groundwaters such as those that exist on the Gnangara Mound presents some difficulties when the parameters to be measured are

sensitive to exposure to the atmosphere. Sulfide and polysulfides are easily oxidised, and their equilibrium distributions are affected by pH changes which may occur upon exposure to the atmosphere. Methylation decreases the sensitivity of polysulfides to oxidation, and it is advantageous if this is carried out as soon as possible after sampling. In order to minimise these effects, groundwater samples were taken by attaching a glass Luer-lock syringe fitted with a Teflon plunger (10 – 100 mL) to the sampling point. The syringe was flushed with sample, then filled to exclude headspace. Methyl iodide (0.5% v/v) was then added to the sample via a microsyringe. The syringe containing the sample was then sealed using a custom made Leur-lock cap and transported on ice to the laboratory. The syringe with Luer-lock fitting allowed direct transfer of the sample to the purge and trap instrument. In this way sampling, derivatisation and transfer of the sample to the extraction system occurred with minimal exposure to the atmosphere and presumably, minimal disruption of analytes.

Sample storage: Stability of DMPS homologues

The stability of DMDS and DMTS in water samples was tested by spiking a sample of water taken from Wanneroo reservoir and a sample of "blank " (Milli-Q) water with these compounds. Their concentrations were monitored over a period of several days.

Results listed in Table 4.14 show that DMTS spiked into water from Wanneroo resevoir appeared to be shortlived in comparison to the same compound in "blank" (Milli-Q) water. The Wanneroo reservoir sample was stored at room temperature in an amber glass Winchester (2.5 L), with headspace ranging from 700 mL to 2000 mL. Losses of DMTS may have been caused by a number of processes, including evaporation into the headspace or during subsampling, adsorption onto the walls of the container, or chemical degradation such as oxidation and disproportionation.

Table 4.14: Stability of DMDS and DMTS spiked into water samples.

Sample	Sample storage time	Compound analysed		% recovery	
		DMDS	DMTS	DMDS	DMTS
		(ng/L)	(ng/L)		
Wanneroo res[a]	1 hour	n.d.	140	n.d.	63
"	72 hours	n.d.	64	n.d.	29
"	7 days	n.d.	22	n.d.	10
Blank (Milli-Q) water [b]	1 hour	79	105	88	91
	24 hours	81	117	90	101
	48 hours	75	95	83	82
	72 hours	86	75	96	64

Notes: (a) Water from Wanneroo reservoir spiked with 220 ng/L DMTS
(b) Blank water spiked with DMDS (90 ng/L) and DMTS (116 ng/L)
n.d. = not determined

Free chlorine, which rapidly oxidises DMTS (discussed in Chapter 3), was not detected in the water prior to spiking with DMTS, but very low levels might have been present, since Wanneroo water is extensively chlorinated. This might have contributed to the observed losses of DMTS in the samples from Wanneroo reservoir.

Subsequent tests on samples of "blank" water were conducted with the aim of preventing loss of analytes due to the above factors. Samples were prepared separately in glass syringes to avoid losses due to subsampling and excessive headspace and these were stored at 4 °C to minimise chemical processes. As shown in Table 4.14, recoveries up to 48 hours after preparation of the sample were within experimental error. The present work shows that samples should be stored in containers without headspace and refrigerated and analysed as soon as possible after sampling to avoid losses of analytes.

4.4 CONCLUSIONS

A novel analytical procedure for determination of inorganic polysulfides in aqueous samples is described. The procedure, first used in a

semiquantitative manner (Wajon and Heitz, 1995) is based on direct in-situ methylation of polysulfides, followed by analysis of the resulting DMPS homologues by GC-MS. In the present work the applicability of the procedure to the analysis of polysulfides in drinking water and groundwater, and its accuracy, reproducibility and potential interferences were investigated. It was demonstrated that the procedure was quantitative and linear for inorganic polysulfides from 0.15 up to 370 μg/L. Difficulties in preparing polysulfide standard solutions due to instability of the compounds in the presence of even small amounts of oxygen precluded testing the method at lower concentrations.

The method described is relatively robust, simple and free from interferences, despite the sensitivity of polysulfides to oxidation and pH changes. Once methylated, polysulfides are more stable and less susceptible to air oxidation. Potential interferences due to pH change can be avoided by ensuring that samples are treated with methyl iodide as soon as possible after sampling. Although the equilibria determining polysulfide chain length and polysulfide-elemental sulfur speciation are pH dependent, it appeared that pH effects due to methyl iodide hydrolysis were minimal. It was found that sulfoxy-anions, particularly sulfite reacted with DMPSs, but that potential interferences from these compounds can be minimised by ensuring that the quantity of methyl iodide added is in excess of the total molar equivalent of sulfur nucleophile species. The method was tested for interferences from organic polysulfides, and from the limited tests carried out it appears that these are negligible.

Considerable modifications were made to procedures for the extraction from water and pre-concentration of DMPSs, as used by previous workers (Wajon et al, 1988). The new procedure was based on purge and trap, rather than CLSA, as used previously (Wajon et al, 1988), enabling partial automation by utilising a commercially available purge and trap instrument. The combination of semi-automation and decreased sample size, coupled with changes to the procedure used to extract the Grob tube resulted in a method that was far less cumbersome than that previously described. Deuterated analogues of

the analytes were synthesized and used as internal standards, resulting in more robust quantification which was less susceptible to poor chromatography. These changes resulted in a streamlined method, suitable for rapid, routine analysis of large numbers of samples by relatively inexperienced operators.

Several deficiencies in the present analytical procedures remain unresolved; remediation of these problems may improve and widen the applicability of the method. The following further work is suggested;

- Improve chromatographic analysis of DMPeS to avoid disproportionation of this compound, in a practical analysis time.

- Since inorganic polysulfides are analysed as DMPSs, the concentration of any DMPSs in the sample prior to methylation must be accounted for. This may be problematic and cumbersome in cases where the concentrations of DMPSs are high relative to inorganic polysulfides. An alternative procedure is to use ^{13}C-methyl iodide as the methylating agent. This would not interfere with the use of deuterated internal standards, providing adequate chromatographic resolution of the three analogues can be achieved.

It should be noted that interactions between reduced sulfur species in aqueous solution, particularly the processes involving inorganic polysulfides, are extremely complex and poorly understood. Measurement of any individual reduced sulfur species requires a thorough understanding of the entire system, including all equilibrium processes involving the species under examination. To this end, as many as possible of the reduced sulfur species that may be present should be analysed, including those shown in Figures 2.2 (a) and (b). While many uncertainties and ambiguities still exist in the investigation of trace concentrations of inorganic polysulfides in the environment, the present method serves to extend the tools available in the study of such systems.

CHAPTER FIVE

TRANSFORMATIONS OF REDUCED SULFUR COMPOUNDS DURING WATER TREATMENT AND DISTRIBUTION

ABSTRACT

It has previously been proposed that sulfur-containing precursors in source waters might lead to DMTS formation in the Wanneroo distribution system (Wajon and Wilmot, 1992; Wilmot and Burkett, 1992). Wilmot and Burkett (1992) measured total reduced sulfur compounds and non-sulfide reduced sulfur compounds (NSRS) in raw, treated and distributed water from Perth's groundwater schemes and found that a small residual of incompletely oxidised sulfur originating from the sulfidic groundwater entered the distribution systems. They proposed that components of this sulfur fraction, which they termed NSRS, might act as precursors to DMTS in distributed water. However, since NSRS is a bulk measure of reduced sulfur and does not give information on the nature of individual species, the identities of any of these potential DMTS-precursor compounds remained unknown. A primary aim of the present study was to determine the most likely components of NSRS entering the distribution system and thus, to examine the hypothesis that one or more key components of this fraction could act as direct precursors to DMTS. As discussed in Chapter 2, the oxidation of sulfide, and the oxidation of sulfide oxidation products (such as polysulfides and thiosulfate), have been the subject of many previous studies. Largely on the basis of these studies, it was concluded that of all of the possible sulfur species that can contribute to NSRS, iron sulfides, some types of refractory organosulfur compounds (OSC) and elemental sulfur are the most resistant to oxidation, and that the latter two of these would constitute the bulk of the residual incompletely oxidised species entering the distribution system. It was concluded that small amounts of DMPSs (20-80 ng/L) that occasionally formed when methyl iodide was added to water samples probably arose from traces of biofilm or sediment that may have entered the water during sampling.

In a previous study, Wajon and Heitz (1995) observed that DMTS appeared to form in some water samples during storage of the sample. This was further investigated in the present study. Significant increases in DMTS in samples during storage were measured on two occasions and it was concluded that these occurred via microbial activity that could occur in the samples during storage.

The primary method of control of DMTS formation in the distribution system has been to maintain free chlorine residuals. However, the mechanisms by which this occurs have not been studied; the effectiveness of DMTS oxidation by chlorine, or how chlorine affects microbial processes that might form DMTS is not known. These issues are addressed in the final section of the present chapter. Experiments to determine the effectiveness of oxidation of DMDS and DMTS (5 μg/L) by free chlorine (0.2 to 0.6 mg/L) in distributed water showed that these substances are rapidly and completely oxidised in water containing a chlorine residual of more than 0.4 mg/L. However, regeneration of traces of DMDS and DMTS after free chlorine had dissipated to non-detectable levels suggested that these compounds were incompletely oxidised at the lower chlorine concentrations. It was shown that disproportion and recombination of the oxidation products of DMDS and DMTS might lead to regeneration of small amounts of these compounds.

5.1 INTRODUCTION

In previous studies it was suggested that DMTS formed in the distribution system via chemical (abiotic) reactions of residual reduced sulfur compounds originating from sulfidic groundwater, rather than via microbial activity (Wajon and Wilmot, 1992; Wilmot and Burkett, 1992). Wajon et al. (1988) found that while the capability to produce DMTS was widespread in bacteria, it depended largely on the availability of an appropriate sulfur substrate. Further, bacteria that could produce DMTS were not confined to DMTS-prone areas, but occurred

throughout the distribution system. These authors therefore concluded that the crucial factor in whether DMTS formed was the presence of a key sulfur-containing precursor compound, rather than the presence of specific bacteria. It was proposed that DMTS formation might occur through methylation of residual polysulfides or other reduced sulfur compounds originating from groundwater (Wajon and Wilmot, 1992; Wilmot and Wajon 1997; Wilmot and Burkett, 1992, p.64). The latter authors showed that a small proportion of sulfide in the groundwater was not completely oxidised to sulfate during the water treatment process and proposed that this residual reduced sulfur fraction, which they referred to as non-sulfide reduced sulfur (NSRS) could contain DMTS precursor compounds.

Studies of NSRS in the various water types in Perth's distribution systems showed that while concentrations varied considerably, they did not appear to be linked to DMTS problems (Wilmot and Burkett ,1992). The history of swampy odour complaints and concentrations of NSRS and DMTS in four major Perth water sources are summarised in Table 5.1.

Table 5.1: History of swampy odour complaints, concentrations of non-sulfide-reduced sulfur (NSRS) and dimethyltrisulfide (DMTS) from four major Perth water sources.

Water source	History of swampy odour complaints	DMTS (ng/L)	NSRS (µg/L) (Wilmot and Burkett, 1992)
Surface water	*No history of complaints.	<5.	3-10
Wanneroo GWTP	Numerous complaints, intermittent outbreaks	<1 to 900	39-43
Mirrabooka GWTP	*No history of complaints.	<1 to 17	45-59
Jandakot GWTP	Very few complaints	<1 to 130	92

Note: *No history of complaints when only water from the usual source is present, but numerous complaints have occurred when water was mixed with a high proportion (>40%) of water from Wanneroo GWTP.

As shown in Table 5.1, water from groundwater sources, most particularly from Wanneroo GWTP, is strongly associated with DMTS formation; DMTS was rarely detected in water from other GWTPs (Mirrabooka and Jandakot) and has never been detected in distributed surface water. NSRS concentrations in treated groundwaters are higher than those in surface waters, but do not appear to be directly related to DMTS, as evidenced by treated waters from Jandakot and Mirrabooka GWTPs having higher NSRS values but being less prone to DMTS formation than Wanneroo. Water from surface water sources, which is not sulfidic and not prone to DMTS formation, has the lowest NSRS levels.

Analysis of sulfide and NSRS

NSRS comprises sulfur compounds with oxidation states between sulfide (-2) and sulfate (+6), and as discussed in Chapter 2, this incorporates an extensive range of possible organic and inorganic sulfur compounds. The method for analysis of NSRS, as described by Wilmot and Burkett (1992) and Wilmot and Wajon (1997), is based on the alkaline reduction of sulfur compounds (other than sulfate) to sulfide using Raney nickel. Briefly, the acidified (0.05 M HCl) water sample is first purged with nitrogen to expel sulfide, which is then trapped in a zinc acetate solution and determined colorimetrically. Raney alloy is then added to the sample to generate Raney nickel *in-situ* by refluxing in sodium hydroxide (24%) under nitrogen. Sulfur compounds are reduced to sulfide and the hydrogen sulfide generated upon subsequent acidification is swept into a zinc acetate solution in a stream of nitrogen and analysed colorimetrically, as for the original sulfide. It was established that, using this procedure, it was possible to efficiently recover a wide range of organic and inorganic sulfur compounds, including thiosulfate, tetrathionate, elemental sulfur, sulfur amino acids, allylthiourea, sulfanilamide and thiocyanate. (It is not known whether iron sulfides, which might exist as fine particulates in groundwaters, are determined using the method.) However, since the method is non-specific for this compound group, the identities of individual sulfur compounds analysed as NSRS in Perth's

water supply remains unknown. Given the potential importance of these compounds in the swampy odour problem, the discussions and investigations in the present Chapter aim to address this issue.

Wajon and Heitz (1995) found that DMTS was formed when methyl iodide was added to untreated and treated water from Wanneroo and Mirrabooka GWTPs. Methyl iodide reacts with inorganic polysulfides to form DMPSs (Korchevin et al., 1989; Birch et al., 1953) and this reaction formed the basis of a new analytical technique used in a semiquantitative manner by Wajon and Heitz (1995) to determine the presence of polysulfides in raw and treated waters. The potential for these compounds to act as precursors to DMTS in distributed waters was proposed by several authors (Wilmot and Burkett, 1992; Wajon and Heitz, 1995). In the study by Wajon and Heitz (1995) significant concentrations of DMTS were formed in Wanneroo and Mirrabooka raw waters upon the addition of methyl iodide (29 μg/L and 12 μg/L, respectively). Amounts of DMTS formed in treated and distributed waters were much lower, generally less than about 150 ng/L, but one sample, from Wanneroo filter outlet, contained 320 ng/L DMTS after methyl iodide addition. In the present study these observations were investigated further; concentrations of reduced sulfur compounds and DOC were determined in raw water supplying Wanneroo GWTP and samples of Wanneroo treated water (filter outlet), clarifier sludge and distributed water were analysed for the presence of inorganic polysulfides. It should be noted that the analyses for inorganic polysulfides discussed in the present chapter and in the study by Wajon and Heitz (1995) were carried out prior to the method development described in Chapter 4 and were therefore semi-quantitative only.

5.2 EXPERIMENTAL

5.2.1 Materials

Blank water, reagents and glassware were prepared as described in Section 4.2.1.

Buffer (pH 6.86, 0.4 M) was prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4; 6.804 g; BDH, Analar) and disodium hydrogen phosphate (Na_2HPO_4; 7.050 g; BDH, Analar) in Milli-Q water (250 mL).

Polysulfide solution was prepared according to the method of Borchardt and Easty (1984). Sodium sulfide ($Na_2S.9H_2O$, 15 g) was dissolved in Milli-Q water (~70 mL) that had been heated and de-aerated by sparging with nitrogen (high purity). Elemental sulfur (0.6 g) was added to the stirred sodium sulfide solution at 75 °C under nitrogen. Polysulfide formation was indicated by the appearance of a yellow colour. After all the elemental sulfur had dissolved, the solution was cooled and diluted to 100 mL with de-aerated Milli-Q water. This stock solution was stored in tightly capped glass vials (~3 mL volume) without headspace at 4 °C. Each vial was discarded after one use only, since polysulfide deteriorated rapidly after exposure to air, as indicated by the change in solution colour from yellow to clear. In a test of polysulfide stability an aliquot of 100 μL was added to Milli-Q water (2.5 L) to give a solution containing approximately 1 mg/L sulfur.

Quantification standards

A mixture of chloroalkanes was prepared by dissolving *n*-chlorooctane, *n*-chlorodecane, *n*-chlorotetradecane, and *n*-chloro-octadecane (Merck-Schuchardt, AR grade, 100 mg each compound) in hexane (100 mL). A serial dilution resulted in a mixture containing approximately 10 ng/μL of each

compound in methanol, which was used as an internal standard mixture. Similarly, *n*-chlorohexadecane (Merck-Schuchardt, AR grade, 100 mg) was dissolved in hexane (100 mL) and diluted further to give a solution containing 10 ng/μL *n*-$C_{16}Cl$ in hexane. This was used as a normalisation standard.

DMDS was purchased from Aldrich and stored in flame-sealed glass ampoules after the supplied ampoule had been opened, and DMTS was synthesized according to the method of Milligan et al. (1963) as described in Section 4.2.2.

5.2.2 Analyses

Analysis of sulfide and non-sulfide reduced sulfur (NSRS)

Sulfide and NSRS were analysed at Water Corporation (by Water Corporation personnel) using methodology developed by Wilmot and Burkett (1992) and later published by Wajon and Wilmot (1997).

Reactions of methylmethanethiolsulfonate (MMTS)

Methylmethanethiolsulfonate (purity >98.0%, MMTS, $CH_3SSO_2CH_3$) was purchased from Fluka and used without further preparation. In a test to determine the concentrations of DMDS and DMTS formed upon disproportionation of MMTS, the neat compound (3 μL) was added to Milli-Q water (10 mL) or phosphate buffer (pH 6.8; 0.05 M; 10 mL). The pH of the unbuffered solution measured with a pH meter and found to be 4.3. The solutions were allowed to react for 24 hours before analysis for DMDS and DMTS using the procedure described in Chapter 4 (Section 4.2.3).

Analysis of inorganic polysulfides

One of the studies described in the present chapter (Section 5.3.1) was carried

out prior to the method development and validation described in Chapter 4, and in this cases the analytical techniques used for analysis of inorganic polysulfides and DMDS and DMTS were slightly different to those previously discussed. These methods are described below.

Water was sampled in amber glass Winchesters (2.5L), and while no special efforts were made to prevent exposure to air, aeration was minimized by avoiding turbulence and by excluding headspace. Methyl iodide (typically 100 μL) was added to the sample (2.5 L) immediately upon arrival in the laboratory; the time between sampling and addition of methyl iodide was less than six hours. Samples were analysed for DMDS and DMTS, using the procedure described below, 24 hours after addition of methyl iodide. An identical sample, to which methyl iodide had not been added, was also analysed for DMDS and DMTS.

Analysis of DMDS and DMTS

Water samples discussed in Section 5.3.1 were analysed using a closed loop stripping procedure (CLSA) similar to that described by Grob and Zürcher (1976). Modifications to the procedure included replacing stainless steel filter holders and stainless steel tubing with materials made of PTFE. Sodium sulphate (50g) was added to the water sample (1L) to increase stripping efficiency. Chloroalkane internal standards (20 ng of each compound) were added to the sample. Stripping was carried out for 90 minutes at 35 °C and organic compounds were eluted from the carbon filter using 4 x 10 μL aliquots of dichloromethane. An external standard (*n*-chlorohexadecane, 20 ng) was added to the extract prior to analysis. Extracts were analysed by gas chromatography-mass spectrometry (GC-MS) as outlined in Table 5.2.

Water samples discussed in Sections 5.3.2. and 5.3.3 were analysed using the purge and trap and GC-MS procedures described in Section 4.2.3.

Gas chromatography-mass spectrometry (GC-MS)

Analyses by GC-MS for samples discussed in Section 5.3.1 were carried out using equipment and conditions listed in Table 5.2.

Table 5.2: Instrumental conditions for analysis of DMDS and DMTS by GC-MS.

GC	
Instrument make and model	Hewlett Packard 5890
Injector	HP cool on-column
Injector temperature	35 °C
Sample volume injected	1 μL
Column make and phase	J&W DB 5 or DB1
Column phase thickness	0.4 μm
Column length	40 m
Column internal diameter	0.18 mm
Carrier gas	Helium
Carrier gas flow velocity	24.0 cm/sec
GC oven temperature program	
Initial temperature	35 °C
Initial hold time	5 minutes
Rate of temperature increase	5 °C/minute
Final temperature	300 °C
Final hold time	10 minutes
MS	
Make and model	Hewlett Packard 5971 MSD
Operating conditions	70 eV, electron impact
Operating mode	Full-scan or selected ion monitoring (SIM)

Reaction of DMTS with chlorine

A water sample (2.5 L) was taken from Wanneroo Reservoir Outlet and stored in an amber glass Winchester for several days, allowing chlorine to dissipate before being divided into subsamples in 100 mL glass syringes. Glass syringes were used so that subsamples of 25 mL each could be analysed over a time course of several days without exposing the sample to air or allowing volatile sample components to evaporate.

A working aqueous solution of chlorine was prepared by diluting chlorine solution (4 mL) which had contained 12.5% chlorine when purchased to 500 mL with Milli-Q water. Aliquots of the working solution of chlorine were titrated with standard thiosulfate and it was found that the solution contained 776 mg/L chlorine.

Working solutions of DMDS (21.8 ng/μL) and DMTS (23.2 ng/μL) were prepared by serial dilution of the pure materials with methanol. Subsamples of water prepared as above (100 mL) were each spiked with DMDS and DMTS working solution (10 μL), resulting in a concentration of approximately 2180 ng/L of DMDS and 2320 ng/L DMTS in each of the subsamples. One of the spiked subsamples was left untreated as a control, and the remaining three were dosed with chlorine ranging from 0.19 to 0.59 mg/L. Aliquots of the spiked subsamples were analysed on day 0, day 6 and day 9 of the trial. Aliquots (25 mL) were analysed for DMDS and DMTS using the method described in Section 4.2.3.

Concentrations of free chlorine were determined using a Palintest 5000 photometer and DPD tablets. Tests were carried out as described in the Palintest instruction leaflet. All concentrations of chlorine reported are based on those determined by this method, and not on the amounts originally added. This was necessary because the water under test contained significant chlorine demand.

5.3 RESULTS AND DISCUSSION

5.3.1 Fate of sulfide and NSRS during water treatment and distribution

In an extensive study of reduced sulfur compounds in raw, treated and distributed water, Wilmot and Burkett (1992) measured concentrations of sulfide and NSRS at various stages of treatment at Wanneroo GWTP. Sulfide levels in raw water entering the GWTP varied from 170-1160 μg/L. These decreased to less than 200 μg/L after aeration and to levels below the analytical limit of detection (1μg/L) after chlorination. NSRS in raw water entering the treatment plant varied from 59-500 μg/L and according to Wilmot and Burkett (1992), most of this fraction of reduced sulfur was removed during clarification (48% on average). However, since some of the NSRS in post-aeration water was presumably derived from oxidation of sulfide in the raw water, it was difficult to accurately determine how much of the NSRS originally present in raw water was removed at each treatment stage. Overall, the groundwater treatment process removed 80-90% of NSRS and a much larger fraction of the total reduced sulfur entering the GWTP. The study showed that the major processes effecting removal of reduced sulfur compounds from groundwater were oxidation by aeration and chlorination and physical adsorption via clarification.

Water leaving Wanneroo GWTP contained a small amount of residual NSRS (36-43 μg/L) which continued to decrease marginally after detention in Wanneroo reservoir (24-31μg/L). No further decrease in NSRS was observed in the distribution system, even though water was rechlorinated at the reservoir outlet. NSRS levels appeared to be fairly constant from the reservoir outlet to the end of the distribution system, and according to Wilmot and Burkett (1992, p. 21), this residual NSRS fraction was refractory and could not be oxidised further. These authors did not discuss the possibility of NSRS formation within the distribution system.

The nature of residual NSRS in the distribution system is unknown, but was of interest in previous studies because of the possibility that it could be a source of sulfur for DMTS formation. It is presumed that all of the reduced sulfur entering the distribution system was originally present as sulfide in sediments of the Swan Coastal Plain. Some of this sulfide would have undergone transformations of the types discussed in Chapter 2, to form OSC, iron sulfides and partially oxidised inorganic sulfur compounds such as polysulfides, elemental sulfur, thiosulfate and polythionates. All of these could potentially contribute to NSRS.

