Taste and Odour Forming Microorganisms in South African Surface Waters

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RESEARCH ON TASTE AND ODOUR FORMING MICROORGANISMS

IN SOUTH AFRICAN SURFACE WATERS

by

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Final report to the Water Research Commission

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EXECUTIVE SUMMARY

The Water Research Commission sponsored an investigation on tastes and odours in the aquatic environment which was carried out by the Division of Water Technology, CSIR. This report contains the main findings of this investigation. Surface waters serve as a major source for potable water supply in South Africa. Tastes and odours of drinking water are the most important parameters employed by water users to judge its quality. Taste and odour problems are caused principally by microorganisms such as algae, cyanobacteria and actinomycetes, but can also arise as a result of contamination with chemical compounds. In South Africa, most of the complaints concerning bad water odours and tastes stem from eutrophic (enriched) surface water reservoirs which are infested with algal blooms.

The main objectives of this study were as follows:

- to investigate taste and odour incidents found in local water bodies, with special attention given to the identification of microorganisms associated with the incidents, the identification of odorous chemical compounds, the confirmation of odour production in culture and the concomitant testing of toxicity.
- to examine and compare various water treatment methods for the selection of optimal methods suitable for the removal of odorous substances frequently implicated in local conditions.

A literature review included information on taste and odour surveys, chemistry of off-flavours, methods of detection and quantification of odours, reports on case studies and a description of available treatment methods.

Fifty-four water dams, reservoirs or pans, situated mostly in the Transvaal and the Orange Free State, were visited. Bacterial and algal content were estimated for all water samples collected. Samples judged by sensory analysis as odorous were investigated further to identify the chemical compound(s) responsible for odour nuisance and to detect the presence of possible concomitant toxic metabolites, by means of a *Daphnia* toxicity test. Attempts were made to cultivate odour producing algae. Taste and odour incidents were found in 30% of the surveyed water bodies. All odourexperiencing lakes were dominated by cyanobacteria. *Microcystis* was the dominant organism in 90% of the cases. Geosmin was the most common odorous substance, identified in nearly 50% of the incidents. Other important odorous compounds were several sulphur-containing compounds. 2-methylisoborneol, commonly associated with odours in water overseas, was not found during this survey. Microcystins, a family of cyanobacterial toxins were found in four out of five samples collected from odour-experiencing water sources. One sample from an odour-free reservoir also contained microcystins. *Daphnia* toxicity was found in only two out of seventeen tests.

To optimize treatment options for the removal of tastes and odours for local conditions, several methods were screened. They included conventional water treatment (coagulation/ sedimentation, coagulation/flotation), activated carbon adsorption and oxidation (chlorine, chlorine dioxide, hydrogen peroxide, ozone, PEROXONE). Geosmin, methylisoborneol and isopropyl disulphide were chosen as model compounds for treatment experiments. Out of the three, isopropyl disulphide proved to be the easiest to remove, geosmin and 2-methylisoborneol, however, were persistent in water after various treatment methods. Activated carbon adsorption, ozonation and PEROXONE were selected as the most suitable methods for removal of common tastes and odours. Minimum dosages required for the effective removal of selected odorous compounds were established experimentally.

The major objectives of the contract were fulfilled. Only the attempts to cultivate odorous planktonic cyanobacteria in the laboratory were unsuccessful. Therefore, proof of odour production by a given species according to Koch's postulates could not be established because of the lack of axenic (pure) cultures. Curves describing the minimal dosages required for the removal of odorous substances from water were established during this study. These curves may serve as guidelines for treatment schedules for water purification plants. The basis of any successful treatment, however, should be an initial investigation into the cause of the taste and odour incident and assessment of the chemical nature and concentration of the offending substance. Water source management, including early warning systems, may help to prevent the outbreaks of new taste and odour incidents and should be introduced at least at the major potable water sources.

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Further research should concentrate on establishing conclusive evidence on the identity of organisms responsible locally for the production of odorous metabolites. Other aspects which need further study are the standardisation of sensory analysis, biochemistry and physiology of odour production and biotechnologies for the treatment of odour problems.

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Dr M J Pieterse	4	Water Research