During the water treatment process most of these components would be transformed and/or removed through oxidation and/or clarification. The oxidation products of sulfide and polysulfides during aeration and chlorination have not been analysed, but they are probably largely sulfate and elemental sulfur, with smaller quantities of sulfoxyanions. Water is aerated prior to chlorination at Wanneroo, so the initial oxidation products would be those that arise from reaction of oxygen with sulfide. These are elemental sulfur and sulfoxyanions such as thiosulfate and sulfite (as discussed in Chapter 2). Similarly, oxygen oxidation of polysulfides forms thiosulfate and elemental sulfur (Steudel et al., 1986). Tetrathionate and other polythionates may also be formed. Thiosulfate, tetrathionate and sulfite react rapidly in the presence of chlorine and oxygen to form sulfate (Kolthoff et al, 1969 p. 821, 857; Padma, 1971 and references therein). Therefore a high proportion of sulfoxyanions formed during aeration should be oxidised to sulfate during the pre-chlorination step (i.e. chlorination at the inlet of the GWTP).

Any sulfide remaining after aeration is likely to form elemental sulfur or sulfate upon chlorination; the major products of the reaction between sulfide and chlorine are elemental sulfur and sulfate, although the ratio of products depends on pH, temperature and sulfide to chlorine ratio (Montgomery, 1985, p 337; White, 1999, p. 556). At the exit of the treatment plant, water is further chlorinated, to approximately 2 mg/L residual, and it is highly unlikely that

sulfoxyanions would persist in the presence of free chlorine at this level. Consequently, it is unlikely that NSRS entering the distribution system contains inorganic polysulfides or sulfoxyanions such as thiosulfate, sulfite or polythionates.

Further confirmation that these simple sulfoxyanions and many OSCs are oxidised in the presence of chlorine is given by the fact that some classical analytical techniques for these compounds are based on titration (i.e. oxidation) of the sulfur with chlorine. Compounds that can be analysed in this way include dialkyldisulfides and dialkytrisulfides, polythionates, thiosulfates, thiocyanates, inorganic sulfides, carbon disulfide, rubeanic acid, thioglycollic acid and thiobenzoic acid, thioacetamide, tetrasulfur tetranitride, tetrasulfur tetraimide, dithiocarbamates and disulfur dichloride and dibromide (Padma et al.,1971 and references therein). The sulfur is oxidised by chlorine (chloramine-T) to tetravalent (+4) or hexavalent sulfur (+6). Since the determinations are carried out by titration, rapid and stoichiometric reaction between sulfur and chlorine is essential. The existence of analytical techniques based on this principle demonstrates that the sulfur compounds are converted rapidly to stable oxidised forms in the presence of excess chlorine, as exists in water at the exit of Wanneroo GWTP.

Contrary to suggestions by previous authors (Wilmot and Burkett, 1992; Wajon and Heitz, 1995), it is highly unlikely that inorganic polysulfides can survive water treatment processes. Steudel et al (1986) reported that autooxidation of polysulfides in aqueous solution occurs rapidly in the presence of air. In the present study considerable difficulties were encountered with the preparation of polysulfide solutions due to their reactivity with oxygen. A polysulfide solution prepared by adding elemental sulfur (0.6 g) to a stirred solution of sodium sulfide (15 g) at 75 °C under nitrogen was serially diluted in de-aerated water to give a concentration of 1 mg/L as sulfur. Addition of methyl iodide formed DMPSs, but the amount formed was equivalent to only about 1-2% of the polysulfide

originally added. Similar results were obtained on other occasions, despite rigorous de-aeration of reagents and dilution water, showing that inorganic polysulfides were highly susceptible to reaction with oxygen. (The preparation and analysis of polysulfide solutions was discussed in Chapter 4). Although Monscvitz and Ainsworth (1974) reported that "polysulfide odours" persisted upon chlorination of sulfidic groundwaters, the sulfide content of their waters was 70 mg/L, around 50-fold higher than those typically found in Wanneroo raw water.

The oxidation of inorganic polysulfides by chlorine has not been studied in detail, but in the present study it (Section 5.3.3) was found that dimethylpolysulfides (up to 4000 ng/L) were oxidised rapidly by concentrations of chlorine as low as 0.2 mg/L. In the present author's experience DMPSs are far more stable than inorganic polysulfides, so low concentrations of the latter compounds would undoubtedly be destroyed by chlorine at the concentrations present in distributed water. Even if water at the filter outlet did occasionally contain traces of inorganic polysulfides, these would not persist after several subsequent chlorination steps: chlorine is added to the clear water (to a residual of 1-2 mg/L), at the outlet of Wanneroo reservoir (0.5-1 ppm residual) and again at several service reservoirs. Inorganic polysulfides originating from the GWTP outlet are therefore most unlikely to persist into the distribution system, especially not to the extremities, where DMTS problems occur most commonly.

In view of the above discussion almost all of the residual NSRS exiting Wanneroo GWTP probably consists of elemental sulfur, some refractory OSC and possibly particulate iron sulfide. Most of the OSC in the raw water entering the treatment plant would be removed in the clarification step with other organic matter. Iron sulfides and elemental sulfur, being highly insoluble in water and hydrophobic, would also be largely removed in this process. Of all of the possible sulfur species that can constitute NSRS, some OSC (e.g. sulfur-bound humic substances, or heterocyclic compounds such as thiophenes), elemental

sulfur and iron sulfides are the most resistant to oxidation. Therefore the residual portions of these that are not removed during clarification and filtration would constitute the refractory component of NSRS in the distribution system, a total of 20-40 μg/L sulfur.

Theoretically the proportion of NSRS that can be attributed to elemental sulfur (cyclic S_6, S_7 and S_8) is limited, since this form of sulfur is highly insoluble in water. An often-quoted value for elemental sulfur solubility is 5 μg/L (Boulegue, 1978) and, on this basis, a maximum of one quarter of the NSRS in distributed water can be attributed to elemental sulfur. However, the literature on elemental sulfur solubility is somewhat controversial, possibly because the formation of sulfur as sols (such as Raffo, Selmi and Weimarn sols) can increase its effective solubility. According to Steudel (1989; 1996 and references therein), Raffo and Selmi sols are hydrophilic due to the presence of long-chain polythionates that bond onto the surface of the insoluble sulfur particles. Weimarn sols are solubilised in the presence of surfactants, especially if the sulfur particles are in liquid form (Steudel, 1996). In the presence of organic molecules the formation of crystalline sulfur is inhibited, and "dirty" sulfur is formed instead. Dirty sulfur consists of liquid-like clusters of hexacyclic, heptacyclic and octacyclic sulfur, incorporating hydrophobic organic molecules, and does not crystallise and separate from solutions in the same way that pure elemental sulfur does (Steudel, 1996). This form of sulfur is stabilised in solution by surfactants more readily than pure crystalline sulfur.

It is unlikely that elemental sulfur arising during water treatment at Wanneroo GWTP would form as a pure crystalline substance, but it might well be present in the form of a sol as described above, in which case its solubility could be considerably higher than 5 μg/L. The refractory NSRS in the distribution system could therefore consist largely of elemental sulfur, which may be associated with some organic matter. The organic matter could be associated with elemental sulfur as a Weimarn sol, perhaps solubilised by NOM, which is known to have

surfactant properties (Guetzloff and Rice, 1996; Mills et al., 1996). Further, a proportion of the sulfur that is associated with organic matter is probably bound within humic substances.

Formation of DMTS after adding methyl iodide to treated water

Wajon and Heitz (1995) found that when methyl iodide was added to treated water and to water from the distribution system, small quantities of DMTS were occasionally formed. Since DMTS is one of the products of reaction between methyl iodide and inorganic polysulfides, these observations led to the proposal that inorganic polysulfides could resist water treatment processes and that they constituted part of the refractory NSRS in treated waters (Wilmot and Burkett, 1992). In the present study, the work of Wajon and Heitz (1995) was extended, and further samples of Wanneroo treated water were analysed using the methyl iodide derivatisation technique. Results of these analyses (Table 5.3) showed that concentrations of DMPSs greater than 50 ng/L formed in only two out of nine samples of water from the filter outlet (59 and 80 ng/L). Although most values were less than 10 ng/L, it appears certain that very low levels of DMPSs can occasionally be formed by adding methyl iodide to treated waters. The formation of DMPSs upon the addition of methyl iodide to water is indicative of the presence of inorganic polysulfides, but , in view of previous discussions, it is unlikely that these compounds are present in treated water samples. The above observations are rationalised in discussions in the following paragraphs.

It should initially be noted that some doubt exists regarding the values for DMTS concentrations published by Wajon and Heitz (1995). These authors found that samples of water from Wanneroo GWTP (filter outlet) and Wanneroo distribution system produced levels of DMTS up to 320 ng/L after methyl iodide had been added. These analyses were conducted in 1992, prior to the commencement of the present studies, and considerable problems were encountered with the analytical technique at this time. The problems occurred because of reversible

adsorption of sulfur compounds to steel surfaces used within the CLSA system. Components such as the glass-to metal seals, constructed of "Covar" were of particular concern because these appeared to be susceptible to corrosion. These problems were subsequently rectified by replacing most of the steel components with PTFE, and later, by other modifications to the method, as discussed in Chapter 4. Since the analytical problems related largely to dirty blanks and carry-over of analytes from one sample to the next, some values obtained at this time might have been over-estimated.

Table 5.3: DMDS and DMTS in water and clarifier sludge from Wanneroo GWTP and distribution system before and after addition of methyl iodide.

Sample Location	Date	DMDS + DMTS (ng/L)	
		no treatment	methyl iodide added
[a]Raw	1992	<3	29000
[a]Filter Outlet	1992	<5	320
[a]Distribution system	1992	40-120	20-140
Clarifier sludge	13-9-94	<1	5.0
Clarifier sludge	28-9-94	<1	5.0
[b]Filter Outlet	28-9-94	<0.5	1.6
[b]Filter Outlet	18-11-94	<0.5	19
[b]Filter Outlet	22-11-94	<0.5	<2
Filter Outlet	30-11-94	<1	59
Filter Outlet	7-12-94	<1	80
Filter Outlet	27-3-95	<1	4.0
Filter Outlet	31-3-95	1.4	4.5
Filter Outlet	7-4-95	<1	<1
Filter Outlet	27-4-95	<1	<1
Distribution system	25-7-94	3.5	21

[a]From Wajon and Heitz (1995).

[b]In earlier measurements only DMTS (and not DMDS) was quantified.

One possible explanation for the formation of DMPSs after addition of methyl iodide to water samples in these earlier studies (Wajon and Heitz, 1995) is that

these compounds might be derived from inorganic polysulfides adsorbed to particulate iron sulfides; in a recent study (Heitz et al., 2000; discussed in Chapter 6) it was shown that DMPSs were formed upon addition of methyl iodide to iron sulfide minerals such as pyrite (FeS_2) and pyrrhotite (Fe_7S_8). However, insignificant concentrations of DMPSs (5 ng/L) were produced upon addition of methyl iodide to the two samples of clarifier sludge tested (Table 5.3). If fine particulates of these iron sulfide minerals were indeed present in the raw water, the bulk of these would be removed in the clarification step and would thus be concentrated in the clarifier sludge. The absence of DMPSs after addition of methyl iodide to clarifier sludge show that these minerals were probably not present in clarifier sludge, and it is therefore unlikely that they could be present in raw water entering Wanneroo GWTP, or in treated water exiting the plant.

In Chapter 6 of the present thesis it is shown that biofilms and pipewall sediments in distribution systems contain high concentrations of organic and inorganic polysulfides, and that these are stabilised against oxidation by the biofilm matrix. These findings provide an alternative explanation for the presence of DMPSs in treated water, after addition of methyl iodide, as shown in Table 5.3; small amounts of particulate matter originating from pipewall biofilms and sediments could be present in treated and distributed water, and this could potentially give rise to the traces of DMPSs formed upon addition of methyl iodide to the samples.

5.3.2 Formation of DMTS in stored water samples

Wajon and Heitz (1995) observed that very occasionally the intensity of swampy odour in some samples increased after they had been stored for periods of up to eight days. This occurred in water from the Wanneroo distribution system, but not in water from the treatment plant outlet. In most cases the observations related to odour only and, because DMTS measurements were usually carried

out on only one occasion, it was possible to measure an increase in the concentration of DMTS in only one instance (DMTS increased from 6 ng/L to 60 ng/L in six days). In the present study, two further samples were identified in which concentrations of DMTS increased during periods of up to six weeks. These are shown in Table 5.4. It should be noted that several hundred samples were analysed in the period relevant to the present investigation, but that increases in DMTS were shown only in the three samples presently discussed. This was partly because increases in DMTS in individual samples were difficult to identify, since samples were normally analysed only once. It was only in cases where an increase in the odour of a sample was coincidentally noticed that it was re-analysed to confirm the change in DMTS concentration. On one or two occasions swampy odour developed in samples for which it was not possible to demonstrate changes in DMTS concentration, since the samples were analysed only after the odour had developed. It is thought that, in general, odour develops in water after sampling only very rarely, probably in about 1% of samples.

Table 5.4: Change in concentrations of DMTS in water during sample storage.

Sample location	Date sampled	Date analysed	Analyte concentration (ng/L)	
			DMDS	DMTS
U4/50 Dellamarta Rd, Wangara (first flush)	27/01/98	27/01/98	120	70
		11-03-98	550	720
Wanneroo Reservoir outlet (prechlorination)	16/5/96	16/5/96	n.d.	<0.5
		16/6/96	n.d.	60

The mechanism of formation of DMDS and DMTS after sampling, as observed in the samples in Table 5.4, is probably via microbial activity occurring within particulate matter. In Chapters 6 and 7 it is shown that biofilms within distribution systems contain high concentrations of dimethylpolysulfides and inorganic polysulfides and that DMTS is most probably formed within biofilms. It is

possible that parts of biofilms present on inside pipe surfaces sloughed off and entered the water during, or prior to sampling, thus introducing particulate matter containing DMTS-forming organisms into the water. Water samples were not sterilised or filtered upon sampling, and microbial activity would therefire not have been suppressed.

Of the two sample locations listed in Table 5.4, the Wangara site was prone to frequent DMTS occurrences, the subject of numerous complaints by occupants of the factory unit. Water was supplied to the unit via a small diameter (2") service pipe (post-water meter) about 50 m in length. Water use at the unit was minimal and water in the service pipe would have been stagnant for long periods. Anecdotally, DMTS occurrences were associated with stagnant water in pipes. If DMTS is indeed formed within biofilms, small diameter service pipes (2" or less) would be most prone to the problem, simply because, per unit volume, water is exposed to more pipe surface than in larger diameter pipes. Biofilms exert chlorine demand, and this would have greatest effect in small diameter pipes. Similarly, diffusion of DMTS into the bulk water would have the greatest effect in the smallest diameter pipes. Both these factors would exacerbate DMTS problems. Interestingly, tenants of neighbouring factory units which had similar water supply and usage patterns did not complain of swampy odours, an observation which was not uncommon. Examination of biofilm in the service pipe at Unit 4 might provide useful information in future studies.

5.3.3 Oxidation of DMTS by chlorine

In the past, swampy odour in the Wanneroo distribution system has been controlled by maintaining adequate levels of free chlorine, although this method is not always successful, probably because chlorine residual levels sometimes fall below those required for effective control (>0.35 mg/L at Wanneroo reservoir outlet). Chlorine is typically dosed at around 6 mg/L at Wanneroo GWTP, but since this water has a high chlorine demand almost all of the chlorine is

consumed within 48 hours (i.e. at Wanneroo reservoir). A further dose of chlorine is thus added at Wanneroo reservoir outlet, to achieve a residual of 0.5-1.0 mg/L in water entering the distribution system.

The mechanism by which chlorine controls DMTS is not fully understood; it has been suggested that DMTS is either oxidised by chlorine as it forms, or that a certain level of disinfectant prevents DMTS from forming in the first place, possibly by controlling microbial activity. Neither of these hypotheses has been fully tested and it is not known how chlorine affects microbial processes that form DMTS, or exactly how the reaction between DMTS and chlorine proceeds.

In the present study, experiments were conducted to test the effect of free chlorine on DMDS and DMTS in water. DMDS (2200 ng/L) and DMTS (2320 ng/L) were added to water from Wanneroo reservoir outlet that had been dosed to obtain chlorine residuals of 0.19 to 0.59 mg/L. Spiked water samples were analysed for DMDS and DMTS immediately after spiking and on several occasions, up to nine days later. Results in Table 5.5 show that DMDS and DMTS are oxidised immediately by chlorine at all concentrations tested. Combined concentrations of DMDS and DMTS as high as 4500 ng/L were destroyed by chlorine at levels as low as 0.19 mg/L, but after several days some of the DMDS and smaller amounts of DMTS were regenerated (150 and 13 ng/L, respectively, for water dosed with 0.19 mg/L chlorine). This process appeared to occur to the greatest extent at the lowest concentration of chlorine (0.19 mg/L), and was less pronounced, or not observed at all, at the higher chlorine concentrations. In all cases, the amounts of DMDS that formed upon regeneration were much higher than the amounts of DMTS. In cases where chlorine was present above the limit of detection (0.02 mg/L), DMDS and DMTS were not detected, and these compounds were regenerated only after chlorine had decreased to non-detectable levels. Regeneration of DMDS and DMTS was not observed in the control sample (Wanneroo reservoir water without addition of DMDS, DMTS or chlorine in Table 5.5), and it therefore appears unlikely that

they were generated via microbial processes as discussed in the previous section.

Table 5.5: The effect of chlorine on DMDS (2200 ng/L) and DMTS (2320 ng/L) added to water from Wanneroo reservoir (sampled October 1996).

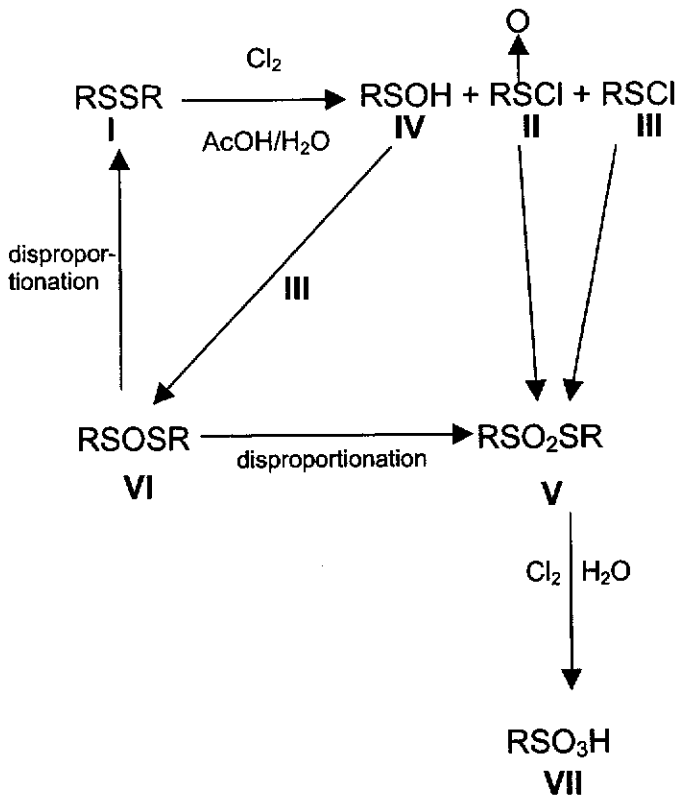
Sample	Free chlorine (mg/L)	Reaction time (days)	DMDS (ng/L)	DMTS (ng/L)
Control: (Milli-Q water + DMPSs, no chlorine addded)	n.d.	0	n.d.	2100
	n.d.	2	n.d.	2000
	n.d.	8	1750	2000
Control: Wanneroo Reservoir (no DMPSs added)	<0.02	0	<0.5	<0.5
	<0.02	9	<0.5	<0.5
Wanneroo Reservoir	<0.02	0	n.d.	2200
	[a]	6	1700	1600
	[a]	9	1600	700
	0.19	0	<0.5	<0.5
	[a]	6	61	10
	[a]	9	150	13
	0.40	0	<0.5	<0.5
	[a]	6	22	2
	[a]	9	58	4
	0.59	0	<0.5	<0.5
	[a]	6	<0.5	<0.5
	[a]	9	<0.5	<0.5

Notes: [a] chlorine decreased to <0.02 mg/L within six days in all cases.
n.d.: not determined

The above observations can be rationalized on the basis of the intermediate products formed during the chlorine oxidation of DMDS (and probably also DMTS). Oxidation of dialkyldisulfides and dialkyltrisulfides by chlorine has been studied by several authors, as discussed in a review by Oae (1991) and by Padma et al. (1971). According to these authors, DMDS and DMTS form methylsulfonylchloride (CH_3SO_2Cl) and methylsulfonic acid (i.e. compounds containing sulfur in the +4 oxidation state) upon reaction with chlorine in aqueous solution. The route to the latter end-product is discussed by Oae (1991,

pp. 241-242) and involves a complex series of steps in which the starting material can re-form via disproportionation of one of the intermediate products as shown in Scheme 5.1. The initial reaction products of the dialkyldisulfide (**I**) and chlorine in acidic aqueous solution are the sulfinyl chloride (**II**), the sulfenyl chloride (**III**) and also some sulfenic acid (**IV**). The sulfinyl chloride (**II**) and the sulfenyl chloride (**III**) go on to produce the thiolsulfonate (**V**), but if the sulfenic acid (**IV**) is produced, this reacts immediately, either with the sulfenyl chloride (**III**), or with itself to produce thiolsulfinate (**VI**). The thiolsulfinate (**VI**) is unstable in acidic solution and disproportionates, forming the original dialkyldisulfide (**I**) and the thiolsulfonate (**V**). Oxidation of the thiolsulfonate (**V**) results in formation of the alkylsulfonic acid (**VII**) which, according to Oae (1991 and references therein), is the end-product of complete oxidation.

Scheme 5.1: Reactions of dialkyldisulfides and chlorine in acidic aqueous medium (Oae, 1991 and references therein).



In the present study it was found that methylmethane thiolsulfonate (MMTS, **V** with R=CH_3) formed small amounts of DMDS and DMTS when dissolved in water, and that the extent of reaction was pH-dependent: when MMTS was dissolved in buffered Milli-Q water (pH 4.3 and 6.9) the solution produced had a strong odour of DMDS and DMTS and subsequent analysis showed that small amounts of these compounds had formed. Reaction of MMTS (48 μM) at pH 6.9 resulted in formation of DMDS (1.7 μM) and DMTS (0.23 μM), equivalent to approximately 4% conversion. At pH 4.3, MMTS formed much lower quantities of DMDS (0.13 μM) and DMTS (0.02 μM), equivalent to about 0.2% conversion to these products. According to Scheme 5.1, MMTS is a product of DMDS oxidation, and might therefore provide an additional route to the regeneration of this compound.

The reactions shown in Scheme 5.1, combined with the above observation that MMTS can form DMDS and DMTS, provide some explanation for the regeneration of DMDS after reaction with small amounts of chlorine. In Scheme 5.1, the formation of (**VII**) represents complete oxidation and it is unlikely that DMDS can be regenerated from this species. The conversion of (**V**) into (**VII**) is probably slow in comparison to the processes that (re-)form DMDS (i.e. the "circular" reaction to regenerate DMDS in Scheme 5.1 and the disproportionation of MMTS to DMDS). In Wanneroo distributed water, chlorine is continually consumed due to unmet "chlorine demand" from the high DOC (typically 3-5 mg/L). If insufficient chlorine is present and it is completely exhausted prior to the exhaustion of DMDS in the "circular" reaction in Scheme 5.1, some DMDS would persist. In the presence of sufficient chlorine, any DMDS that is regenerated from (**VI**) would continue to react, and all of the DMDS would eventually form methylsulfonic acid (**VII**), preventing further regeneration. The quantity of chlorine required to fully oxidise DMDS would vary, depending on the magnitude of the "chlorine demand" and on the rate of chlorine decay relative to the reactions in Scheme 5.1. The present findings offer

an alternative explanation for the above observations that DMTS is generated in some stored water samples.

5.4 CONCLUSIONS

Previous studies (Wilmot and Burkett, 1992) have determined that most of the reduced sulfur in raw water entering Wanneroo GWTP is removed by aeration, chlorination and clarification. In the present study it was concluded that the residual fraction of refractory NSRS that is not removed by water treatment processes (20-40 μg/L) most probably comprises non-crystalline elemental sulfur and some refractory OSC. These are the sulfur compounds that are detected by the NSRS assay and are known to be most resistant to oxidation by chlorine and oxygen. It is unlikely that traces of particulate iron sulfide minerals that can give rise to DMPSs by direct methylation are present in treated water, since the clarifier sludge itself did not produce DMPSs upon addition of methyl iodide.

Contrary to suggestions by previous authors (Wilmot and Burkett, 1992; Wajon and Heitz, 1995), due to their reactivity with oxygen and chlorine, inorganic polysulfides should not survive water treatment processes. While traces of inorganic polysulfides were detected intermittently in treated water, in the present study it is concluded that these could have originated from fine particulate material in the water, probably originating from pipewall biofilms and sediments. This is discussed further in Chapter 6.

The observation that DMTS can be generated in water after it has been sampled, as reported by Wajon and Heitz (1995), was confirmed in the present study. Increases in DMTS were measured over periods of up to six weeks in two samples of distributed water. It was concluded that the most likely explanation for these observations was that DMTS was generated by microbial action,

probably from microbes contained within pipewall biofilm and/or sediment particulates that could have entered the flowing water before or during sampling.

Studies of the reactions of chlorine with DMDS and DMTS showed that these compounds were rapidly and completely oxidised by the levels of chlorine present at the outlet of Wanneroo GWTP. However it was found that in the presence of low concentrations of chlorine, such as those that might exist at extremities of the distribution system (<0.4 mg/L), small amounts of DMDS and DMTS (up to 10 % of the original amount) could be regenerated over a period of several days. Oxidation of DMDS initially forms methylmethanethiolsulfinate and MMTS, both of which can disproportionate to re-form DMDS, and possibly DMTS. In the presence of sufficiently high concentrations of chlorine, the end-products of DMDS and DMTS oxidation, methylsulfonic acid and methylsulfonyl chloride are formed, preventing regeneration of the starting materials.

CHAPTER SIX

POLYSULFIDE SULFUR IN PIPEWALL BIOFILMS: ITS ROLE IN THE FORMATION OF SWAMPY ODOUR IN DISTRIBUTION SYSTEMS

ABSTRACT

Biofilms and pipewall sediments in drinking water distribution systems were analysed for dimethylpolysulfides (DMPSs) and inorganic polysulfides in an effort to determine the origin of dimethyltrisulfide, which causes unpleasant swampy odours in drinking water. Inorganic polysulfides were determined using the technique of methyl iodide derivatisation, and subsequent analysis as DMPSs by gas chromatography-mass spectrometry. The polysulfide-rich biofilms and sediments occurred in pipes constructed from a variety of different materials, and fed by water from several different surface and groundwater sources. The biofilm/sediment matrix appeared to retard oxidation of polysulfides, by preventing their diffusion into the oxic water and by providing a barrier within the biofilm, against the oxidative action of chlorine and dissolved oxygen.

6.1 INTRODUCTION

6.1.1 Biofilms in drinking water distribution systems

The formation of biofilms and slimes due to biological regrowth within drinking water distribution systems is the cause of a host of water quality problems, including high heterotrophic plate counts, corrosion problems, "dirty water", excessive consumption of chlorine disinfectant and taste and odour problems (Volk and Le Chevalier, 2000; Flemming et al, 2001). Biofilms have been defined as "an organic or inorganic surface deposit consisting of micro-organisms, microbial products or detritus" (LeChevalier, 1990) and "adherent microbial communities surrounded by a matrix composed of microbial polymer and components originating from the environment" (Jass and Walker, 2000). Biofilms most often form at solid-

liquid interfaces in aquatic environments, but may also form at air-liquid or liquid-liquid interfaces (Jass and Walker, 2000 and references therein). Locations that support microbial growth within distribution systems include pipe walls (Camper and McFeters, 2000, and references therein), inorganic tubercles or particles (Tuovinen and Hsu, 1982) sediments and reservoir walls and floors (Kerneis et al., 1995). Microorganisms associated with biofilm formation in drinking water distribution systems include pathogenic and non-pathogenic bacteria, protozoa, algae and fungi (Neil, 1987; Jass and Walker, 2000; Flemming, 2001). According to Costerton et al. (1987) more than 99% of the earth's microorganisms exist within biofilms.

An integral part of a biofilm structure consists of extracellular polysaccharide substances, sometimes called extracellular polymeric substances (EPS). These are the "slimes", consisting of polysaccharides, proteins, nucleic acids and lipids excreted by biofilm-forming organisms (Walker et al., 2000; Flemming et al., 2001). Together with water, EPS compose approximately 98% of the biofilm structure, and these two components are referred to as the glycocalyx (Jass and Walker, 2000). The glycocalyx allows the biofilm to adhere to surfaces and provides structure for the microbial assemblage. EPS are also important for protection of microbes from antimicrobial substances and disinfectants such as chlorine in drinking water distribution systems (Le Chevalier 1990; van der Kooij, 1990; Jass and Walker, 2000 and references therein).

Biofilm growth and structure is highly variable and is influenced by several physical and chemical factors. Major determinants influencing biofilm structure in water distribution systems are the low nutrient status and the high hydrodynamic shear forces. In this environment the biofilm is often a highly hydrated and porous structure containing microbial cell clusters separated by voids and channels. The cell clusters are held together by EPS, and the voids contain water moving at a much slower flow rate than in the bulk phase (Stoodley et al., 1994; Stoodley et al., 1999). Biofilms in drinking water distribution systems are usually thin and patchy and associated with inorganic deposits and corrosion products (Flemming et al., 2001). The rate

of biofilm growth depends on the physicochemical properties of the solid-liquid interface, the physical roughness of the surface and the physiological characteristics of the attached organism (van der Kooij, 1990). Also important are shear forces (Donlan and Pipes, 1988) and the effects of disinfectants, both of which may result in detachment of biofilms from surfaces (Le Chevalier 1990; van der Kooij, 1990; Herson et al., 1991). This sloughing off of the biofilm layer is a common cause of consumer "dirty water" complaints.

6.1.2 The potential role of biofilms in DMTS formation

Formation of DMTS occurs almost exclusively in parts of the Perth distribution system receiving water from shallow groundwater sources, especially that from Wanneroo GWTP, and has never been observed in areas not receiving this water type. The odour problem therefore appears to be strongly related to the groundwater source. Groundwater is sulfidic, unlike other local water sources, and previous authors (Wajon and Wilmot, 1992; Wajon and Heitz, 1995) proposed that DMTS may arise from traces of reduced sulfur species, which are incompletely oxidised during treatment, but were not able to present conclusive evidence in support of this.

In previous studies aimed at identifying the chemical and/or biochemical mechanisms of DMTS formation, the focus has been on the formation of DMTS in the bulk water, and studies of the possible role of the biofilm have been largely ignored (Wajon and Wilmot, 1992; Wajon and Heitz, 1995). Neil (1987) found that pipewall sediments contained SRB, but did not associate their presence with DMTS formation. In a series of very limited studies Barton (1995 a, b, c) confirmed the presence of SRB in biofilms, but the results were inconclusive because the SRB were not shown to be viable, and their presence did not appear to correlate with DMTS formation. Even though it was recognised that pipewall deposits contained significant concentrations of sulfur (0.03 to 0.09% by weight), the possibility of any association with DMTS was dismissed..."It is unlikely that sulphur in pipe sediments is the determining factor in the production of swampy odours" (Wajon et al., 1988, p. 41).

The sulfur in DMTS is present as polysulfide with an average oxidation state of $-^{2}/_{3}$, and hence a prerequisite to the formation of this compound is a source of reduced sulfur in a sufficiently low oxidation state. Although raw groundwater contains appreciable levels of sulfide and polysulfide (sulfur in a sufficiently reduced form), this is removed, or converted into more highly oxidised forms during treatment. Insufficient residual sulfide/polysulfide remains in the treated water to form DMTS, even at the low levels (ng/L) which can cause odour problems. Sulfur reduction processes must therefore occur within the distribution system to form the sulfide/polysulfide species required to generate DMTS. These processes are most likely to occur within the biofilm rather than the bulk water phase, since the latter is comprehensively aerated and chlorinated during treatment.

In the present study, pipewall sediments and biofilms in Perth's distribution systems were analysed for DMPSs and inorganic polysulfides. It is shown that the occurrence of both groups of these highly reduced sulfur species within pipewall biofilms is widespread amongst the distribution systems sampled, and does not appear to be strongly related to the source of the water.

6.2 EXPERIMENTAL

Sample collection

Pipewall sediment and reservoir sediment samples were collected in sterile glass or plastic vials, filled with water from the site to exclude headspace. On most occasions samples were collected opportunistically, when pipes were opened due to maintenance or accidental pipe bursts.

Groundwater was sampled using a syringe (20-100 mL), and where inorganic polysulfides were analysed, methyl iodide was added immediately to the groundwater sample in the syringe, as described in Chapter 4. Samples were analysed twenty-four hours after collection.

Analysis of inorganic polysulfides and dimethylpolysulfides (DMPSs) in water and biofilm materials

Analytical techniques used in the analysis of DMDS, DMTS and inorganic polysulfides are described in detail in Chapter 4 (Section 4.2). Briefly, inorganic polysulfides were converted into their methylated analogues by adding methyl iodide (0.05% v/v) directly to water and sediment/biofilm samples, and the mixtures were allowed to react in darkness for 24 hours. DMPSs were isolated from biofilm materials and groundwater using a nitrogen purge to transfer the analytes onto a Grob activated carbon trap. They were then eluted from the trap with dichloromethane and the resulting extract analysed by GC-MS. The concentration of inorganic polysulfide sulfur was calculated by subtracting DMPSs originally present in the samples from the concentration of DMPSs resulting from reaction with methyl iodide.

Analysis of biofilm/sediment samples for moisture content and metals

The dry weight of biofilm/sediment samples was determined by drying of the whole sample to constant weight (105 °C), after it had been analysed by purge and trap.

Dried samples were examined for the presence iron sulfides by scanning electron microscopy (SEM), using the EDS technique on a Philips XL30 instrument. Metal concentrations were analysed using flame atomic absorption spectrophotometry (FAAS) after digestion in nitric acid.

Preparation of mineral sulfide (pyrite and pyrrhotite) samples

Samples of pyrite (Peru pyrite; FeS_2) and pyrrhotite (Fe_7S_8) were obtained from Soklich Trading (Perth, WA). Minerals were ground in a mortar and pestle, then sieved to obtain a particle size between 710 μm and 125 μm. Sieved mineral samples were soaked in Milli-Q water overnight. The wash water was removed by vacuum filtration using a sintered glass funnel and the sample was washed with three further aliquots of Milli-Q water. Samples of

the washed minerals (~ 0.2 g) were added to buffered water (10 mL; phosphate buffer; 0.01 M; pH 6.86 prepared as described in Section 3.2), to which methyl iodide (0.05% v/v) was added. After standing in the dark for 24 hours a portion of the supernatant water (5 mL) was removed and analysed for DMPSs.

Control samples were also analysed. These included buffer and methyl iodide without the mineral samples and mineral samples in buffered water without methyl iodide. Further, methyl iodide was added to a portion of the supernatant (5 mL) taken from the latter experiment (i.e. such that there was no direct contact between methyl iodide and the mineral).

6.3 RESULTS AND DISCUSSION

6.3.1 Polysulfides in pipewall sediments in Perth distribution systems

Pipewall biofilms/sediments in many parts of Perth's distribution system contain significant concentrations of inorganic polysulfide sulfur (up to 79000 μg/Kg, dry weight, equivalent to 2,000,000 ng/L wet weight), and lower levels of the corresponding methylated organic species (DMPSs) (up to 1500 μg/Kg, dry weight), as shown by results listed in Table 6.1. The samples were taken from a variety of pipes of different construction materials and diameters, that contained water from either surface or groundwater sources. Inorganic polysulfides, and very low levels of DMPSs, were also found in sediment samples taken from the floors of roofed service reservoirs.

Effect of water source on polysulfides in distribution systems

Previous studies (Wajon and Wilmot, 1992) have found a strong correlation between sulfidic groundwater sources and DMTS-related odour problems, and the presence of DMPSs in pipe sediments where only surface water is supplied was therefore unexpected. These source waters are not sulfidic, and have never generated DMTS odours, but relatively high concentrations of methylated organic polysulfides were found in at least one biofilm sample

from a site receiving this water type (up to 95 μg/Kg DMTS, see Table 6.1). This sample also contained very high levels of inorganic polysulfides (42000 μg/Kg), comparable to samples from areas receiving only groundwater.

Table 6.1: DMDS and DMTS, inorganic polysulfide sulfur and total iron in pipewall biofilms and service reservoir sediments in the distribution systems of Perth. Concentrations are expressed both in μg/Kg (dry weight) and ng/L (wet weight) for DMDS and DMTS and inorganic polysulfides, and in g/Kg dry weight for total iron. In the case of supernatants from SW1 and SW2, concentrations are expressed in ng/L only.

Sample number/ [a]Water source	[c]Sample type, pipe ø (mm), material	DMDS μg/Kg, dry [ng/L, wet]	DMTS μg/Kg, dry [ng/L, wet]	polysulfide μg/Kg, dry [ng/L, wet]	total iron (g/Kg, dry)
SW 1	B, 100 mm, AC	27 [310]	95 [1100]	42000 [480000]	n.d.
SW 2	B, 100 mm, RC	50 [1200]	13 [320]	2400 [58000]	175
SW 3	B 600 mm, CLS	4 [48]	2 [20]	120 [1500]	200
GW 1	B 250 mm, AC	9 [230]	10 [250]	79000 [2000000]	270
[b]GW 2	B 50 mm, Cu	640 [40]	48 [3]	45000 [2800]	n.d.
GW 3	B 100 mm, RC	1300 [4800]	160 [590]	15000 [51000]	91
GW 4	B 100 mm, RC	45 [10]	6 [1]	4900 [1100]	n.d.
GW 5	B 400 mm, RC	2 [63]	2 [65]	1640 [56000]	n.d.
GW 6	B 100 mm, RC	2 [74]	0.2 [6]	520 [18000]	n.d.
GW 7	B 600 mm, CLS	41 [950]	4 [85]	14000 [340000]	n.d.
GW 8	B 600 mm, CLS	4 [22]	2 [12]	300 [1800]	n.d.
GW 9	B 600 mm, CLS	4 [92]	2 [48]	300 [7000]	n.d.

Table 6.1 (continued)

Sample number/ * Water source	pipe material and ø (mm)	DMDS µg/Kg, dry [ng/L, wet]	DMTS µg/Kg, dry [ng/L, wet]	polysulfide µg/Kg, dry [ng/L, wet]	total iron (g/Kg, dry)
GW 10	B 100 mm, RC	1.2 [59]	0.2 [8]	79 [1250]	n.d.
SW 4	R.S	0.4 [240]	0.1 [92]	570 [200000]	n.d.
GW 11	R.S	<1 [<1]	<1 [<1]	930 [2800]	n.d.
GW 12	R.S	1 [<10]	<1 [<1]	630 [5700]	n.d.
GW 13	R.S	51 [68]	4 [5]	320 [420]	n.d.
GW 11	R.S	<1 [<10]	<1 [<10]	120 [1200]	n.d.
Supernatant water in contact with biofilm SW 1 (see SW1)		<1 ng/L	<1 ng/L	<50 ng/L	n.d.
Supernatant water in contact with biofilm SW 2 (see SW2)		<1 ng/L	<1 ng/L	<50 ng/L	n.d.

Notes:

[a]Major water source supplied at site: SW = surface water; GW = shallow groundwater

[b]All samples except for the pipewall biofilm sample from the 50mm Cu main consisted of thick, brown sludge. The sample from the copper main was thin (<1mm) and translucent.

[c]B = pipewall biofilm; R.S = sediment from the base of roofed storage reservoirs within distribution systems.

AC = asbestos cement; RC = reinforced concrete; Cu = copper; CLS = cement lined steel

n.d. = not determined.

Despite the small data set in Table 6.1, the results indicate that production of inorganic polysulfide and DMPSs in pipewall sediments is probably not strongly linked to sulfidic source waters. Water flowing through the pipes and reservoirs from which the biofilms and sediments were sampled originated from at least five different groundwater and surface water sources, suggesting that the occurrence of these compounds is widespread in distribution systems. Almost all of the samples studied contained detectable concentrations of inorganic polysulfides and DMPSs.

6.3.2 The ocurrence of anoxic microniches within oxic environments

The observed polysulfides are most likely generated in anoxic microenvironments within the biofilm, via assimilatory and/or dissimilatory sulfur reduction processes (see Section 2.1). The observation that polysulfide production occurs in relatively thin biofilms growing in water distribution systems, where the bulk water may be saturated with respect to dissolved oxygen and contains some free chlorine, requires some consideration. The thicknesses of pipewall biofilms varied at around an estimated thickness of 1 mm, or less, in small diameter (50 mm) polyethylene and copper pipes, to up to about 3 mm thick in large diameter (600 mm) trunk mains. (These biofilm thicknesses are similar to those observed earlier in Perth distribution systems by Neil (1987, p. 53)). Most studies of sulfate reduction processes in natural ecosystems where sediments are sampled on a centimeter scale do not address the possible existence of reduced microenvironments on a millimetre, or micrometre scale. In the present study (Section 4.3.1) it was observed that even small traces of oxygen would rapidly destroy inorganic polysulfides at concentrations below 4.5 nM, despite the presence of reducing agents such as ferrous sulfate, ascorbic acid and hydrazine hydrate. However as discussed in Chapter 2, it has been clearly established that sulfate reduction can occur in anoxic micro-environments within apparently oxic environments. Kühl and Jorgensen (1992) demonstrated microzonation of oxygen respiration, sulfide oxidation and sulfate reduction in the top 2 mm of biofilms between 3-5 mm thick. Although the biofilms were in an oxic environment, oxygen depletion commenced within the water at the liquid-biofilm boundary and conditions were totally anoxic 0.4 mm below the biofilm surface. Sulfate reduction took place within the biofilm, between 1-2 mm from the surface, and sulfide oxidation occurred in a narrow zone at a depth of 0.2 to 0.4 mm. Similarly, Jorgensen (1977) showed that sulfate reduction by obligate anaerobes could occur within microniches in the uppermost layers of oxic marine sediments. The bulk redox potential in some sediments where sulfate reduction occurred was up to + 400 mV. It was shown that reducing conditions could exist within sediment particles of

diameter as small as 100 μm. It is therefore quite likely that similar processes might exist within biofilms growing in our water distribution systems.

Effect of pipe material

The nature of biofilms in distribution systems is determined by many factors, including the availability of growth-promoting substances, temperature, liquid-surface shear, and age and type of pipe materials (van der Kooij, 1992; Ridgeway and Olson, 1981). The samples listed in Table 6.1 were taken from diverse sites, receiving water from a variety of sources, from four different pipewall materials and pipe diameters ranging from 50-600 mm. Due to the limited data set it is not possible to conclusively determine whether any of these factors affected polysulfide production in biofilms, but there is some evidence of effects from variability in pipe material, water quality (i.e.nutrient flux and free chlorine concentration) and flow characteristics (shear). The highest levels of inorganic polysulfides (dry wt) were found in biofilms taken from asbestos cement (AC) pipes, while the material taken from reinforced concrete (RC) pipes generally contained lower polysulfide levels, possibly indicating an effect from pipewall material. Levels of inorganic polysulfides and DMPSs in material sampled from pipewalls were highly variable, while those from sediments from roofed storage reservoirs in the distribution system appear to be lower and more constant. This may be because the environment near reservoir floors is more stable than that at the water-pipewall interface. The reservoir floor would be less susceptible to changes in physical and chemical parameters, such as water flow velocity (and therefore shear and nutrient flux) and free chlorine residual. These factors would affect biofilm growth and metabolism, and therefore, the rate and extent to which sulfur reduction processes and polysulfide generation can occur. Higher free chlorine residuals, would also result in more rapid oxidation of polysulfide. Biofilms in larger diameter pipes (400-600 mm) are probably in continuous contact with water containing higher free chlorine residuals, because these would generally be closer to re-chlorination points, thus accounting for the lower polysulfide levels found in larger diameter pipes.

Adsorption of organic and inorganic polysulfides on biofilm/sediment particles

In several of the sediment and biofilm samples examined, the high concentrations of DMTS would have been sufficient to impart an odour to the supernatant (surrounding) water, if DMTS had been able to partition between the water and the sediment phases. However, none of the wet samples had a perceptible odour, suggesting that DMPSs were sequestered within the biofilm or sediments, and were only released when subjected to strong gas stripping during the analytical processes. This was further investigated by carrying out a simple experiment in which the water and sediment phases from two samples (SW1 and SW2) containing high concentrations (1100 and 320 ng/L) of DMTS were analysed separately (Table 6.1). The water and sediment phases (approximately equal volumes of each phase) were first initimately mixed by vigorous shaking, the sediment was then allowed to settle, and an aliquot of supernatant water was carefully removed using a pasteur pipette. Significant concentrations of DMPSs remained in the sediment layer, but none were detected in the supernatant, confirming that these compounds are very strongly adsorbed into the sediment matrix, and do not partition readily into water. Inorganic polysulfides appeared to behave similarly; these compounds were never detected in concentrations greater than 100 ng/L in any treated water, nor in any water in intimate contact with polysulfide-rich biofilms. The residual amounts of inorganic polysulfides, which were occasionally detected, probably originated from suspended biofilm/sediment particulate material in the water. The results show that concentrations of methylated and non-methylated polysulfides within biofilm/sediment materials are orders of magnitude higher than the waters with which they are in contact, and that these compounds are sequestered within the biofilm/sediment matrix.

Previous authors have observed the binding, or adsorption of organic and inorganic sulfur compounds onto particulates. Kiene and Taylor (1988) attributed abiotic losses of the thiol, 3-mercaptopropionic acid in coastal marine sediment porewaters to physical adsorption onto particulates. It is

conceivable that similar processes might occur within the biofilms studied, that is, that polysulfides are adsorbed onto either inorganic particulates within sediments, or onto the organic biofilm (i.e. EPS) matrix.

6.3.3 The effect of biofilm/sediment matrix on polysulfide oxidation

Dilute solutions of inorganic polysulfides are extremely unstable in the presence of oxygen, although oxidation rates can be considerably affected by a number of reagents (Pasiuk-Bronikowska et al., 1992). The susceptibility of these compounds to oxidation was demonstrated in the present study (Chapter 4), where preparation of polysulfide solutions was not possible when the dilution water contained even trace amounts of dissolved oxygen. Under the conditions used during sampling and analysis, which did not include precautions to prevent exposure to air, dilute solutions of polysulfide would have been extremely unstable, and would have deteriorated within hours. However, it was found that polysulfide concentrations in the biofilm/sediment samples under examination did not deteriorate significantly (<15%) over periods of more than two weeks. Similar results were obtained in samples in which microbial activity had been quenched by the addition of sodium chloride (20% w/v), eliminating the possibility of continuing polysulfide production by microbes. The observations indicate that the biofilm/pipewall sediment matrix attenuates (or prevents), oxidation of the inorganic polysulfides detected with the present method.

A possible explanation to account for the stability of inorganic polysulfides under apparently oxic conditions in our studies is that oxidation is inhibited by ferric or ferrous iron, or by organic anti-oxidants. Many organic compounds (e.g. ethylenediaminetetraacetic acid (EDTA), mannitol and phenolic compounds), sometimes catalysed by ferrous or cuprous salts, are known to inhibit auto-oxidation of sulfur compounds (Pasiuk-Bronikowska et al., 1992). The biofilm/sediment materials contained very high concentrations of total iron, up to 27% (dry weight) as shown in Table 6.1. Examination of the material by SEM and optical microscopy indicated that iron was probably predominantly present in the form of oxides, rather than sulfides. It is

possible that polysulfides may simply be adsorbed onto the surface of iron oxides, and that this hinders their oxidation by preventing diffusion from the biofilm/sediment matrix into the oxic water phase. Alternatively, other components of the biofilm/sediment matrix, such as extracellular mucopolysaccharide substances (i.e. EPS) could also protect polysulfides from oxidation, possibly by forming weak complexes, or by acting as antioxidants. The effect of this slime layer in protecting microbial populations from oxidants and disinfectants is well documented (e.g. Ridgeway and Olson, 1981, van der Kooij, 1992).

The suggestion that inorganic polysulfides may be protected from oxidation in some way is supported by observations of other workers who detected these polysulfides in cultures of the obligate aerobe *Acinetobacter lwoffii* (Ginzburg et al., 1999) in an apparently oxic environment. Similar apparent stability of inorganic polysulfide compounds in an oxic freshwater lacustrine environment was reported by Gun et al. (2000). Using thermodynamic calculations the latter authors showed that, theoretically, the lower polysulfide (n=2) homologues dominate over higher homologues (n=4,5) in dilute systems, but did not demonstrate reasons for their existence in oxic environments. The possibility that binding or adsorption of polysulfides to particulate or cellular material could inhibit oxidation was not discussed.

The distribution of individual inorganic polysulfide homologues in pipewall biofilm/sediment samples further indicates that these compounds might be present as sorbed species, rather than as polysulfides in a true solution. In aqueous systems inorganic polysulfides exist as equilibrium mixtures of di-anionic chains of sulfur atoms (Pasiuk-Bronikowska et al., 1992), with the number of sulfur atoms in the chains, n, varying from 2-5 (Schoonen and Barnes, 1988 and references therein). Under the conditions applying to the present samples, that is, in dilute solutions, at near neutral pH and ambient temperature, the predicted average value of $\bar{n}$ is approximately 3.6 (Giggenbach, 1972). As discussed in Chapter 4, it is assumed that addition of methyl iodide to water and biofilm samples quenches polysulfide interreactions and freezes the equilibrium, providing a snapshot of the

distribution of di-anionic polysulfides present in the original sample. A typical chromatogram showing the distribution of DMPSs after treatment of pipewall biofilm/sediment samples with methyl iodide is shown in Figure 6.1(a). The most abundant homologues in these samples are the disulfides and trisulfides, with very minor amounts of tetrasulfide, and no pentasulfide. The value for $\bar{n}$, of 3.6, as predicted by Giggenbach (1972), requires much greater proportions of the higher polysulfides (tetrasulfide and pentasulfide) than those observed in the biofilm/sediment samples. Other sample types, such as pre-filtered sulfidic groundwater samples (Figure 6.1(d)), consistently yielded DMPS distributions where the value of $\bar{n}$ was much closer to the expected value of 3.6. This observation confirmed that it was reasonable to expect a value of around 3.6 for $\bar{n}$ in samples where polysulfides are in solution (at near-neutral pH and ambient temperature), and that the observed deviation from this value in the biofilm/sediment samples suggests that polysulfides are, in fact, not in solution, but might be sorbed to the solid matrix.

The hypothesis that inorganic polysulfides might be sorbed onto solid surfaces in biofilm/sediment samples was further investigated by treating an aqueous slurry of an iron sulfide mineral (pyrite or pyrrhotite) with methyl iodide, then analysing for DMPSs. Previous studies have shown that inorganic polysulfides can be adsorbed onto the surfaces of these, and other sulfide minerals (Schoonen and Barnes, 1991 and references therein; Luther, 1991), and it has been shown that methyl iodide reacts on the surfaces of some of these minerals to produce traces of DMS and DMDS (Thayer et al., 1984). Luther (1991) proposed that pyrite formation involved the formation of iron-polysulfide intermediates, possibly complexes of the form $Fe\text{-}S_x^{2-}$, which might be present either in solution or at the mineral surface. Observations made in the present study confirm the findings of these previous authors; significant amounts of DMPSs were obtained when methyl iodide was added to water in contact with pyrite (FeS_2) and pyrrhotite (Fe_7S_8), as shown in Table 6.2. Inorganic polysulfides were not detected in water that had been in contact with the sulfide minerals after the mineral had been removed by

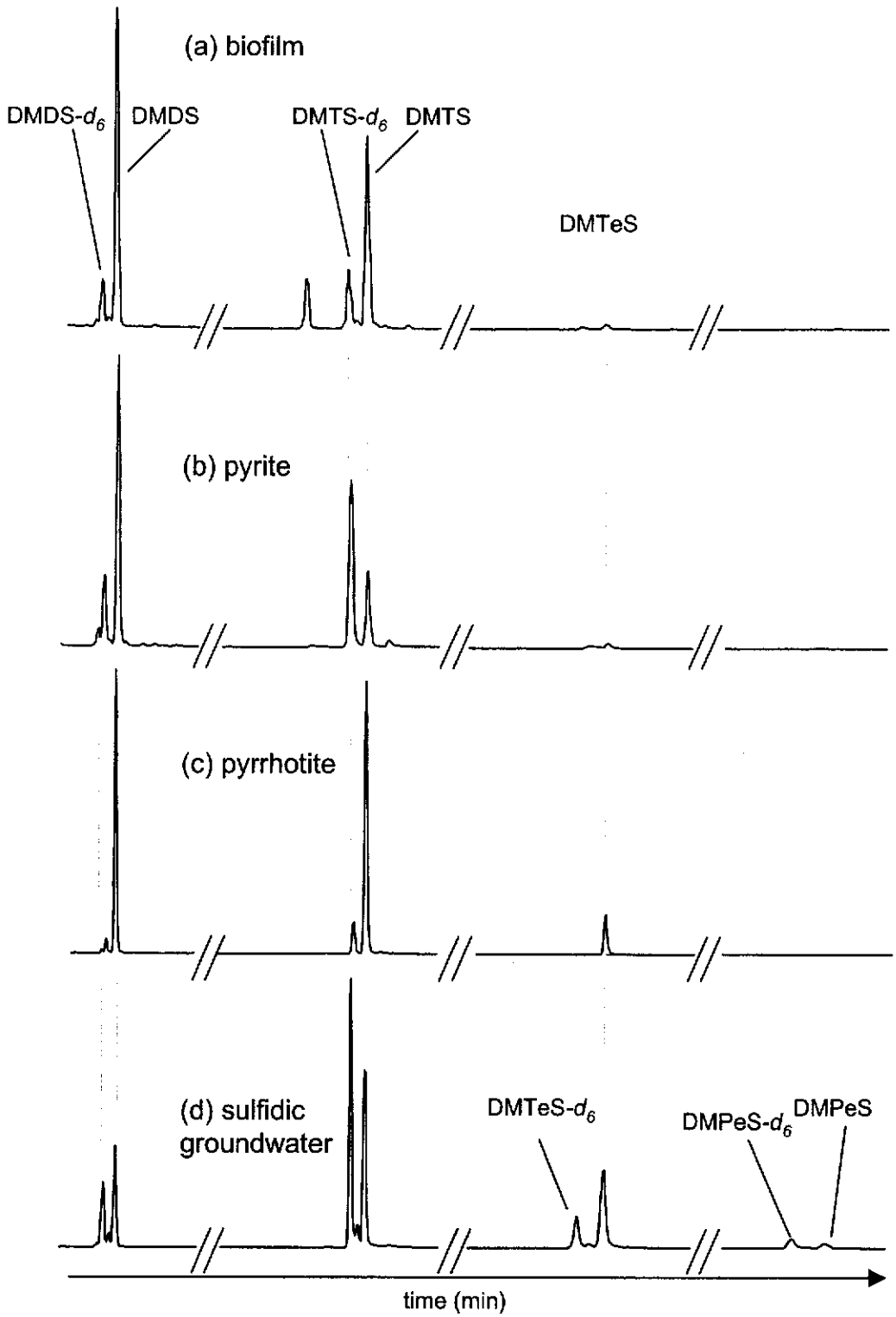


Figure 6.1: Mass chromatograms showing the distribution of DMPSs obtained from (a) biofilm, (b) pyrite, and (c) pyrrhotite, (d) filtered sulfidic groundwater, after derivatisation with methyl iodide. Perdeuterated DMPSs (DMDS-d_6, DMTS-d_6, DMTeS-d_6 and DMPeS-d_6) were used as internal standards. The GC-MS conditions used are listed in Table 4.3.

filtration, showing that methylation of these compounds must have occurred at the mineral surface. Chromatograms of DMPSs obtained upon the reaction of methyl iodide with pyrite and pyrrhotite in water at pH 6.9 (Figure 6.1 (b) and (c)) show that these minerals yielded a DMPS mixture with a distribution very similar to that yielded by the biofilm/sediment samples. In all cases the disulfides and trisulfides are the dominant homologues, with the tetrasulfides and pentasulfides as very minor constituents, or not detectable. It therefore appears that where inorganic polysulfides are sorbed onto mineral surfaces, the equilibrium distribution of these homologues is altered, resulting in a lower value of $\tilde{n}$. In the present study this observation has provided important insights into the nature and behaviour of inorganic polysulfides within the biofilm and sediment samples found in drinking water distribution systems.

Table 6.2: Concentrations of DMPSs formed after addition of methyl iodide (0.05 % v/v) to mixtures of crushed pyrite or pyrrhotite in unbuffered Milli-Q water, or buffered Milli-Q water (phosphate buffer, 0.01M; pH 6.86).

Sample tested	pH	DMDS (ng/L)	DMTS (ng/L)
Pyrite in contact with buffered Milli-Q water	6.86	970	380
Pyrrhotite in contact with buffered Milli-Q water	6.86	1200	2600
Pyrrhotite in contact with unbuffered Milli-Q water	4.72	870	1200
*Buffered Milli-Q water after contacting pyrrhotite for 24 hours.	6.86	<20	<10

Note: * Methyl iodide was added to an aliquot of the buffered Milli-Q water after it had been removed from the mixture containing the pyrrhotite solid.

Although it has not been possible to identify pyrite or pyrrhotite in the biofilm/sediment samples, the polysulfides detected may be associated with amorphous iron sulfide species. In the present study, analysis by SEM showed that although most of the iron existed as oxides, some sulfides were present. If polysulfides were adsorbed onto the surfaces of iron oxides or sulfides, these could be released and methylated by methyl iodide, resulting in the observed DMPSs. Insoluble iron-sulfide species have also been observed previously in biofilms: in a study of the distribution of sulfur species (elemental sulfur, FeS_2 and FeS) in aerobic wastewater biofilms, Okabe et al.

(1998) found that all three species occurred in a biofilm 600 μm thick. Elemental sulfur and iron monosulfide constituted the greatest proportion of the sulfur species, and FeS_2 was present at relatively low concentrations.

6.3.4 Mechanism of DMTS formation via methylation of inorganic polysulfide in distribution systems

In the present chapter it has been shown that inorganic polysulfides exist within biofilms in drinking water distribution systems at concentrations up to 2 000 000 ng/L (2 mg/L, wet weight), many thousands of times higher than the levels of DMPSs found in the bulk water. It is possible therefore that the DMPSs found in distributed water could be formed by methylation of these inorganic polysulfides. Several plausible mechanisms exist for the methylation of inorganic polysulfides in distribution systems, and these are discussed briefly. A mechanism involving direct chemical methylation by compounds such as methyl halides, or methyl moieties in humic substances has been proposed previously (Wajon and Wilmot, 1992). In biological systems, compounds possessing methane thiol, or dimethyl-sulfonio moieties, such as methionine and dimethylsulfoniopropionate, undergo transformations resulting in the formation of DMDS and methane thiol (Taylor, 1993; Segal and Starkey, 1969). It is conceivable that the S-methyl moieties released during these reactions can incorporate sulfide and polysulfide, to form the higher DMPSs. Alternatively, Nystrom et al. (1992) found that several species of actinomycetes isolated from tap water could methylate trichlorophenol to produce trichloroanisole. Actinomycetes are common in drinking water supplies, and have been detected in the systems under study (Neil, 1987). Biomethylation of polysulfide via these organisms, is a highly plausible mechanism for the formation of DMTS. The possibility that DMDS and DMTS are formed in distribution systems via these processes is explored in Chapter 7.

6.4 CONCLUSIONS

Microbial sulfate-reducing and/or sulfur-reducing mechanisms occurring within pipewall biofilms provide a pool of reduced sulfur that is potentially important in the formation of DMTS and swampy odour. The pipewall biofilm/sediment appears to adsorb organic and inorganic polysulfides, and can act as a protective barrier against the oxidative action of chlorine and dissolved oxygen in the bulk water. The mechanisms by which this occurs have not been fully investigated, but may be related to formation of complexes between polysulfide and biofilm organic matter and/or iron species. Results of the present study are being applied to the study of organosulfur-related organoleptic problems (i.e. DMTS), but may also have important implications in other sulfide-related problems in distribution systems, such as sulfide-induced corrosion (Hao et al., 1996) and consumption of chlorine due to sulfide production.

CHAPTER SEVEN

*FORMATION OF DIMETHYLPOLYSULFIDES IN BIOREACTORS SIMULATING DRINKING WATER DISTRIBUTION SYSTEMS

*Note: Most of the work described in the present Chapter was carried out as a collaborative study between groups from CSIRO Land and Water (WA), Curtin University, Water Corporation and Brown and Root. The parts of the experimental work that involved the use of bioreactors were carried out jointly by CSIRO Land and Water (Dr Peter Franzmann and Mr Luke Zappia) and Curtin University (Ms Anna Heitz and Mr Jason Self (technical assistant)). Specifically, the bioreactors were designed, built, maintained and operated by the CSIRO group, while the samples generated from these bioreactors were analysed by the Curtin group. While the study was a collaborative effort, as shown in publications arising from it (Franzmann et al., 2000; Franzmann et al., 2001), the present author was a major participant in its planning, design and execution, and in the interpretation of the results. The study arose largely from findings of investigations described in Chapter 6 of the present Thesis, and its aims were based, in part, on hypotheses proposed in Section 6.3.4.

ABSTRACT

Studies described in Chapter 6 linked the occurrence of DMTS in the bulk water phase to sediments and biofilms growing within distribution systems. In the present Chapter, consideration is given to the microbial processes that might lead to the formation of inorganic polysulfides and dimethylpolysulfides under conditions approximately representative of those in distribution systems. The aim was to investigate the possible role of biofilms in the formation of DMTS and to determine the nature of chemical precursors which might stimulate, or contribute to these processes. Biofilms, artificially generated on high-surface-area synthetic supports (bioballs) within chambers filled with water from Wanneroo GWTP, were exposed to compounds thought to be potential DMTS precursors. The response of the systems in terms of production of methylated sulfur compounds was monitored. The mechanisms investigated included; (a) direct chemical (abiotic) methylation by methylating agents such as methyl halides, or methyltoluenesulfonate; (b) methiolation via compounds possessing methane thiol moieties, such as methionine and dimethylsulfonio-propionate (Taylor, 1993); (c) transfer by sulfate reducing

bacteria of methyl groups in methoxy-aromatic compounds to form methylated sulfides; and (d) biomethylation as a response to microbial toxins such as the methylation of trichlorophenol to produce trichloroanisole, or methylation of metals by actinomycetes and fungi. Production of DMTS was also stimulated by the presence of high concentrations of easily bioavailable dissolved organic carbon (BDOC), sodium sulfide and by allowing the water to stagnate. These experiments showed that, under the test conditions, production of DMDS and DMTS could occur via several mechanisms, and dimethylsulfur compounds could be formed even without the addition of compounds containing sulfur or methyl moieties. DMTS did not form in the absence of biofilms and it was therefore concluded that minimisation of microbial activity within biofilms was a key in preventing DMTS formation.

7.1 INTRODUCTION

Recent work (Heitz et al., 2000; discussed in Chapter 6) has linked the occurrence of DMTS in the bulk water to biofilms and sediments occurring within distribution systems. Samples of pipewall biofilms and sediments collected from floors of service reservoirs were found to contain dimethylpolysulfides (DMPSs) and inorganic polysulfides far in excess of the concentrations of these compounds in the surrounding water. Inorganic polysulfides are potential precursors to DMTS since they react rapidly with chemical methylating reagents such as methyl halides (Barbash and Reinhard, 1989a), and can be methylated microbially via transfer of methyl groups from methoxy-aromatic compounds (Bak et al., 1992; Bak and Finster, 1993). The presence of inorganic polysulfides is indicative of activity by sulfate reducing, or sulfur reducing microorganisms. The purpose of the present investigation was to determine whether biofilms could form DMTS from specific precursor compounds under conditions which as closely as possible represented those in the distribution system.

Several mechanisms for the methylation of polysulfides in biofilms to form DMTS were investigated using bioreactors to simulate distribution system conditions. The mechanisms investigated include; (a) direct chemical

(abiotic) methylation by methyl-donating compounds such as methyl halides, or methyltoluenesulfonate; (b) methiolation via compounds possessing methane thiol moieties, such as methionine and dimethylsulfoniopropionate (Taylor, 1993); (c) transmethylation of methyl groups in methoxy-aromatic compounds to form methylated sulfides by sulfate reducing bacteria; and (d) biomethylation as a response to microbial toxins such as the methylation of trichlorophenol to produce trichloroanisole, or methylation of metals by actinomycetes and fungi (Nystrom et al., 1992; Fatoki, 1997; Harper, 1993). Experiments to determine whether these mechanisms could occur under distribution system conditions were conducted using bioreactors fed with water from DMTS-prone areas and a wide range of precursor compounds representative of the above groups.

7.2 EXPERIMENTAL

7.2.1 Biofilm reactors

Three biofilm reactors were constructed and set up by Mr. Luke Zappia and Dr. Peter Franzmann (of CSIRO Land and Water, Floreat, Western Australia) as described by Franzmann et al. (2000; 2001). Briefly, each biofilm reactor (or bioreactor) consisted of a polycarbonate and stainless steel chamber filled with floating solid supports of a high surface area on which biofilm could grow, termed "bioballs". Each bioreactor was fitted with inlets to receive feed water (from Wanneroo GWTP), nutrient feeds and nitrogen purge, as shown in Figure 7.1. Biofilms were established rapidly over an initial four-week period by conducting six-hourly cycles (including one hour of stagnation) of feeding with Wanneroo water containing ammonium chloride (NH_4Cl; 0.1g/L), potassium dihydrogen phosphate (KH_2PO_4; 0.01 g/L) and ethanol (1 g/L). After the four-week phase of establishing the biofilms, the reactors were fed on Wanneroo water only for a period of three weeks. Three biofilm reactors were run in parallel, one as a control, where no precursor compounds were added. The other two were amended with potential DMTS precursor compounds, which were added to Wanneroo water at pre-determined concentrations. Precursor experiments were typically conducted over a

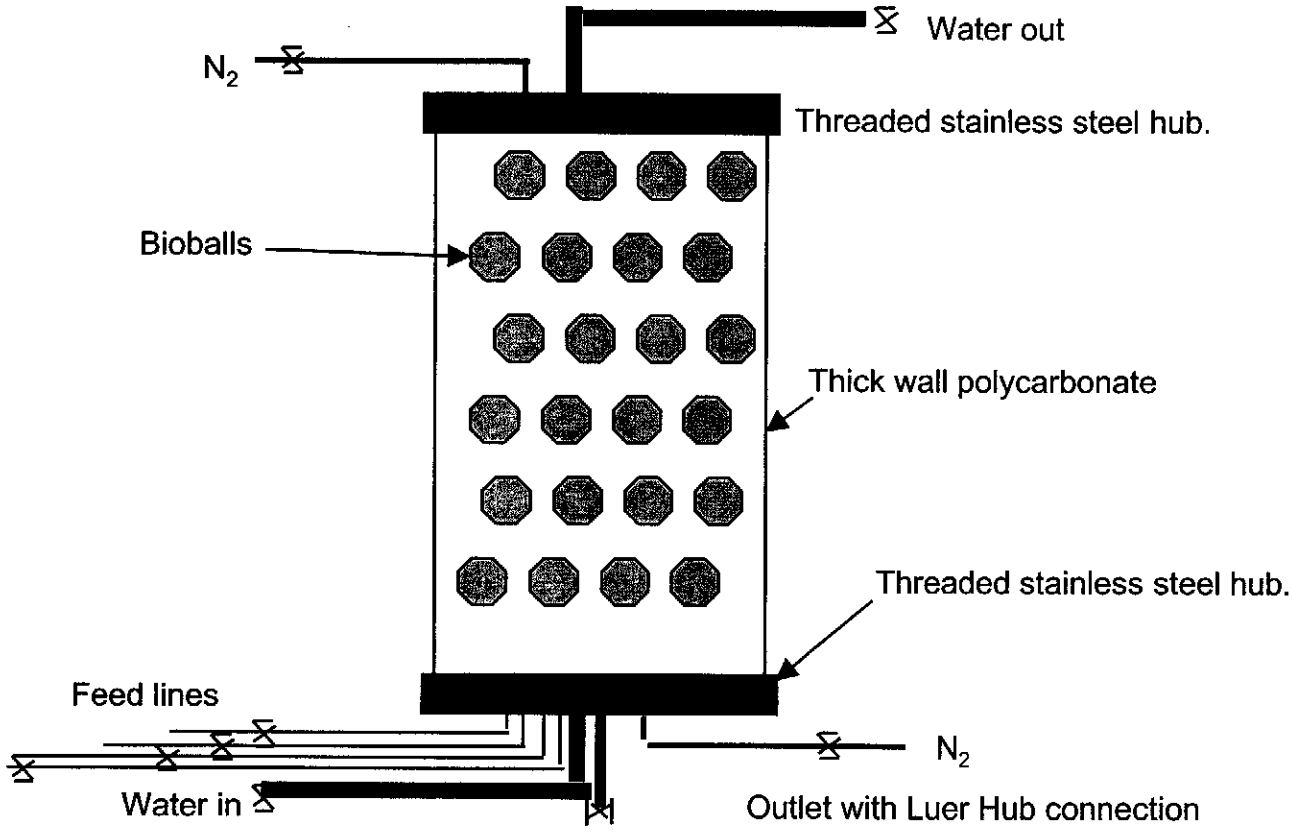


Figure 7.1: Schematic of biofilm reactor used to test Wanneroo water containing various added substrate compounds for their capacity to stimulate the formation of DMTS by biofilms. Biofilms were grown on the solid supports or "bioballs" contained in the reactor chamber (see Franzmann et al., 2001).

period of four hours, during which the flow was stopped (i.e. four hour stagnation). Samples were collected from each reactor prior to adding the test chemical, at time, t = 0 hr (immediately after adding the test chemical), and at time, t = 4 hr (four hours after adding the test chemical). Prior to analysis, samples were stored in 10% sodium chloride at 4 °C to prevent further microbial activity.

7.2.2 Analysis of dimethylpolysulfides and inorganic polysulfides

Analytical techniques for dimethyldisulfide (DMDS), dimethyltrisulfide (DMTS) and inorganic polysulfides are described in detail in Chapter 4. In summary, DMDS and DMTS were isolated from water (up to 100 mL), sediments and biofilms (5 mL) using a purge and trap technique. Sample extracts were analysed by GC-MS (Hewlett Packard 5890/5971 GC-MSD) equipped with a cool on-column injector. Quantification of analytes was carried out using perdeuterated DMDS and DMTS internal standards normalised against *n*-chlorohexadecane. Inorganic polysulfide sulfur was analysed by in-situ methylation of polysulfides with methyl iodide (0.05% v/v), followed by analysis of the resulting DMPSs as described above. The concentration of inorganic polysulfides was calculated by subtracting DMDS and DMTS originally present in the samples from the concentration of DMDS and DMTS resulting from reaction with methyl iodide. The dry weight of biofilm samples was determined by drying of the whole sample to constant weight (105 °C), after it had been analysed by purge and trap.

7.2.3 Test of abiotic methylating reactivity

Several methylated sulfur compounds and methylating reagents (as listed in Table 7.8) were tested for their reactivity with inorganic polysulfides to form DMPSs abiotically. A solution of disodium polysulfide was prepared by adding elemental sulfur to a solution of sodium hydroxide and hydrazine hydrate (3:2:1 molar ratio) in de-oxygenated water (100mL), while stirring and heating to 85 °C under nitrogen (Korchevin et al., 1989). The polysulfide solution was diluted in oxygen-free water at pH 6.86 to produce solutions

containing 0.015 mM sulfur as polysulfide, to which was added an approximately two-fold molar excess of the methylated compound under test. The mixture was allowed to react for 24 hours before analysis for DMPSs. All preparation and handling of polysulfide solutions was carried out in a nitrogen-filled glovebag.

7.2.4 Analysis of the metal content of biofilms

Biofilm material was dried at 105 °C and weighed. Whole samples were digested with redistilled, hot, concentrated nitric acid in acid-washed PTFE beakers. The material was digested for 16 hours, cooled, and diluted to 10 mL with Milli-Q water. Hydrochloric acid (100 µL) was added to prevent precipitation of ferric iron. Digested samples were analysed using ICP-MS (carried out by Mr. David Pritchard and Professor John Watling of Curtin University) and flame-AAS after appropriate dilution with Milli-Q water.

7.3 RESULTS AND DISCUSSION

7.3.1 Initial experimentation

An initial experiment was conducted to determine an appropriate incubation time for subsequent experiments. This was done by adding methionine (10 mM) to a bioreactor and monitoring the formation of DMDS and DMTS at hourly intervals over a four-hour period. Methionine was chosen because in a previous study (Wajon et al., 1988) it had consistently produced DMTS in bacterial cultures isolated from a distribution system, and it is a ubiquitous component of proteins (Taylor and Keine, 1989). Results, listed in Table 7.1, show that methionine-containing Wanneroo water which had not been exposed to biofilms, or had only been exposed to biofilms for 10 minutes did not produce significant amounts of DMDS or DMTS, even though the sample was not analysed until after completion of the time-course experiments. That is, methionine in Wanneroo water, in the absence of biofilms, did not produce appreciable DMDS and DMTS, whereas in the presence of biofilms (in methionine-containing Wanneroo water) the concentrations of these

compounds continued to increase for up to three hours. Production of DMDS and DMTS peaked at three hours (160 000 ng/L) and two hours (17 000 ng/L) respectively. Since the production of DMTS appeared to plateau after 3 hours it was decided to use a reaction time of four hours for all bioreactor experiments.

Approximately thirty different compounds were tested for their potential to form DMDS and DMTS in the presence of biofilms. In most cases the compounds were added to Wanneroo water at concentrations far in excess of those that would be expected in the distribution system in order to obtain a result as unambiguous as possible. However, some selected compounds including methionine, methyl iodide and methylparatoluenesulfonate were added at more realistic concentrations, as low as 7×10^{-6} mM. All of the test compounds are numbered in the text (structures are listed in Section 7.5).

Table 7.1: Concentrations of DMDS and DMTS formed in bioreactors after the addition of methionine (10 mM).

	DMDS (ng/L)	DMTS (ng/L)
Control (unamended)		
Before addition	70	9.2
10 minutes	100	9.2
4 hours	140	7.8
Methionine added (10 mM)		
Before addition	99	13
10 minutes	1700	370
1 hour	55000	8400
2 hours	72000	17000
3 hours	160000	8900
4 hours	130000	9100

7.3.2 Formation of DMDS and DMTS from methanethiol-containing compounds

Of all the compounds tested for their potential to form DMPSs when in contact with biofilm, those possessing a methanethiol moiety formed by far

the highest concentrations. As can be seen in Table 7.2, various concentrations of methionine [1], ranging from $7x10^{-6}$ mM to 10 mM, produced varying amounts of DMDS and DMTS, and the yield of these products was highly dependent on the concentration of substrate added, but not directly proportional. The percentage yield of DMDS and DMTS as listed in Table 7.2 is a measure of the amount of dimethylsulfur compounds produced from the added substrate (i.e. the percentage of moles of substrate added versus moles of DMDS and DMTS produced). At the very high concentration of methionine (10 mM), a much smaller proportion of this compound was converted to dimethylsulfur compounds than at concentrations less than 7×10^{-3} mM (% yield DMDS and DMTS = 0.028 for 10 mM and 12.6 for 7×10^{-4} mM). This suggests that production of DMDS and DMTS is probably limited by some factor other than the availability of methionine. Since methionine did not form appreciable amounts of DMPSs in the absence of biofilms (i.e. "before addition" in Table 7.2), it can be assumed that the limiting factor must be biofilm activity. In reviews of the biochemistry of methyl sulfur compounds Taylor and Keine (1989) and Taylor (1993) state that methionine is demethiolated by anaerobic and aerobic micro-organisms, producing methanethiol (MT), which may then undergo oxidation to form DMDS (Lacombe et al., 1998). The enzyme responsible for release MT from methionine is methionine γ-lyase and this may be the limiting factor in the present case.

In the case of methyl-3-methylmercaptopropionate [5], methyl(methyl-mercapto)-acetate [2] and (methylmercapto)acetate [6], significant concentrations of DMDS and DMTS were present at t = 0 hours. However, in all cases DMDS and DMTS concentrations were much higher in the solutions in contact with biofilm (t = 4 hours) than in solutions which had not contacted biofilm, or only contacted biofilm for a short time (ie. t = 0 hours). This indicates that these compounds must have been formed by microbial processes in the biofilm, rather than via abiotic reactions.

Table 7.2: Concentration of DMDS and DMTS formed in bioreactors after addition of compounds containing methanethiol-moieties.

Substrate (concentration)	Before addition		t = 0 hours		t = 4 hours (*t = 8 hours)		** % yield DMDS+DMTS
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	
[1] Methionine (10 mM)	<200	10	1700	370	130000	9100	0.028
Methionine (7×10^{-3} mM)	22	48	30	9.5	22000	4400	7.6
Methionine (7×10^{-4} mM)			10	1.0	4000	210	13
Methionine (7×10^{-5} mM)	14	1.6	34	2.6	*60	*4.8	2.0
Methionine (7×10^{-6} mM)	28	1.3	47	1.4	*16	*<1	4.8
[2] Selenium methionine (7×10^{-3} mM)	-	-	16	<1	1600	230	not relevant
[3] S-Methyl methionine (1 mM)	-	-	70	70	43000	1400	0.094
[4] S-methyl cysteine (1 mM)	<100	<50	110	70	110000	32000	0.28
[5] Methyl-3-(methylmercapto)propionate (1 mM)	<200	<50	33000	2300	400000	54000	0.94
[6] (Methylmercapto)acetate (1 mM)	<20	<10	9500	730	35000	11000	0.092
[7] Methyl(methylmercapto)-acetate (1 mM)	<50	<10	6800	750	33000	3700	0.076
[8] 4-(methylmercapto)benzoic acid (1 mM)	<20	<10	<20	<10	70	40	0.0002

* Concentration after 8 hours, not 4 hours, as for other experiments.

** % yield (DMDS + DMTS) = $\frac{\text{[moles DMDS + moles DMTS formed after 4 hours]} \times 100 \times 2}{\text{[moles substrate added]}}$

Note that for all substrates two moles of substrate are required to form one mole of DMDS or DMTS. For DMTS an additional sulfur atom is required. 1 mM of each of the compounds in Table 7.2 is equivalent to 32 mg/L sulfur.

Every methanethiol-containing precursor compound tested, yielded significantly greater concentrations of disulfide than trisulfide (e.g. the highest ratio of DMDS/DMTS was 30, formed from S-methylmethionine [3]; the lowest was 3.1, formed from (methylmercapto)acetate [6]. In most cases these ratios were quite different to the relative proportions of these compounds produced in the distribution system. All samples from the distribution system analysed in 1998 which contained measurable DMTS are listed in Table 7.3. In many of these samples almost equal proportions of DMDS and DMTS were produced, and in some cases more of the trisulfide was produced than the disulfide. Although the data set is not extensive, this apparent disparity in the proportions of disulfide versus trisulfide suggests that the mechanism of DMTS formation in the distribution system is not the same as that in bioreactors fed with methanethiol-containing compounds.

Table 7.3: Concentrations of DMDS and DMTS in water samples analysed during 1998. The ratio of DMDS/DMTS is listed for each sample, showing that approximately equal concentrations of these compounds are formed in most cases.

Sample location	Sampling Date	DMDS	DMTS	DMDS/DMTS
		(ng/L)	(ng/L)	
Balcatta	12/10/98	180	120	1.5
Alexander Heights	12/10/98	33	39	0.9
Morley	5/01/98	11	12	0.9
Wangara 1	15/01/98	18	17	1.1
[a]Wangara 1	27/01/98	120	70	1.7
[b]Wangara 1	11-03-98	550	720	0.8
Padbury 1	12/03/98	64	94	0.7
Padbury 2	12/03/98	140	130	1.1
Gnangara 1	6/03/98	37	19	2.0
Gnangara 2	6/03/98	18	8	2.2
Gnangara 3	6/03/98	23	16	1.5
Leederville 1	3/08/98	17	38	0.4

Notes: [a] Sampled and initially analysed 27-01-98 and

[b] same sample re-analysed 11-03-98.

The strong preference of disulfide over trisulfide in the catabolism of methanethiol-containing compounds suggested that the primary mechanism of formation of the dimethylsulfur compounds (i.e. DMDS and DMTS) was enzymatic cleavage of the ω–thiomethane bond to release methanethiol, followed by oxidation of methanethiol, forming DMDS. In order to confirm the predominance of this mechanism, an experiment was conducted where the selenium analogue of methionine, selenium methionine [2] was added to bioreactors. The chromatogram obtained from analysis of bioreactor water 4 hours after the addition of selenium methionine is shown in Figure 7.2. The sample extract contained a high abundance of a compound eluting at 16.5 minutes, that had not been observed previously in other samples and was not present in the corresponding sample taken immediately after adding selenium methionine to the bioreactor. The mass spectrum of this compound (Figure 7.2) was not found in the Wiley mass spectral database, but the cluster of ions at *m/z* 186 to 192 was as expected for dimethyldiselenide. The mass spectrum of another compound of lesser abundance, eluting at 14.1 minutes, had similar characteristic clusters separated by two atomic mass units, including a cluster around *m/z* 93 which was common to both compounds. Again, the compound could not be identified by matching the mass spectrum with data from the Wiley database, but the cluster at *m/z* 140 to 144 matched molecular ions expected from dimethylselenenylsulfide (CH_3SeSCH_3). Mass spectra for dimethyldiselenide and dimethylselenenyl-sulfide published by Fan et al. (1998) compare well with the compounds eluting at 16.5 minutes and 14.1 minutes respectively, confirming their identities as shown in Figure 7.2. The high abundance of dimethyldiselenide formed from catabolism of selenium methionine is analogous to the fomation of DMDS from methionine and other methanethiol-containing compounds. The results of this experiment support the suggestion that the predominant mechanism for formation of dimethylsulfur compounds from methanethiol-containing compounds is cleavage of ω–thiomethane to form MT, which then forms primarily DMDS.

The formation of dimethylselenenylsulfide (CH_3SeSCH_3) showed that a range of reactions probably occurred, with some of the components of the

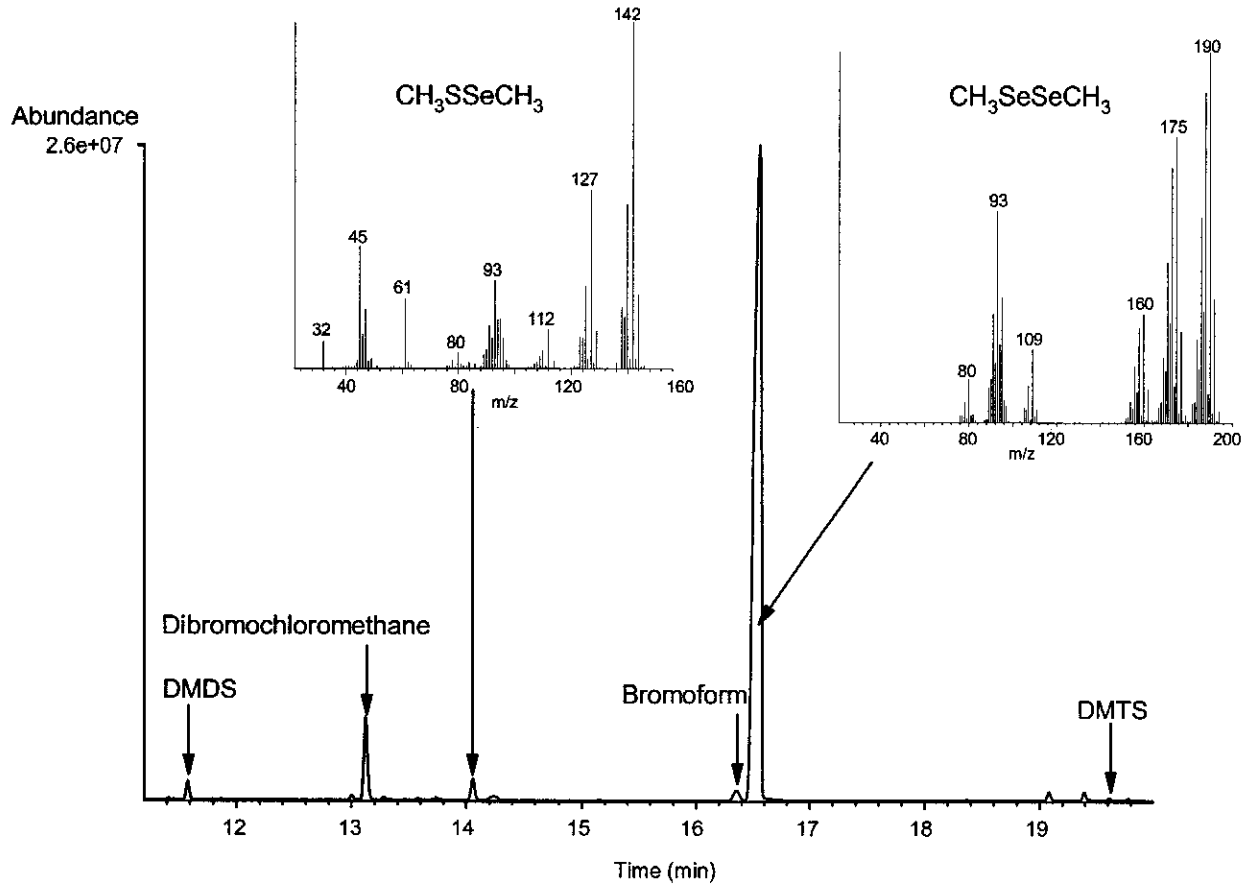


Figure 7.2 : Total ion chromatogram of purge and trap extract of bioreactor water 4 hours after adding selenium methionine (7×10^{-3} mM).

dimethylsulfides/selenides probably originating from biochemicals such as methionine that naturally occur within growing biofilms. Similar production of mixed sulfur/selenium species has been previously observed; Fan et al., (1998) found evidence that methylselenomethionine and methylseleno-cysteine were precursors of dimethylselenide, dimethyldiselenide and dimethylselenenylsulfide in cyanophyte mats cultured on agricultural drainage waters.

The formation of additional DMTS (230 ng/L *vs* 5 ng/L DMTS in the control, Table 7.2) devoid of selenium atoms is interesting. This may have formed through transfer of a methyl group from selenium methionine. Alternatively the presence of high concentrations of methylselenide may have stimulated indiscriminate methylation, such as that which occurs as a detoxification response (see discussion later). As estimated by peak areas in Figure 7.2, the amount of dimethyldiselenide formed was about fifty times the amount of DMDS and about 350 times the amount of DMTS, suggesting that these were minor processes.

In many studies where the formation of DMDS from MT is observed, the presence of DMTS is not reported (Taylor, 1993 and references therein). In order for the trisulfide to form, an additional sulfur atom is required, which must be incorporated into the sulfur chain. This additional sulfur could be derived from the inorganic polysulfides, which are present in high concentrations in some biofilms in the distribution system, as discussed in Chapter 6. Milligan et al. (1963) demonstrated the interreactions between nucleophilic sulfur species, such as methanethiol, and dialkylpolysulfides as shown in eq 7.1 (a). In these reactions nucleophilic alkylthiols such as CH_3S^- and CH_3SS^- attack a sulfur atom within DMDS, resulting in exchange of $CH_3S_n^-$ moieties (n = 1,2). Since bisulfide and polysulfide ions are stronger nucleophiles than alkylthiols (Barbash and Reinhard, 1989a), interactions of the type shown in eq 7.1(b) to (f), where sulfide or polysulfide are incorporated into dimethylpolysulfides, are likely to occur. In the presence of an amine catalyst methanethiol will react with cyclo-octasulfur (S_8) (Vineyard,

1966), and this may be an alternative route for the formation of DMTS from MT.

$CH_3SS^- + CH_3SSCH_3 \Leftrightarrow CH_3S^- + CH_3SSSCH_3$ ...[7.1(a)]

$HS^- + CH_3SSCH_3 \Leftrightarrow CH_3SS^- + CH_3S^-$ ...[7.1(b)]

$CH_3SS^- + CH_3SSCH_3 \Leftrightarrow CH_3SSSCH_3 + CH_3S^-$ ...[7.1(c)]

$S_n^{2-} + CH_3SSCH_3 \Leftrightarrow CH_3S\ S_n^- + CH_3S^-$ ...[7.1(d)]

$CH_3S\ S_n^- + CH_3SSCH_3 \Leftrightarrow CH_3S\ S_nSCH_3 + CH_3S^-$ ...[7.1(e)]

$CH_3S\ S_nSCH_3 \Rightarrow$ disproportionation to equilibrium mixture of DMPSs. (n = 2, 3) ...[7.1(f)]

The only methanethiol-containing compound which did not form significant concentrations of DMDS or DMTS was 4-(methylmercapto)benzoic acid [8]. Whereas in all the other listed methanethiol-containing compounds the sulfur atom is bound to an alkyl group, in 4-(methylmercapto)benzoic acid it is bound to an aromatic ring. These results suggest that only enzyme systems capable of cleaving alkyl-sulfur bonds, and not aryl-sulfur bonds were active in the bioreactors. Indeed, in an extensive review of biogenic production of DMS and MT from organosulfur compounds Taylor (1993) listed only enzymes capable of cleaving alkyl-S bonds and did not mention aryl-S bonds. Aryl-S moieties are probably much more biologically stable than the alkyl species and CH_3-S-aryl compounds are therefore less likely to be involved in DMTS formation in the distribution system.

As discussed above, the concentrations of many of the test compounds added to the bioreactors were orders of magnitude in excess of those which would realistically be expected in the distribution system, or even in the raw water. The following experiments were conducted in order to gauge whether methanethiol-containing compounds could form relevant amounts of DMTS at the concentrations which might exist in the bulk water phase in the distribution system. Methionine was added to the bioreactors at

concentrations between 7 x 10^{-3} and 7 x 10^{-6} mM, as shown in Table 7.2. These concentrations correspond to 200 and 0.2 μg/L S respectively and were chosen on the basis of the concentrations of non-sulfide reduced sulfur (NSRS) compounds known to exist in the Wanneroo distribution system (Wilmot and Burkett, 1992). NSRS is a gross measure of the total concentration of non-volatile sulfur compounds, not including sulfide and sulfate (Wilmot and Wajon, 1997), but including most, if not all of the test compounds listed in Table 7.2. Concentrations of NSRS in the Wanneroo distribution system ranged from 23-31 μg/L in a 1992 study (Wilmot and Burkett, 1992). In a more recent study the NSRS concentration in twenty determinations carried out over a six-month period ranged from 10 to 35 μg/L, with an average value of 22.4 μg/L (Water Corporation, 1998, unpublished data). NSRS comprises a large number of possible sulfur compounds, especially those not easily oxidised by chlorine such as elemental sulfur and perhaps heterocyclic organosulfur compounds (discussed in Chapter 5), and it can be assumed that methanethiol-containing compounds would not comprise more than 10% of NSRS. At a typical concentration of 20 μg/L NSRS the maximum concentration of methanethiol-containing compounds can be estimated at around 2 μg/L or 7 x 10^{-5} mM. As shown in Table 7.2, methionine at this concentration did not form appreciable levels of DMTS (4.8 ng/L), although 60 ng/L DMDS was formed. At the lower concentration of 7 x 10^{-6} mM, the concentration of DMDS formed was negligible (16 ng/L), and DMTS was not detected. In the bioreactor tests, concentrations of around 20 μg/L (7 x 10^{-4} mM) methionine were required to obtain concentrations of DMTS that would be of concern in distributed water (>20 ng/L). From the known NSRS concentrations, typically 20 μg/L, it is unlikely that methanethiol-containing compounds would be present at the required levels in the bulk water in the Wanneroo distribution system.

7.3.3 Formation of DMDS and DMTS from partially oxidised methyl-sulfur compounds

In several previous studies it has been suggested that one or more sulfur-containing precursor compounds originating from the raw water may be responsible for DMTS formation in the distribution system. It was proposed that reduced sulfur compounds in the raw water are incompletely oxidised during water treatment and could enter the distribution system to produce DMTS (Wajon and Wilmot, 1992; Wilmot and Burkett, 1992; Wajon and Heitz, 1995). As discussed in previous chapters, organic and inorganic polysulfides and sulfides are the only forms of sulfur of sufficiently low oxidation states to directly form DMTS, and it was shown that sulfide and polysulfide are unlikely to be present in water leaving Wanneroo GWTP. However, the possibility that methanethiol-containing compounds in the raw water might play a role has not yet been fully investigated.

During conventional water treatment processes involving aeration and chlorination, the expected products from oxidation of methanethiol compounds would include partially oxidised methyl sulfur compounds, where the sulfur atom is bound to one or two oxygen atoms. For example, Miles (1991) reported that aldicarb, a methanethiol-containing compound, is rapidly oxidised, first to aldicarb sulfone and then to aldicarb sulfoxide in chlorinated water (Figure 7.3 (a)). Lacombe et al. (1998) list the possible oxidation products of dialkyldisulfides as oxysulfur compounds where oxygen has been added to one or both sulfur atoms. These are thiosulfinates, thiosulfonates, *vic*-disulfones and several unstable oxy-sulfur products (structures are shown in Figure 7.3 (b)). Partially oxidised methyl sulfur compounds such as dimethylsulfone [13] and dimethylsulfoxide (DMSO) [12], are known to undergo microbial reduction in environmental systems, to produce methylsulfides (Taylor, 1993; Kiene and Capone, 1988), and this could be a potential route to DMTS. DMSO is chemically reduced to DMS by sulfide (Keine and Capone, 1988). Partially oxidised sulfur compounds could therefore potentially contribute to DMTS formation if they are microbially or chemically reduced, or possibly, by acting as methylating agents.

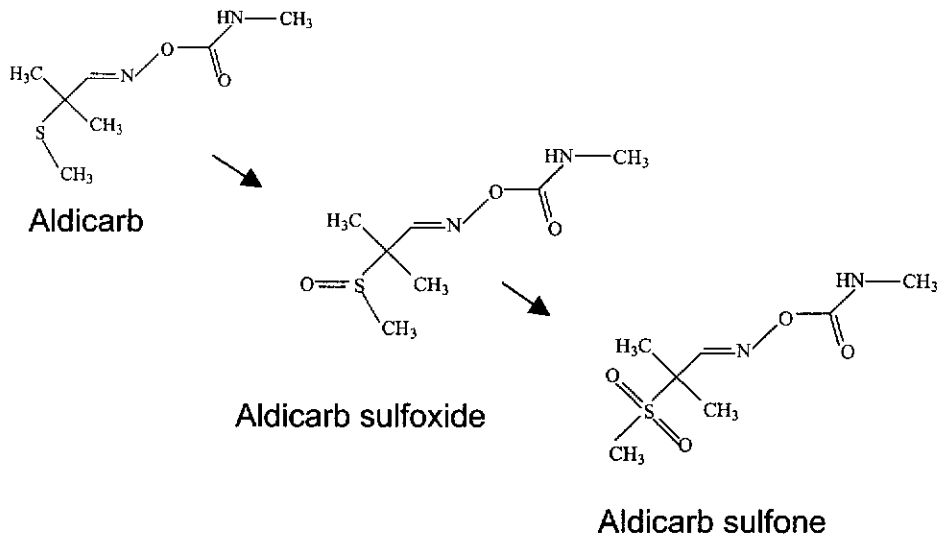


Figure 7.3 (a). Oxidation of aldicarb in chlorinated water (Miles, 1991)

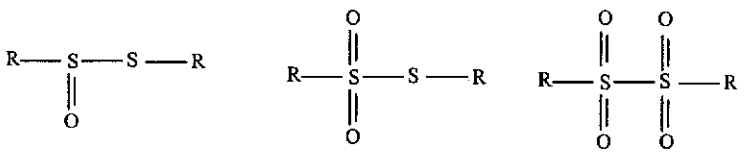


Figure 7.3 (b). Stuctures of oxidation products of dialkyldisulfides (Lacombe et al, 1998)

The possibility that methylated oxysulfur compounds could take part in DMTS formation was examined by testing dimethylsulfone and DMSO for their reactivity in bioreactors. As shown in Table 7.4, the concentrations of DMDS and DMTS did not increase in the case of either compound, and in fact, in the case of DMSO, which was added at a very high concentration of 10 mM, the concentrations decreased. (In the case of DMSO, some DMDS and DMTS was present at t = 0, probably formed from MT, an impurity in the reagent.) The results showed that neither reduction to produce dimethylsulfides, nor methylation of polysulfides occurred in biofilms in the presence of these methylated oxysulfur compounds, and that they appear to be essentially unreactive in the bioreactors.

Table 7.4: Concentration of dimethyldisulfide (DMDS) and dimethyltrisulfide (DMTS) formed in bioreactors after addition of compounds containing partially oxidised methylsulfur (sulfonyl and sulfinyl) moieties.

Compound added (concentration)	t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
[12] Dimethylsulfoxide (10 mM)	3000	86	1800	47
[13] Methyl sulfone (1 mM)	<20	<20	<10	<10

Note: Numbers in square parentheses relate to structures in Section 7.5.

Effect of chlorine on methionine

A further test was conducted to confirm that existing water treatment processes at Wanneroo would oxidise methanethiol-containing compounds and thereby prevent them forming DMTS in the distribution system (i.e. if these were indeed present in the raw water). Methionine was used as a model for other methanethiol-containing compounds, since this may be one of the compounds most likely to be present in raw water. Methionine was added to Wanneroo clearwater containing typical concentrations of free chlorine (1.45 mg/L, Table 7.5.) and this was then brought into contact with biofilms in a bioreactor. Control experiments were conducted, where

bioreactors were fed with Wanneroo water with added methionine, but no chlorine, and with Wanneroo water only. None of the mixtures contained measureable free chlorine at the time of addition to the bioreactors.

Results, listed in Table 7.5 show that when chlorine-treated methionine is added to bioreactors the amount of DMDS and DMTS formed is significantly less than when only methionine is added. Despite using a concentration of methionine of 3.5×10^{-3} mM (100 μg/L - S), fifty times in excess of realistic concentrations, and allowing chlorine to react with it for only a few minutes before adding to bioreactors, only 29 ng/L DMTS was formed. This is close to the odour threshold of 10 ng/L for DMTS and is much less than the concentrations of several hundred nanograms per litre sometimes found in the distribution system. These results indicate that it is highly unlikely that methionine at realistic concentrations (7×10^{-5} mM) in contact with chlorine in excess of 2 mg/L for several hours, as would occur post-treatment, could produce DMTS.

Table 7.5: Effect of chlorine on the formation of DMDS and DMTS in Wanneroo water amended with methionine (3.5×10^{-3} mM)

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
Control (Wanneroo water only)	22	<1	20	1.3	15	<1
[a]Methionine + Wanneroo clearwater (dechlorinated)	17	<1	20	2.4	5000	220
[b]Methionine + Wanneroo clearwater	25	3.4	170	5.4	250	29

Notes: [a] The concentration of free chlorine in Wanneroo clearwater at the time of sampling was 1.67 mg/L.

[b] The concentration of free chlorine was 1.45 mg/L just prior to adding methionine.

These experiments show that even if methanethiol-containing compounds, such as methionine were present in source waters, they probably do not contribute directly to odour formation since they would be oxidised to

unreactive compounds. However, methionine and possibly other methanethiol-containing compounds are almost certainly biosynthesized by microorganisms within the biofilm and these could form dimethylsulfur compounds as discussed above. The formation and persistence of methanethiol-containing compounds in the distribution system requires microbial activity and an environment that provides a barrier against chlorine oxidation, as occurs within the biofilm.

7.3.4 Abiotic methylation of inorganic polysulfides

Polysulfides are strong nucleophiles and react rapidly with alkylating agents such as alkyl halides or alkyl-p-toluenesulfonate (Barbash and Reinhard, 1989a). Methyl iodide, methyl-p-toluenesulfonate [9], methyl sulfate [10] or methyl methanesulfonate [11] were added to bioreactors as examples of methylating agents. The experiments were conducted to determine the extent of DMDS and DMTS formation by direct abiotic methylation of inorganic polysulfides in biofilms, under conditions approximately representing those in distribution systems. Results obtained after adding these chemical methylating reagents to bioreactors (Table 7.6) showed that DMDS and DMTS formed at concentrations up to 2800 ng/L and 490 ng/L respectively. In these experiments, the primary mechanism of DMDS and DMTS formation was probably an abiotic reaction, involving nucleophilic attack of polysulfide anion on the methylating agent, rather than a microbially mediated reaction. However, the process of DMTS formation still requires the presence of biofilms, since inorganic polysulfides themselves are almost certainly the products of microbial activity (i.e. inorganic polysulfides are formed in biofilms via assimilatory or dissimilatory sulfate reduction).

The methylating agents formed lower concentrations of DMDS and DMTS than the methanethiol-containing compounds in Table 7.2, but these concentrations were still much higher than the odour threshold of DMTS (10 ng/L). In a previous study (Wajon and Heitz, 1995) methyl iodide was not detected in samples of distributed water, but the limit of detection of the analytical procedure used was relatively high (1 mg/L). Methyl iodide could

therefore have been present in these samples at concentrations up to this level, and methyl iodide and methyl p-toluenesulfonate were added to biofilms at approximately 1 mg/L (7 x 10^{-3} mM) and 0.1 mg/L (7 x 10^{-4} mM), concentrations approaching those that could reasonably be present in distribution systems. Even at these low concentrations DMTS was formed well above its odour threshold, showing that methylation of polysulfides by this route is a plausible mechanism of formation of DMTS in the distribution system.

Table 7.6: Concentration of DMDS and DMTS formed in bioreactors after addition of methylating reagents.

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
Control (Wanneroo water only)	8.6	1.5	10	1.5	9.0	3.0
Methyl iodide (7 x 10^{-3} mM)	13	4.4	19	9.4	310	130
Methyl iodide (7 x 10^{-4} mM)	20	5	7.3	2.0	110	44
[9] Methyl–p-toluenesulfonate (7 x 10^{-3} mM)	22	13	32	12	220	130
Methyl–p-toluenesulfonate (7 x 10^{-4} mM)	39	83	21	12	80	220
[10] Methyl sulfate (1 mM)	<200	<50	240	90	530	160
[11] Methylmethanesulfonate (1mM)	<50	<10	360	170	2800	490

Note: Numbers in square parentheses relate to structures in Section 7.5.

The content of inorganic polysulfide in biofilms was tested further by analysing biofilm that had adhered to one of the bioballs contained in the bioreactor. A bioball was removed from each of three bioreactors, suspended in 130 mL of reactor water in 10% sodium chloride (added to quench microbial activity), to which was added methyl iodide (0.05% v/v). Yields of DMDS were 3200-9800 ng/L and yields of DMTS were 2000-12000 ng/L, as listed in Table 7.7, showing that the biofilms contained significant levels of inorganic polysulfides.

Table 7.7: Concentrations of DMDS and DMTS formed from reaction of methyl iodide with a bioball selected from each of three bioreactors.

Reactor #	DMDS	DMTS	DMDS + DMTS
	(ng/L)	(ng/L)	(nM)
1	5400	1900	73
2	9800	9300	180
3	3200	12000	130

Abiotic reactions of some methylated sulfur compounds with polysulfide

The reactivity of some methylated oxysulfur compounds with inorganic polysulfides was investigated in order to determine which, if any of these would form DMPSs in the absence of biofilms. Polysulfide solutions (0.015 mM S) at pH 6.86 were added to aliquots of each compound listed in Table 7.8 under oxygen-free conditions. The mixtures were allowed to react for twenty four hours before analysis for DMDS and DMTS. The structures of all compounds are shown in at the end of the present Chapter.

The results listed in Table 7.8 show that under the test conditions, methylsulfoxy compounds can act as methylating agents where a methyl group is bound to an oxygen atom, but not where the methyl group is bound to a sulfur atom. In dimethylsulfoxide, methanesulfonic acid (MSA) and methanesulfonylchloride all methyl carbon atoms are bound directly to a sulfur atom. None of these compounds produced DMDS and DMTS when reacted with the solution containing inorganic polysulfides. However, both dimethylsulfate and methylmethanesulfonate produced significant concentrations of DMDS and DMTS, comparable to methyl iodide. In both of these compounds at least one methyl carbon atom is bound to sulfur via an oxygen atom. In the case of methyl iodide around 100% of the polysulfide was methylated and recovered as DMDS and DMTS, as shown by the amount of sulfur recovered in mM (0.017 mM sulfur recovered vs 0.015 mM sulfur originally added as polysulfide). Methylations of polysulfide involving

dimethylsulfate and methylmethanesulfonate were not as efficient, with only 0.013 and 0.009 mM, respectively, of sulfur recovered as DMDS and DMTS.

Table 7.8: Concentration of DMDS and DMTS formed from the abiotic reaction of methyl iodide or methylated sulfur compounds with disodium-polysulfide (0.015 mM).

	Concentration of DMPS formed					
	Control (no polysulfide added)		Polysulfide added			
Compound added (concentration)	DMDS (µg/L)	DMTS (µg/L)	DMDS (µg/L)	DMTS (µg/L)	DMDS +DMTS (mM)	S (mM)
[a][12] Dimethylsulfoxide (0.02 mM)	0.37	0.155	0.37	0.052	<0.0001	<0.0001
[a][14] Methanesulfonic acid (0.02 mM)	0.05	0.043	0.057	0.049	<0.0001	<0.0001
[a][15] Methanesulfonyl chloride (0.02 mM)	0.045	<0.02	0.030	0.025	<0.0001	<0.0001
[b][16] Dimethylsulfate (0.02mM)	0.06	0.022	265	315	0.0053	0.013
[ab][11] Methylmethane-sulfonate (0.02 mM)	0.063	<0.02	185	210	0.0036	0.009
Methyl iodide (0.03 mM)	0.06	0.030	300	455	0.0068	0.017

Notes: [a] Compounds where methyl group(s) are bound to a sulfur atom.

[b] Compounds where methyl groups are bound to an oxygen atom.

Numbers in square parentheses relate to structures in Section 7.5.

These results support the suggestion that in the case of the tests listed in Table 7.6 (methylating reagents), the dominant mechanism of DMDS and DMTS formation was probably abiotic methylation of polysulfides rather than a microbially mediated process.They also explain why DMSO and methyl sulfone do not form DMPSs; the compounds do not react abiotically with polysulfides and the enzyme systems required for their reduction and transformation do not exist in the biofilm populations used for the experiments. The results also confirm that partially oxidised sulfur compounds which might form if methanethiol-containing species were

subjected to water treatment processes could not form DMPSs without some kind of microbial reduction.

7.3.5 Formation of DMTS via biomethylation and detoxification

Biomethylation occurs as a response to many substances that are toxic to organisms. These include metals such as mercury, arsenic and tin, and organic compounds such as phenols and chlorophenols (Nystrom et al., 1992; Fatoki, 1997). Sulfide may also be methylated in a direct response to its toxicity to some organisms (Watling and Harper, 1998). The most common enzyme to effect methylation of anionic species such as sulfide is the sulfonium compound S-adenosylmethionine (SAM). This enzyme can donate a methyl carbocation and therefore reacts readily with strong nucleophiles, such as sulfide and polysulfides (Fatoki, 1997). It has been shown that these types of methylation reactions are competitive and non-specific (Harper, 1993), and consequently, strongly nucleophilic substances will be methylated as a result of an organism's attempt to detoxify some other substance.

Selenate, sulfide and trichlorophenol [17] are among compounds known to induce biomethylation in a variety of organisms, including fungi, actinomycetes and other microorganisms (Montiel et al., 1999; Nystrom et al., 1992; Fatoki, 1997). Both fungi and actinomycetes are known to be present in biofilms within Perth distribution systems (Neil, 1987). These substances were added to bioreactors in order to determine whether organisms capable of biomethylation might be active in the biofilms and whether DMDS and DMTS could form as a side-reaction to biomethylation of non-sulfur compounds. Results of experiments using sodium selenate (Na_2SeO_4) and trichlorophenol are shown in chromatograms in Figures 7.4 (a-d) and production of DMDS and DMTS in each of these three experiments is shown in Table 7.9.

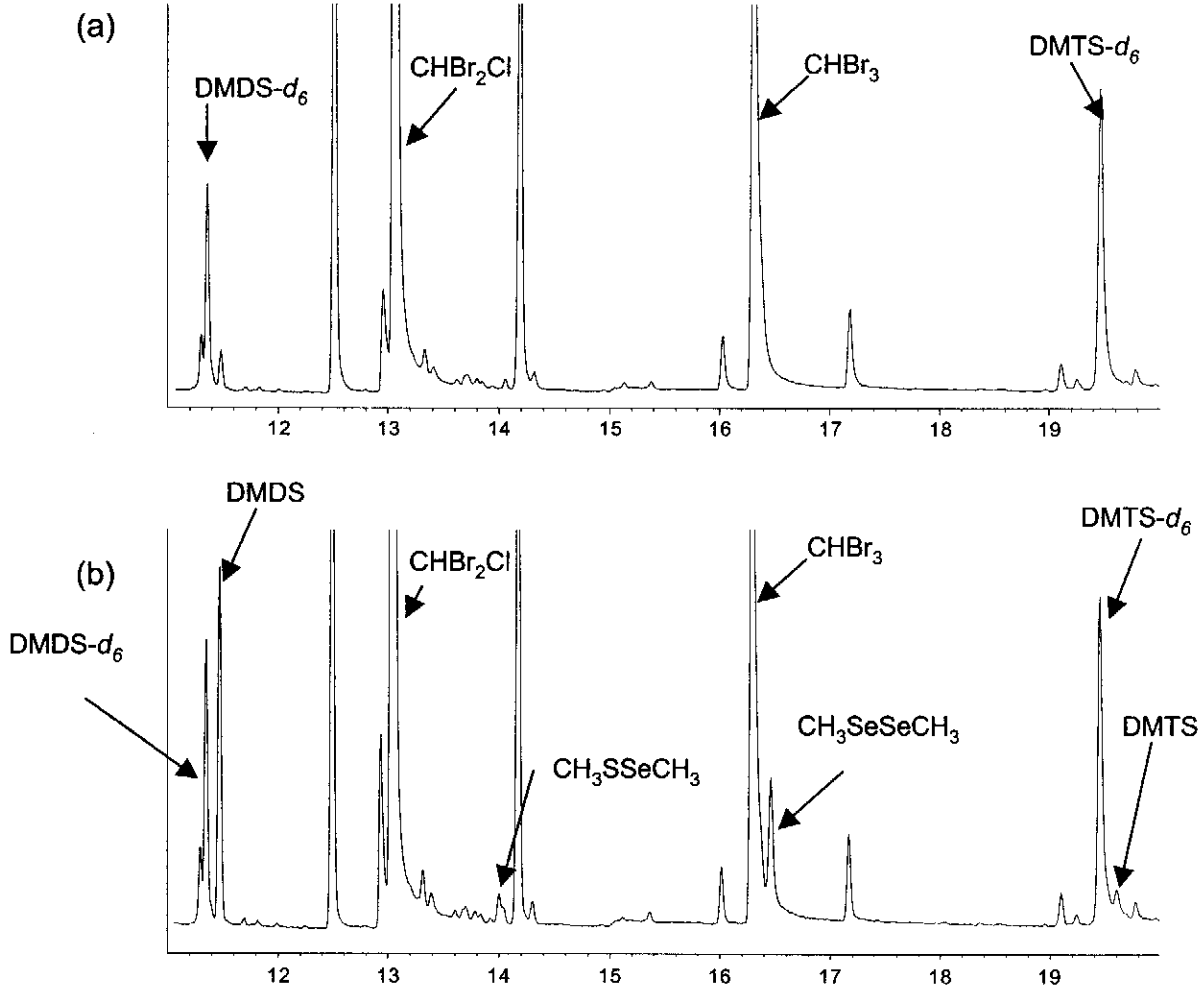


Figure 7.4 (a) and (b): Total ion chromatograms of purge and trap extracts of water from bioreactors to which sodium selenate (1mM, duplicate 2 in Table 7.9) had been added. (a) at time = 0 hours; (b) at time = 4 hours.

Figure 7.4 c,d

Table 7.9: Concentrations of DMDS and DMTS formed in bioreactors after the addition of compounds known to induce biomethylation.

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
Control (Wanneroo water only)	24	3.1	23	1.5	24	2.6
*Sodium sulfide (10 mM)	*34	*43	*41	*90	*120	*1300
Sodium selenate (1 mM) (duplicate 1)	56	13	6.1	<1	250	47
Sodium selenate (1 mM) (duplicate 2)	2.8	1	6.6	<1	50	7.4
Selenium methionine (7×10^{-3} mM)	n.d.	n.d.	16	<1	1600	230
[17] 2,4,6-Trichlorophenol (1 mM)	40	9.6	27	4.3	117	35
2,4,6-Trichlorphenol (7×10^{-3} mM)	15	2.9	15	3.6	1100	43

Notes: * Results are questionable due to interference of sulfide with the analytical method, as discussed in text.
n.d. = not determined.

Methylation of sulfide

Addition of sodium sulfide (10 mM) to bioreactors induced methylation to produce DMDS and DMTS at 120 and 1300 ng/L respectively. It should be noted that sulfide at these high concentrations interferes with quantification because it reacts with internal standards. This is discussed further in Section 7.3.6. Nevertheless, it appears certain that contact of sulfide with biofilm did induce some degree of methylation since concentrations of DMDS and DMTS in samples at t = 4 hours are much higher than those at t = 0 hours and those in the control experiment.

Drotar et al. (1987) established that a wide variety of bacteria from numerous sources were able to methylate sulfide added to cultures, forming MT. The study showed that methyltransferase activities occur in many heterotrophic

aerobic bacteria and facultative anaerobes isolated from soil, water, sediment, vegetation and algal cultures and that in at least some of these organisms methyltransferase activity was S-adenosylmethionine dependent. Wilkins (1996) reported the production of DMDS, DMTS and DMTeS by seven isolates of actinomycetes cultured on agar, but did not mention possible formation mechanisms. As discussed in Chapter 6, the activity of SRB in biofilms in contact with aerated water can produce sulfide within the biofilm at concentrations up to 200 μM (Okabe et al., 1998; Kühl and Jorgensen, 1992). Neil (1987) reported the presence of many types of heterotrophic bacteria, SRB and actinomycetes in biofilms in the Perth distribution system. Barton (1995a,b,c) found further evidence of the presence of SRB. As discussed in Chapter 6, inorganic polysulfide, which would behave analogously to sulfide in the presence of methyltransferases, occurs at concentrations of up to 62 μM in biofilms in Perth distribution systems. There is therefore a strong possibility that the combined activity of SRB and methytransferase-containing bacteria in biofilms contributes to DMDS and DMTS formation in the distribution systems of Perth.

Reduction and methylation of selenate

Chromatograms in Figures 7.4 (a) and (b) show that bioreactors to which selenate had been added produced small but significant amounts of methylated selenium and sulfur compounds including dimethyldiselenide, DMDS, DMTS and dimethylselenylsulfide (CH_3SeSCH_3). Selenate (SeO_4^{2-}) is chemically similar to sulfate and, like molybdate, acts as a sulfate analogue in the metabolism of sulfate reducing bacteria (Banat and Nedwell, 1984). The reduction of selenate to the organoselenium compounds $CH_3SeSeCH_3$ and CH_3SeSCH_3 may be indicative of activity by sulfate reducing bacteria. Methylation presumably occurred as a response to the toxicity of selenium (Greenwood and Earnshaw, 1984) and may well have been effected by a different group of microorganisms. The production of these methylated selenium and sulfur compounds demonstrates the capacity of the biofilm population to perform both reductions and methylations, two transformations that must occur in the distribution system to form DMTS.

Methylation of 2,4,6-trichlorophenol (TCP)

Chromatograms in Figures 7.4 (c) and (d) and results in Table 7.9 show that bioreactors to which 2,4,6-TCP had been added produced 2,4,6-trichloroanisole as well as DMDS and DMTS. As in the case of selenate, it is presumed that the addition of TCP induced methylation in the biofilm as a detoxification response. An increase in the concentration of TCP from 7×10^{-3} mM to 1 mM did not substantially alter the production of TCA or DMDS and DMTS, and from this it can be inferred that the limiting factor for production of the methylated compounds is biofilm activity.

Biomethylation targets a wide range of substrates

The formation of DMDS and DMTS, concomitant with that of methylselenides and trichloroanisole, shows that methylation was indiscriminate, targeting polysulfides as well as selenium and trichlorophenol. Several studies have shown that enzyme systems responsible for transmethylation are not highly specific. Harper (1993) reported the production of methyl chloride and concomitant methylation of various aromatic acids by the fungus, *Phellinus pomaceus* when mixtures were incubated with labeled methionine. It appears that methionine was converted to methyl chloride, which was possibly a metabolic intermediate in methylation reactions in fungi and higher plants. In a similar study Harper and Kennedy (1986) found that thiocyanate ion (SCN^-) inhibited production of methyl halides and aromatic acids, presumably because thiocyanate is the stronger nucleophile and was able to compete for methyl groups. The results of these studies suggest that there is broad substrate specificity for methylation by fungi and other organisms, and this also appears to be the case in the present experiments, although in the present studies it is not known which microorganisms might be involved. As reported by Neil (1987), biofilms and sediments sampled from Perth water mains contained large numbers of heterotrophic bacteria, SRB and iron bacteria as well as actinomycetes and fungi. Many of these microorganisms may have the capacity for biomethylation. The activities of these microbes in

the presence of high concentrations of polysulfides in the biofilm would result in the formation of DMDS and DMTS.

7.3.6 Formation of DMDS and DMTS from other organic and inorganic sulfur compounds

A range of organic sulfur compounds including several thiols, thiodipropionic acid [21] and thiophene [23] were tested for their propensity to form DMPSs in bioreactors. The response of the biofilm to these compounds was variable, although all compounds produced some DMDS and DMTS (Table 7.10). Compounds possessing an ω-thio group such as cysteine [18] and 3-mercaptopropionate (3-MPA) [19] formed copious quantities of DMDS and DMTS (1300 and 1400 ng/L, 460 and 210 ng/L, for cysteine and 3-MPA respectively).

Table 7.10: Concentrations of DMDS and DMTS formed in bioreactors after addition of some organic and inorganic sulfur compounds.

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
[18] Cysteine (1 mM)	<100	<50	<100	<50	*1300	*1400
[19] 3-Mercaptopropionate (1 mM)	160	40	150	30	*460	*210
[20] Thiolactic acid (1 mM)	<20	<10	<20	<10	40	10
[21] Thiodipropionic acid (1 mM)	<20	<10	<20	<10	120	40
[22] Methylthiosalicylate (7×10^{-4} mM)	*5.8	*3.0	*1.4	*<1	*63	*150
[23] Thiophene (1 mM)	<50	<10	<50	<10	130	10
Tetrathionate (1 mM)	<50	<20	<50	<20	50	20

Note: * Results are questionable due to the interference of sulfide with the analytical method. Deuterated DMPSs used as internal standards were partially converted to compounds containing one deuterated and one non-deuterated methyl group. This suggests that methyl mercaptam and/or hydropolysulfide anion were produced during the course of the reaction, resulting in rearrangement of DMPSs during analysis. Samples emitted a strong odour of hydrogen sulfide.

Cysteine, 3-MPA and methylthiosalicylate [22], the compounds that produced the most DMDS and DMTS also appeared to produce large quantities of sulfide in the bioreactors. Water samples from bioreactors to which these compounds had been added had a strong odour of hydrogen sulfide. Upon analysis for DMDS and DMTS it was found that methyl groups of the perdeuterated DMDS and DMTS added as internal standards prior to extraction, had been partly exchanged with native methyl (see chromatogram in Figure 7.5). This did not occur in the case of any of the other compounds tested except for sulfide, and it was inferred that catabolism of the thiols resulted in production of sulfide. As discussed in Section 7.3.2, sulfide is able to displace methyl sulfide moieties from DMPSs [eq 7.1 (b)], and the presence of sulfide in mixtures of native and perdeuterated DMDS and DMTS would therefore result in the mixed species observed in Figure 7.5.

The production of hydrogen sulfide via breakdown of protein-derived thiols such as cysteine and 3-MPA by heterotrophic bacterial metabolism is well accepted (Dunette, 1989). Dunette (1989) investigated conversion of proteinaceous sulfur to sulfide by proteolytic bacteria in freshwater sediments. It was established that putrefaction of endogenous protein contributed to between 5% and 57% of the total hydrogen sulfide production in the lake sediments studied. Minami et al. (1993) measured release of hydrogen sulfide, carbonyl sulfide and carbon disulfide from waterlogged rice paddy soils after incubation with cysteine and cystine. Kiene et al. (1990) showed that cysteine was transformed to mercaptopyruvate and mercaptoethanol and then oxidised to mercaptoacetate in anoxic coastal sediments. Mercaptoacetate was further metabolised, and probably eventually mineralised to carbon dioxide, with the release of hydrogen sulfide. Taylor (1993) postulated that SRB might effect the latter transformation. Generation of hydrogen sulfide and pyruvate from cysteine, with mercaptopyruvate as an intermediate also occurs in mammalian tissues (Taylor, 1993, and references therein). Ginzburg et al. (1999) suggested that microbial digestion of cysteine in cultures of aerobic bacteria from lake water produced sulfide, which then produced dimethylsulfides upon addition of methyl iodide. Catabolism of 3-MPA, an intermediate in the breakdown of

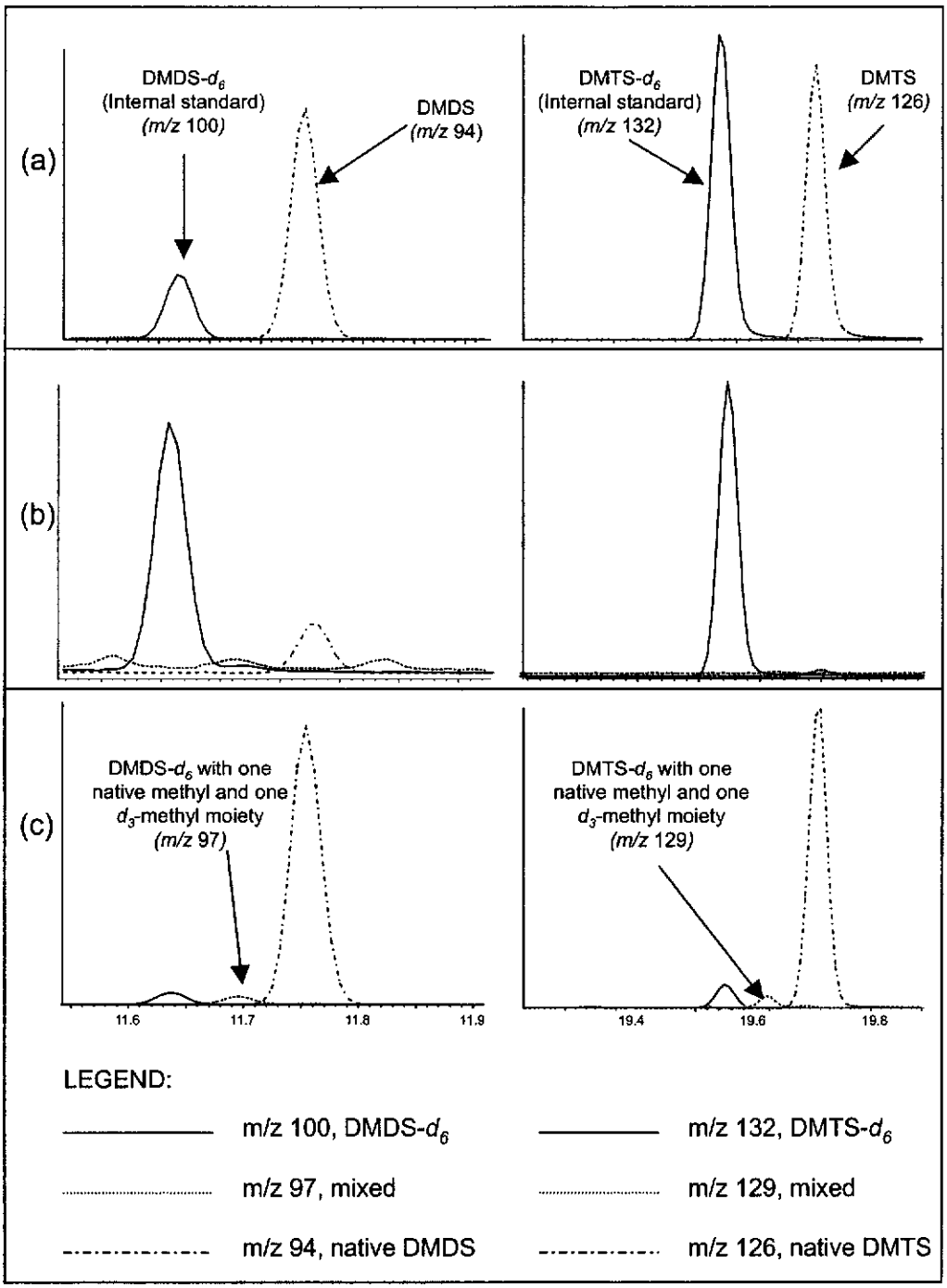


Figure 7.5. Selected ion chromatograms of (a) quantification standards, (b and c) extracts isolated from water from bioreactor amended with cysteine after 0 hours (b) and after 4 hours (c). Chromatograms in (c) show that DMDS (molecular ion m/z 97) and DMTS (molecular ion m/z 129) species possessing mixtures of native methyl and methyl-d_3 moieties were produced.

homocysteine, methionine and DMSP results in the formation of acrylate and the elimination of hydrogen sulfide (Taylor, 1993; Kiene et al., 1990).

As discussed in Section 7.3.5, the addition of sulfide induced the generation of dimethylpolysulfides in bioreactors, possibly as a response to its toxicity. The formation of DMDS and DMTS in the present experiments appears to be associated with release of sulfide from the added thiol precursors. This probably stimulated methylation in the same way as in the previous experiment, where only sulfide was added. Further experimentation using labelled thiols (e.g. with ^{34}S) might help to clarify the mechanisms involved.

Other compounds such as thiophene, in which sulfur is bound as a heterocycle, and thiodipropionic acid where sulfur is bound to two carbon atoms, produced only low concentrations of DMDS and DMTS. These compounds do not contain the biologically labile terminal sulfhydryl groups present in cysteine and 3-MPA and did not appear to produce significant quantities of sulfide. Notably, the yield of dimethylsulfur compounds was markedly different for the two mercaptopropionic acid isomers, 3-MPA and thiolactic acid (2-mercaptopropionate). The latter produced only 40 and 10 ng/L of DMDS and DMTS respectively, compared with 460 and 210 ng/L for 3-MPA. This observation demonstrates the significance of the ω–thiol group in production of dimethylsulfur compounds. Interestingly methylthiosalicylate, in which the sulfhydryl group is bound to an aromatic ring, produced sulfide and some DMDS and DMTS (63 and 150 ng/L, respectively).

Tetrathionate was included in the tests of potential DMTS precursor compounds as an example of a relatively highly oxidised inorganic sulfur compound. Almost negligible concentrations of DMDS and DMTS were produced from this compound.

7.3.7 Transmethylation reactions involving methoxyaromatic moieties

Some strains of SRB are able to mediate transmethylation reactions, where methyl groups in methoxyaromatic compounds such as syringic acid and

trimethoxybenzoate react with sulfide to form DMS and MT. These are obligate anaerobes that inhabit both freshwater and marine environments. The organisms utilise the organic carbon in the methoxyaromatic compounds for growth and may also use analogous hydroxyaromatics as substrates, in which case methylated sulfur compounds do not form. The major metabolic end-products of the substrate degradation were acetate and also, in the case of methoxyaromatics, methylated sulfur compounds (Bak et al., 1992; Bak and Finster, 1993).

Syringic acid [26] and 1,2,3-trimethoxybenzene [27] were added to bioreactors to investigate the possibility that methylation of sulfide and polysulfide might occur in our biofilms via metabolism of methoxyaromatic compounds. Results listed in Table 7.11 show that only minimal production of DMDS and DMTS occurred. Even when the experiment using trimethoxybenzene was repeated with tetrathionate as an added source of sulfur, production of methylated polysulfides was very low at 70 ng/L for DMDS and <20 ng/L for DMTS. The results indicate that methoxyaromatic compounds are probably not significant in the production of DMTS in the present biofilm systems. This is in agreement with discussions in Chapter 5, where it was found that methoxyaromatic moieties were not abundant in aquatic NOM entering Wanneroo GWTP.

Table 7.11: Concentrations of DMDS and DMTS formed in bioreactors after addition of methoxyaromatic compounds.

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
[27] 1,2,3-Trimethoxybenzene	-	-	24	5.5	40	18
Tetrathionate (1mM) + 1,2,3 trimethoxybenzene (1mM)	<50	<20	<50	<20	70	<20
[26] Syringic acid			<50	<10	<50	<10

7.3.8 Effect of increase in dissolved organic carbon (DOC) on production of DMDS and DMTS

Several studies have established that biofilm activity and growth are stimulated by increases in bioavailable organic matter in the bulk water phase (Le Chevalier et al., 1990; van der Kooij, 1992; Volk and Le Chevalier, 2000; Escobar and Randall, 2001). According to Le Chevalier (1990) optimum conditions for microbial growth in distribution systems are the presence of accumulated organic matter and sediment in pipes, low chlorine residuals, high water temperatures and the availability of nutrients (N, P, C). For heterotrophic plate count (HPC) bacteria the required ratio of these is 100:10:1 (C:N:P) and organic carbon is therefore usually the growth limiting nutrient (Le Chevalier, 1990). Bioavailable organic matter is generally divided into two fractions, assimilable organic carbon (AOC) and biodegradable organic carbon (BDOC). AOC is considered to be the fraction of dissolved organic carbon (DOC) which is assimilated and utilised for growth by microbes, while BDOC is the fraction which is mineralised through respiration (i.e. converted to CO_2) (Volk and LeChevalier, 2000). These fractions usually comprise only a few percent of the total dissolved organic carbon (Le Chevalier, 1990; van der Kooij, 1990).

Most of the organic matter in drinking water sources originates from highly degraded plant and animal material and consists of humic substances, hydrophilic acids, carbohydrates, amino acids and carboxylic acids (Volk and LeChevalier, 2000). However, the organic matter that eventually finds itself into the distribution system may have been substantially altered by treatment and disinfection processes and may be quite different to the original material. The exact nature of the organic molecules comprising AOC and BDOC is poorly understood, but it is expected that water-soluble compounds such as carbohydrates, carboxylic acids and amino acids would predominate (Volk and Le Chevalier, 2000). In the present study glucose [25] and betaine [24] were tested in bioreactors, as compounds which might represent easily available organic matter. Also tested were Aldrich humic acid and tannins

extracted from E. *camaldulensis*. These materials are broadly representative of humic substances in raw water, as discussed in Chapter 3.

Betaine, in addition to being a source of easily biodegradable organic carbon (as are most of the other precursors tested, for that matter), has been shown to stimulate DMS formation in seawater samples (Kiene and Service, 1993). Betaine is an algal osmolyte and a functional analogue of DMSP. Like DMSP it is present as an extracellular substance in seawater as a result of algal exudation and cell disruption and may be consumed by organisms either for osmotic purposes or as a methyl substrate. Kiene and Service (1993) found that the increase in DMS formation was not attributed to a general increase in microbial activity because non-methylated organic substrates such as glucose, acrylate and glycine had no effect. Other methylated quaternary ammonium compounds also stimulated production of methyl sulfur compounds. Betaine was tested in our systems to see whether methylated quaternary ammonium compounds could act as methyl donors to produce DMDS and DMTS from the polysulfide known to be present in biofilms.

Results of the addition of glucose, betaine, Aldrich humic acid and tannin are shown in Table 7.12. In the case of glucose the flow in the bioreactor was halted for twenty-four hours, instead of four hours, as was done in all other cases. The amount of DOC added was 60 mg/L (1 mM glucose), compared with a typical concentration of 2-5 mg/L DOC in Wanneroo water. In the glucose experiment considerable quantities of DMDS and DMTS were produced after eight hours, and these continued to increase for up to twenty-four hours (220 ng/L DMDS, 630 ng/L DMTS). Significantly, the control bioreactor, containing unamended Wanneroo water, stagnant for twenty-four hours, also produced appreciable quantities of DMDS and DMTS (290 ng/L and 470 ng/L respectively), although somewhat less than the glucose-treated experiment.

It appears that long periods of stagnation, as well as increased biofilm activity due to added organic carbon stimulate DMTS formation. Betaine stimulated

Table 7.12: Concentrations of DMDS and DMTS formed in bioreactors after the addition of compounds that increase DOC.

Compound added (concentration)	Before addition		t = 0 hours		t = 4 hours		t = 8 hours		t = 24 hours	
	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)	DMDS (ng/L)	DMTS (ng/L)
Control	7.6	1.9	13	2.0	-	-	82	39	290	470
[25] Glucose (1 mM, 60 mg/L DOC)	24	12	16	19	-	-	99	220	220	630
[24] Betaine (1 mM,48 mg/L DOC, 14 mg/L N)	<50	<10	340	110	1200	320	-	-	-	-
Tannins from E. *camaldulensis*	13	4.9	5.0	<1	29	7.7	-	-	-	-
Aldrich humic acid	13	3.2	24	1.0	120	26	-	-	-	-

formation of DMDS and DMTS to a greater degree than glucose (1200 and 320 ng/L for DMDS and DMTS respectively for betaine, versus 99 ng/L and 220 ng/L after 8 hours for glucose). This may have been due to a combination of an increase in AOC and the presence of methyl groups in betaine. These methyl moieties might participate in some kind of microbially mediated methylation process, such as that demonstrated by Keine and Service (1993).

Aldrich humic acid and the eucalypt tannin did not induce formation of DMDS and DMTS in bioreactors to any great extent, although there was a minor increase in activity in the Aldrich humic acid experiment. Humic substances are generally significantly biodegraded, and the organic carbon in these materials is probably not easily bioavailable. However reactions of humic substances with oxidants used in water treatment, such as ozone and possibly chlorine, result in increased AOC (Kuo et al., 1996). In order to gain a more complete understanding of the effect of these substances on DMTS formation, further experiments could be conducted, where NOM is subjected to chlorination prior to adding it to bioreactors.

Consumption of dissolved oxygen (D.O.) by biofilm activity

In the control experiment discussed above, where unamended Wanneroo water was held stagnant in the bioreactor, small amounts of hydrogen sulfide and elemental sulfur were also produced. Sulfide production probably occurred within the biofilm and this would have been oxidised to elemental sulfur. Bacterial respiration and sulfide oxidation would have resulted in depletion of oxygen from the bulk water in the bioreactor. The consumption of D.O. in the bulk water was further investigated by repeating the stagnation experiment with a D.O. probe installed in the bioreactor. The results of this experiment are plotted in Figure 7.6 and, although the amount of DMDS and DMTS produced is less than previously, there was clearly a concomitant decrease in D.O. with increasing DMDS and DMTS production.

Depletion of oxygen in the water distribution system is caused by respiration of BDOC by microorganisms and probably also by microbially driven nitrification and by oxidation of microbially produced sulfide. In the present study it was calculated that the rate of consumption of oxygen in the bulk water was 0.33 mg/L/hr between 8 and 12 hours after the commencement of stagnation (see Franzmann et al., 2001). Okabe et al. (1998) showed that in pipes containing water with 1.6 mg/L oxygen in the bulk water, the oxygen concentration within the biofilm can reach zero at about 0.4 mm depth. (Refer also to Kühl and Jorgensen, 1992; Kerneis et al., 1995). In the present experiment the oxygen concentration in the bulk water was less than 1.6 mg/L for some hours, decreasing to less than 0.4 mg/L and, therefore, oxygen must have been absent within the biofilms for at least some time. Anaerobic biofilm processes, such as sulfate reduction, are likely to increase in the absence of oxygen upon extended stagnation.

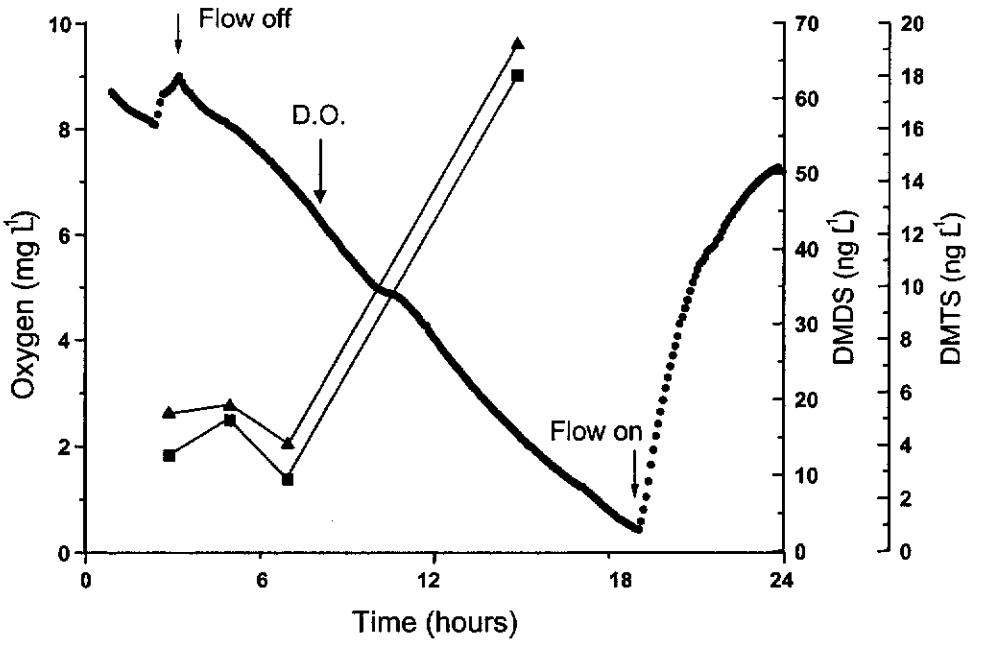


Figure 7.6. Change in concentrations of dissolved oxygen (D.O.) (●), DMDS (▲) and DMTS (■) within bioreactor containing unsupplemented Wanneroo water. Flow of water through the bioreactor was terminated for 16 hours.

In previous experiments where sulfide was added to bioreactors,

considerable DMTS production occurred. Since sulfide appears to be generated in the presence of biofilms during stagnation, increased anaerobic biofilm activity linked to oxygen depletion within the biofilms is probably partially responsible for DMTS formation. Measurements of D.O. in water from various points in the distribution system, especially at dead ends where stagnation is likely to occur, might therefore provide useful insights in further studies on the causes of DMTS formation.

7.3.9 Accumulation of trace metals in biofilms

The content of metal ions in biofilms is of interest since many metal ions are also known to undergo biomethylation in environmental systems (Fatoki, 1997). Further, it is thought that some metals, such as ferrous iron, can affect rates of sulfide oxidation in anaerobic systems (e.g. Steudel, 1996). Therefore, knowledge of metal concentrations in biofilms could provide useful insights into factors which might affect the chemistry and biochemistry of sulfur in these systems.

Concentrations of metals in biofilms collected from bioreactors on two occasions (23rd April 1999 and 12th May 1999) are listed in Table 7.13. A total of forty seven metals were measured, but only those metals detected at concentrations greater than 10 μg/g and those known to induce biomethylation, such as arsenic, antimony, tin and mercury are reported. Samples of biofilm were collected from suspended bioballs and from the material that had accumulated at the base of each of the three bioreactors. Bioreactor 1 had been fed with Wanneroo water only, and Bioreactors 2 and 3 had been fed with the various test compounds as discussed in previous sections. The samples of biofilm were dried, subjected to acid digestion and analysed by inductively coupled plasma-mass spectrometry (ICP-MS) and flame atomic absorption spectroscopy (FAAS).

Many metals were present in biofilms at concentrations far in excess of those that would be expected in the bulk water, demonstrating the immense capacity of the biofilm to sequester these substances from the flowing water.

Table 7.13: Concentrations of metals in biofilm samples taken from bioreactors (23-4-99, 12-5-99). Concentrations in $\mu g\ g^{-1}$ (ppm), dry weight.

Bioreactor #	Date	Ti	Cr	Co	Ni	Cu	Zn	As	Se
(location)									
1 (bioball)	23-4-99	420	160	19	41	2000	2900	9.1	2.2
1 (base)	23-4-99	400	170	15	37	2000	3300	4.5	2.4
1 (bioball)	12-5-99	470	120	22	23	1100	2500	95	0.8
1 (base)	12-5-99	480	220	16	71	2200	3500	11	2.7
2 (bioball)	23-4-99	450	1300	40	370	1100	4000	8.6	0.9
2 (base)	23-4-99	210	100	40	2800	2800	8300	5.9	1.2
2 (bioball)	12-5-99	350	190	100	28	1500	1700	33	0.6
2 (base)	12-5-99	350	670	94	120	1400	1900	13	1.0
3 (bioball)	23-4-99	330	100	16	14	2200	2300	5.0	38
3 (base)	23-4-99	230	150	19	220	3400	7500	5.3	240
3 (bioball)	12-5-99	540	260	33	59	2900	2800	9.7	26
3 (base)	12-5-99	360	120	28	44	2600	4500	4.8	150

Table 7.13 (continued)

Bioreactor #	Date	Mo	Ag	Cd	Sn	Sb	Hg	Pb	Fe	Mn
(location)										
1 (bioball)	23-4-99	12	13	1.8	14	0.4	1.1	700	3200	1700
1 (base)	23-4-99	11	19	1.8	9.6	1.2	0.84	660	34000	850
1 (bioball)	12-5-99	5.1	10	2.0	41	8.3	7.1	680	51000	7200
1 (base)	12-5-99	17	31	3.3	24	1.6	0.83	790	44000	1800
2 (bioball)	23-4-99	42	10	1.6	0.9	0.5	0.53	730	39000	4800
2 (base)	23-4-99	400	26	5.9	2.8	0.9	1.1	460	16000	480
2 (bioball)	12-5-99	5.9	6.6	0.8	16	1.9	3.6	570	33000	1900
2 (base)	12-5-99	28	13	1.6	0.7	2.2	2.7	700	38000	1700
3 (bioball)	23-4-99	6.7	12	1.3	0.0	0.2	0.55	530	21000	2100
3 (base)	23-4-99	22	21	6.3	12	0.5	0.64	470	17000	2100
3 (bioball)	12-5-99	9.2	13	4.7	6.6	0.3	0.53	730	38000	5700
3 (base)	12-5-99	7.8	17	7.9	17	0.6	1.8	530	26000	5600

Iron was the most abundant metal, constituting up to 4% dry weight in some samples. Other metals, including copper, zinc, lead and manganese were also present at significant levels. The high concentrations of iron and manganese were in agreement with the high (percentage) levels of these elements found in distribution system biofilms (Chapter 6). Concentrations of metals between samples were quite variable, probably reflecting the heterogenous nature of biofilms. For example, concentrations of chromium were less than 200 μg/g in five out of six samples, but increased to 1300 μg/g in one of the samples. The nickel content of samples also varied widely, from 14 to 2800 μg/g, as did that of molybdenum, tin, tungsten and manganese.

The concentrations of arsenic in samples taken from bioballs in Bioreactors 1 and 2 on 12th May are notable since they are considerably higher than in the other samples. Arsenic, like antimony, tin, tellurium and selenium is significant because the mechanism for methylation of this compound involves the enzyme S-adenosylmethionine, which is relatively non-specific and can also methylate sulfides and polysulfides (Fatoki, 1997; Drotar et al., 1987). Background concentrations of arsenic and selenium in groundwater feeding Wanneroo are around 2 μg/L or less, but up to 15 μg/L arsenic in water feeding Gwelup GWTP (Water Corporation, 1999). Concentrations of antimony, tin and tellurium have not been measured. It is conceivable that the presence of these elements in source waters might result in bioaccumulation in biofilms, to levels that induce methylation, and this in turn could form DMTS.

A significant memory effect was demonstrated in biofilms which had been exposed to selenium. Biofilm in Bioreactor 3 had been exposed to selenium, in the forms of selenium methionine and sodium selenate, added as test compounds. Biofilms in this reactor contained much higher concentrations of selenium than the unexposed biofilms in Bioreactors 1 and 2. Selenium compounds were added on three occasions, Se-methionine (1mM) on 9th March, and selenate on 11th and 18th March (1mM), but selenium concentrations were still significantly higher than in the unexposed biofilms

more than one month later, on 23rd April. This demonstrates the capacity of biofilms to sequester metals and also shows that relatively long periods of flushing are required to remove these substances completely from the systems. Selenium levels in samples taken on 12th May were only slightly lower than those taken three weeks earlier on 23rd April (150 *vs* 240 µg/g respectively, in samples taken from base of bioreactor). Interestingly, most of the selenium accumulated in sloughed off materials sampled from the base of the bioreactor.

7.3.10 Inconsistencies in bioreactor experiments

Reactivity of apparently structurally similar compounds appeared to vary considerably in the bioreactors, casting some doubt over reproducibility of the experiments. For example, methyl-3-(methylmercapto)propionate and methylmercaptoacetate yielded 0.47 and 0.038 % DMDS and DMTS respectively (Table 7.2). These two compounds differ in their structure only by one carbon atom, and it was therefore not expected that their reactivity would vary so much. Similarly, when the experiment with sodium selenate was repeated, concentrations of DMDS and DMTS were much lower than in the initial experiment (Table 7.9). Visual inspection of biofilms revealed that the amount of biomass varied between different reactors at different times and on occasions considerable quantities of biofilm sloughed off the bioball support and collected on the floor of the reactor.

The observed inconsistencies in the amounts of DMDS and DMTS produced were probably caused by a combination of factors including variation in biofilm activity and structure and in the amount of biomass present. Biofilm structure and growth would have been influenced by the recurrent periods of stagnation in the bioreactors, which were an integral part of the experiment, and possibly by the relatively high concentrations of some of the test compounds that were added. Some of the test compounds such as methionine and glucose would have acted as nutrients and others, such as the selenium compounds and trichlorophenol were toxicants. Even though bioreactors were flushed with Wanneroo water for at least three days after

each experiment to allow biofilms to stabilise and recover, it is apparent (e.g. from the concentration of selenium found in biofilms to which this element had been added) that memory effects exist in these systems for considerable periods. Changes in other conditions such as quality and temperature of the feed water may also have had some influence. Despite the apparent problems with reproducibility the outcomes of experiments were generally sufficiently clear to meet the aims of the study.

7.4 CONCLUSIONS

The odorous DMTS was formed in biofilms which are likely to be similar to those in distribution systems upon the addition of a wide variety of precursor compounds. The addition of these potential precursors showed that the DMPSs can be formed via a number of mechanisms.

1. Microbial metabolism of methanethiol-containing compounds such as methionine to release methanethiol, with subsequent oxidation, dimerisation and incorporation of an addition sulfur atom to form DMDS and DMTS. Methanethiol-containing compounds are likely to be rapidly oxidised by chlorine during water treatment and converted to compounds which do not form DMDS and DMTS in the bioreactors. If methanethiol-containing compounds are indeed involved in the formation of DMTS in the distribution system, they must be formed within the biofilm, and probably do not originate from the source water.

2. Inorganic polysulfides formed by sulfate reducing bacteria in the biofilm can undergo abiotic methylation with chemical methylating reagents originating from source water, and these precursors have the potential to form low concentrations of DMDS and DMTS. It is, however, not known whether methylating reagents exist in the source water at the required concentrations.

3. Biomethylation of polysulfides formed by sulfur or sulfate reducing bacteria in the biofilm may occur as a detoxification reaction (i.e. as a response to an agent that is toxic to part, or all of the microbial population). Selenium was shown to induce biomethylation reactions, but other metals such as arsenic could have a similar effect. It was demonstrated that biofilms have considerable capacity to sequester a wide range of metals including those which might induce biomethylation.

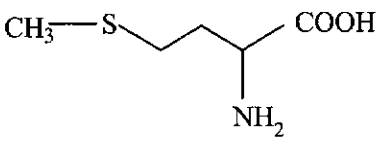
4. High concentrations of sulfide added to the bulk water can stimulate methylation, presumably due to the toxicity of sulfide. Sulfide generated from within the biofilm as a result of increased microbial activity, which may be stimulated by increasing the input of bioavailable organic carbon, appears to have a comparable effect. Sulfide is generated by catabolism of thiols containing an ω-thio moiety and probably also by sulfate reducing bacteria.

5. DMDS and DMTS also formed in bioreactors after long periods of stagnation (>16 hours), even when no precursor compounds were added. However, sulfide was produced in the biofilm in these experiments, so DMDS and DMTS might have been produced as a response to sulfide accumulation within biofilms, and not as a direct response to stagnant conditions.

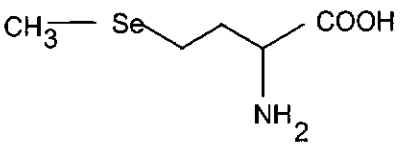
Potential precursor compound groups which were tested but did not form significant concentrations of DMDS and DMTS were (a) organic sulfur compounds where the sulfur atom was not bound as either an ω-thio or an ω-methanethiol moiety; (b) tetrathionate, a relatively highly oxidised inorganic sulfur compound; (c) partly oxidised methylsulfur compounds such as DMSO, where the sulfur atom is bound to one or more oxygen atoms and the methyl group is bound to sulfur, not oxygen; (d) methoxyaromatic compounds; and (e) humic substances and tannins.

In all cases formation of DMDS and DMTS required the involvement of biofilms. Hence it should be possible to limit DMTS production by reducing biofilm growth and activity through reduction of BDOC and AOC in water supplied to areas where the swampy odour problem occurs

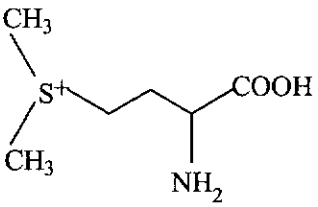
7.5 STRUCTURES OF TEST COMPOUNDS ADDED TO BIOREACTORS



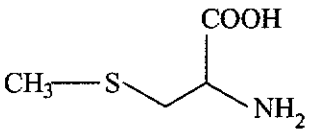
[1] methionine



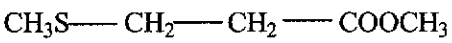
[2] selenium methionine



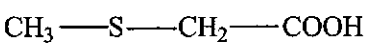
[3] S-methyl methionine



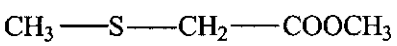
[4] S-methyl cysteine



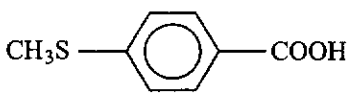
[5] methyl-3-(methylmercapto) propionate



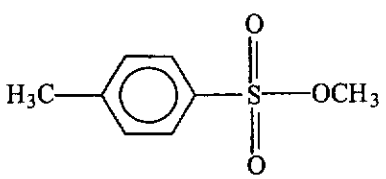
[6] (methylmercapto)acetate



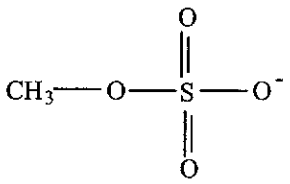
[7] methyl(methylmercapto)acetate



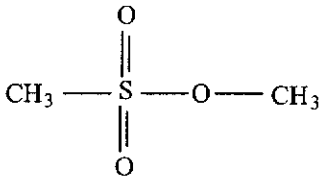
[8] 4-methylmercapto benzoic acid



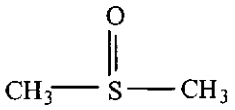
[9] methyl-para-toluenesulfonate



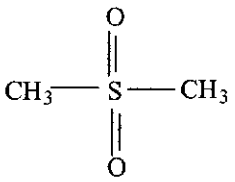
[10] methylsulfate



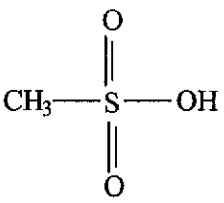
[11] methylmethanesulfonate



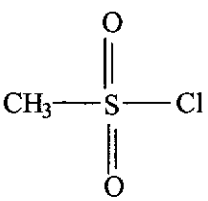
[12] dimethylsulfoxide



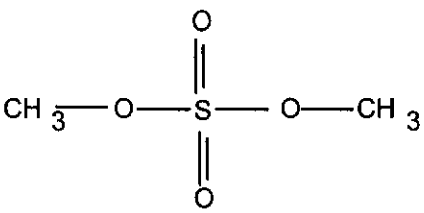
[13] dimethylsulfone



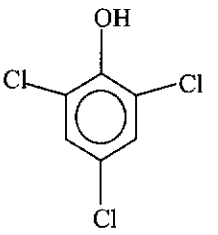
[14] methanesulfonic acid



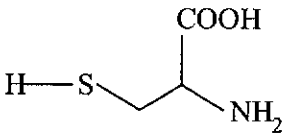
[15] methanesulfonyl chloride



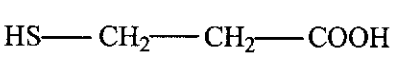
[16] dimethylsulfate



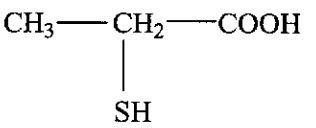
[17] 2,4,6-trichlorophenol



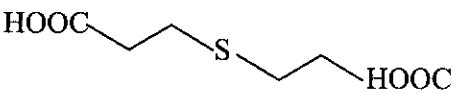
[18] cysteine



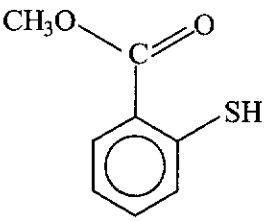
[19] 3-mercaptopropionate



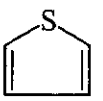
[20] thiolactic acid



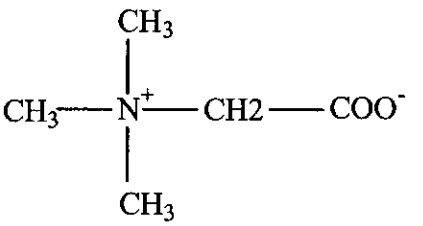
[21] thiodipropionic acid



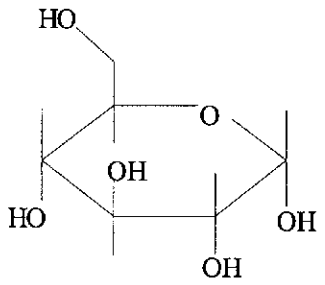
[22] methylthiosalicylate



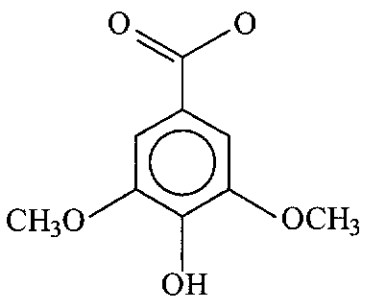
[23] thiophene



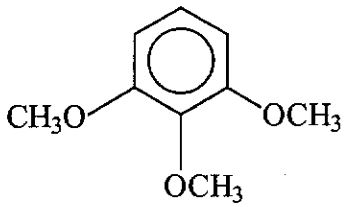
[24] betaine



[25] glucose



[26] syringic acid



[27] 1,2,3-trimethoxybenzene

CHAPTER 8

CONCLUSIONS

The research described in this Thesis has clearly linked the problem of DMTS formation with biofilm processes in the distribution system. While its exact formation mechanisms and the reasons for its occurrence only in distribution systems supplied with water from a few specific sources remain unknown, it was clearly established that the formation of DMTS requires the presence of biofilms. A new analytical method was developed that enabled quantitative determination of inorganic polysulfides in water and biofilm samples, and improvements were made to an existing method for analysis of DMPSs, to make it more robust, rapid and accurate. Using these new methods, it was demonstrated that inorganic polysulfides and their methylated analogues existed in pipewall biofilms in concentrations several orders of magnitude greater than those ever detected in the bulk water phase (Chapter 6). This finding provided strong evidence that DMTS detected in distributed water was produced within biofilms, rather than in the bulk water. DMTS was never detected in the absence of inorganic polysulfides in pipewall biofilm samples, suggesting that inorganic polysulfides are precursors to DMTS, or at least, that a strong link exists between production of DMPSs and inorganic polysulfides. Subsequent work, described in Chapter 7, showed that DMTS could form in the presence of biofilms when given a number of organic and sulfur-containing substrates, and under conditions of stagnation when no substrate was added. It was concluded that, while DMTS could form via a number of mechanisms, including from methanethiol-containing compounds, via direct methylation by abiotic methylating reagents and by biomethylation, the compound was not formed in the absence of biofilms.

It was also shown that several of the initially proposed hypotheses were probably not significant in DMTS formation. For example, no evidence was

found to support suggestions that methoxyl moieties in aquatic humic substances might contribute to the problem. Aquatic humic substances were isolated from surface water and groundwater samples, and analyses were carried out to determine some of the types of structural moieties present within these substances (Chapter 3). Although methoxyl moieties were not detected, compounds thought to be derived from 3-MPA were found, and these might indicate the presence of microbes that produce the osmolyte DMSP, which could conceivably affect DMTS formation in distributed water. Further, DMTS did not form in bioreactors to which compounds containing methoxyl moieties had been added (Chapter 7), showing that, even if these types of compounds were present in distributed water, it was unlikely that DMTS could be generated from them.

Although no strong evidence for a direct contribution to DMTS formation from aquatic humic substances was found, it was shown that distinct structural differences existed between humic substances originating from groundwater and surface water: For example, organosulfur moieties appeared to be far more abundant in NOM derived from groundwater, while surface water NOM appeared to have greater carbohydrate character. The existence of these differences in the chemical nature of NOM supports the hypothesis that a component of NOM might be a key factor in whether or not DMTS is formed in distributed water. Chemical and structural characterisation of aquatic humic substances from Western Australian environments had not been carried out previously, and useful new insights on the nature, chemistry and origin of these materials were obtained.

Another previously proposed hypothesis to explain DMTS formation in distributed water was that DMTS was formed from the traces of residual reduced sulfur originating from the source water, that were not removed at Wanneroo GWTP. Studies of the likely forms of reduced sulfur in groundwater, and their likely oxidation products (i.e. compounds that would be formed during water

treatment), found that the residual fraction of refractory reduced sulfur that is not removed by water treatment processes most probably comprises non-crystalline elemental sulfur and some OSC (Chapter 5). No evidence of any possible association between DMTS formation and any of these substances was found. Studies in Chapter 5, supported by experiments in Chapter 7, showed that, even if compounds containing methanethiol moieties (potential DMTS precursors) were present in raw water, these would be oxidised to substances that could not form DMTS. It is therefore unlikely that residual, incompletely oxidised forms of reduced sulfur (collectively termed NSRS) that enter the distribution system from Wanneroo GWTP have any effect on DMTS formation. This is significant because considerable efforts have previously been made towards the development of water treatment procedures that can remove NSRS from source waters, with the view that these might prevent DMTS problems (Kinhill 1992, 1994, 1996, 1998).

The present work has laid the foundations for proposed new treatment solutions to the DMTS problem. Pilot-scale water treatment processes designed to reduce concentrations of DOC, especially the bioavailable fractions (AOC and BDOC) and eliminate DMTS problems in distributed water are presently being tested at Wanneroo GWTP. The processes of sulfate reduction and biofilm growth are driven by the availability of organic carbon and other factors controlling biological regrowth. Decreases in DOC, AOC and BDOC in the bulk water are expected to minimise or eliminate DMTS problems in several ways; (a) less microbial activity would limit oxygen depletion in biofilms (oxygen depletion was shown to be linked to DMTS formation in Chapter 7); (b) decreasing the amount of AOC and BDOC should hinder formation of inorganic polysulfide (probable DMTS precursor) by limiting microbial activity; (c) decreasing DOC will allow chlorine residuals to persist for longer by reducing the chlorine demand of the water, and this will, in turn, limit microbial activity and oxidise any DMTS that is formed.

It is planned to treat drinking water to decrease the levels of DOC, AOC and BDOC entering the distribution system using either biological filtration with or without ozone, or by proprietary ion exchange processes (MIEX® resin), or by a combination of one or more of these. Consulaqua Consultants of Hamburg, Germany, commissioned by Water Corporation to independently assess research on DMTS commented "This understanding (i.e. the outcomes of the present Thesis) forms the basis for the strategy for optimising the treatment process at Wanneroo GWTP by enhancing NOM removal to significantly reduce the regrowth and biofilm formation potential."

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APPENDIX 1: Peak areas of pyrolysis products obtained from Py-GC-MS analysis of samples of aquatic NOM.

Table A1: Peak areas obtained from Py-GC-MS analyses of NOM samples isolated from groundwater and surface water.

Pyrolysis fragment	**Group in Figure 3.4**	**Peak areas**				
		Sample name (as shown in Figure 3.4)				
		W110	**W90**	**W50**	**Wanneroo clarifier sluge**	**Mundaring Weir**
		(a)	(b)	(c)	(d)	(e)
2-methylphenol	Tannin	16735	5134	18085	27221	18082
2,6-dimethylphenol	Tannin	1864	344	1878	2306	1958
2-ethylphenol	Tannin	1269	253	1482	1273	1410
dimethylphenol	Tannin	9305	1971	10545	8602	10790
dimethylphenol	Tannin	6422	2237	8319	9875	9527
dimethylphenol	Tannin	0	0	0	0	0
dimethylphenol	Tannin	0	0	0	0	0
dimethylphenol	Tannin	0	0	0	0	0
dimethylphenol	Tannin	1954	439	3485	0	2500
4-methylphenol	Tannin	37314	14757	38839	44779	58602
phenol	Tannin	71382	38780	67761	133917	107789
benzene	Benzene	83481	41029	29588	141578	32065
acetamidofuran	C	0	0	0	0	10280
furan	C	1571	1355	1847	12563	8944
1,3-cyclopentadiene	C	7612	3102	8839	28225	12210
2-methylfuran	C	2417	1449	2246	7700	8828
2-methylfuran	C	852	510	627	4943	1662
acetic acid	C	22334	14444	22548	90239	334888
dimethylfuran	C	1141	539	1159	1986	3304
3-methyl-cyclopentene-one	C	6497	1973	6230	4883	14068
2-acetylfuran	C	0	0	0	27	4421
methylfurancarboxaldehyde	C	0	0	0	95	10159
methylfurancarboxaldehyde	C	0	0	0	281	31062

Table A1 (continued)

Pyrolysis fragment	Group in Figure 3.4	Peak areas				
		Sample name (as shown in Figure 3.4)				
		W110	W90	W50	Wanneroo clarifier sluge	Mundaring Weir
methylfurancarboxaldehyde	C	0	0	0	80	101666
hydroxymethylcyclopenteneo	C	0	0	0	18	11313
furancarboxaldehyde	C	0	0	0	41	17129
furancarboxaldehyde	C	0	0	0	135	41888
trimethylfuran	C	0	0	612	93	970
methylcyclopenten-1-one	C	5569	1462	8171	14865	14030
toluene	Toluene	117820	48935	70079	122120	97976
propenenitrile	Protein	0	0	0	19	0
propanenitrile	Protein	8056	2819	4155	76	11411
2-methylpropenenitrile	Protein	0	0	0	178	763
pyridine	Protein	0	3467	0	277	14690
1H-pyrrole	Protein	5585	4528	6840	18181	30512
methyl-1H-pyrrole	Protein	3843	1557	3509	5975	6828
methyl-1Hpyrrole	Protein	2896	2013	3549	4820	5341
styrene	Protein	3265	2310	4098	10696	8854
2,3-dimethyl-1H-pyrrole	Protein	0	0	1071	1227	2499
methyl-benzonitrile	Protein	0	0	0	5007	1306
benzenepropanenitrile	Protein	0	0	285	0	2075
1H-indole	Protein	5093	3087	6815	8282	16560
3-methyl-1H-indole	Protein	3329	1898	3522	2280	7291
benzeneacetonitrile	Protein	1628	1513	2354	1963	6981
benzonitrile	Protein	0	2729	3151	27967	7798
ethylbenzene	Xylenes	3716	1102	2743	2826	3615
p-xylene	Xylenes	58312	14752	35291	35572	31896
m-xylene	Xylenes	13735	3546	10215	12141	9836
methylbenzothiophene	OSC	0	0	0	1465	0
COS	OSC	18503	36320	10395	22940	4776
SO_2	OSC	0	0	0	257719	0

Table A1 (continued)

Pyrolysis fragment	Group in Figure 3.4	Peak areas				
		Sample name (as shown in Figure 3.4)				
		W110	W90	W50	Wanneroo clarifier sluge	Mundaring Weir
MeSH	OSC	24907	35313	12440	16482	20621
dimethylsulfide	OSC	2870	3102	748	0	2387
thiophene	OSC	10493	7257	3923	11447	1196
methylthiophene	OSC	7819	2988	3354	5358	817
methylthiophene	OSC	6620	2651	2518	4455	582
ethylthiophene	OSC	2627	662	1097	973	0
2,5-dimethylthiophene	OSC	1852	770	858	1350	0
2,3-dimethylthiophene	OSC	3174	499	1304	997	0
ethenylthiophene	OSC	65	0	0	1002	0
benzothiophene	OSC	2964	1226	1644	8787	0
dibenzothiophene	OSC	0	0	0	3314	0
acetophenone	*aromatic ketone	0	0	1254	1658	1484
2,3-dihydro1-H-indene-1-one	*aromatic ketone	2524	610	3038	4681	3146
benzofuran	BF	17	819	1302	16852	0
7-methylbenzofuran	BF	1067	1187	870	2808	1158
2-methylbenzofuran	BF	4633	0	2136	6473	476
2-methylbenzofuran	BF	0	0	0	11115	0
dimethylbenzofuran	BF	547	162	429	4166	0
dimethylbenzofuran	BF	2157	513	1558	1414	1487
dibenzofuran	BF	0	1458	1619	19358	2670
C_3-benzene	C_3 B/HC	8257	2761	10083	2804	174
C_3-benzene	C_3 B/HC	6695	538	2366	1740	8260
C_3-benzene	C_3 B/HC	2563	2512	1788	1877	1908
ethenyl-methylbenzene	C_3 B/HC	2625	845	1260	3451	2171
C_3-benzene	C_3 B/HC	2011	909	12116	8117	9968
ethenylmethylbenzene	C_3 B/HC	0	380	2954	2227	1369
C_3-benzene	C_3 B/HC	15406	1472	4384	3809	4560

Table A1 (continued)

Pyrolysis fragment	Group in Figure 3.4	Peak areas				
		Sample name (as shown in Figure 3.4)				
		W110	W90	W50	Wanneroo clarifier sluge	Mundaring Weir
1H-indene	C_3 B/HC	7673	2825	8347	32016	5042
methyl-dihydro-indene	C_3 B/HC	671	0	556	470	481
methyl-indene	C_3 B/HC	5132	1119	5530	11402	0
methyl-indene	C_3 B/HC	5485	1228	6107	12983	4030
1-hexene	C_3 B/HC	0	193	583	27	936
1,3-cyclohexadiene	C_3 B/HC	0	0	3708	8141	2966
hexadiene-5-yne	C_3 B/HC	0	183	1248	3829	438
methylcyclopentadiene	C_3 B/HC	2837	896	4341	6229	0
methylcyclopentadiene	C_3 B/HC	2610	757	0	0	2622
cycloheptatriene	C_3 B/HC	0	0	1247	5424	0
methyl-1,3,5-hexatriene	C_3 B/HC	0	238	1408	2462	0
1,3,5-cycloheptatriene	C_3 B/HC	0	0	0	2657	0
azulene	PAH	0	1027	0	13252	94
naphthalene	PAH	23355	11021	15935	99221	12360
1-methylnaphthalene	PAH	10650	3387	8431	25328	6817
2-methylnaphthalene	PAH	5115	1484	5113	11892	4219
biphenyl	PAH	0	1261	1615	8117	2574
dimethylnaphthalene	PAH	412	387	944	2256	548
dimethylnaphthalene	PAH	4037	565	2085	2166	3380
dimethylnaphthalene	PAH	2677	609	1610	3738	2252
dimethylnaphthalene	PAH	2329	574	2816	3047	2556
dimethylnaphthalene	PAH	16263	693	6711	41037	9567
acenaphthylene	PAH	0	0	492	8351	0
dimethylnaphthalene	PAH	828	0	1028	1358	608
9H-fluorene	PAH	4177	1706	4711	18143	2856
1-methylfluorene	PAH	2688	625	2685	4984	1126
2-methylfluorene	PAH	1114	259	1455	2763	472
methylfluorene	PAH	604	171	941	2087	269
phenanthrene	PAH	3566	1763	3633	22277	2563

Table A1 (continued)

Pyrolysis fragment	Group in Figure 3.4	Peak areas				
		Sample name (as shown in Figure 3.4)				
		W110	W90	W50	Wanneroo clarifier sluge	Mundaring Weir
anthracene	PAH	1687	614	1381	13546	919
dimethylfluorene	PAH	0	0	1190	586	0
methyl-phen/anthrene	PAH	0	0	0	2801	0
methyl-phenanthrene	PAH	0	0	0	10251	0
methyl-phenanthrene	PAH	0	0	0	2801	0
4H-cyclopenta(def)phenanth	PAH	0	0	0	3303	0
methyl-phenanthrene	PAH	0	0	0	1387	0
methyl-phenanthrene	PAH	0	0	0	2885	0
phenyl-naphthalene	PAH	0	238	0	2695	0
fluoranthene	PAH	0	0	0	7149	0
pyrene	PAH	1096	0	1166	7150	0
benzo(b)fluorene	PAH	0	0	0	3138	0
1-methyl-pyrene	PAH	0	0	0	2146	0

Notes: 0 = not detected;

C = carbohydrate;

BF = benzofurans (included in Tannin group);

C_3 B/HC = C_3 alkylbenzenes and hydrocarbons;

OSC = organosulfur compounds;

PAH = polycyclic aromatic hydrocarbons.

* Data for aromatic ketones were not included in graphs in Figure 3.4. This was because (bio)polymer origins of these compounds were unknown, and because their contribution to the total amount of pyrolysis products was considered to be negligible.

Table A2: Total of all peak areas in each group, and total of all peak areas, as used to construct pie charts in Figure 3.4.

	W110 (a)	**W90** (b)	**W50** (c)	**Wanneroo clarifier sluge** (d)	**Mundaring Weir** (e)
Total of all peak areas	586891	314822	428219	1131621	1166156
Carbohydrate	47993	24834	52279	166174	616542
Tannin	154666	68054	158308	290159	216449
Benzofuran (included in Tannin group)	8421	4139	7914	62186	5791
Organosulfur	81894	90788	38281	336289	30379
Protein	33695	25921	39349	86948	122909
Benzene	83481	41029	29588	141578	32065
Toluene	117820	48935	70079	122120	97976
C_2 Benzene	75763	19400	48249	50539	45347
C_3 Benzene/Hydrocarbon	53708	14095	57943	106861	44751
PAH	80598	26384	63942	329855	53180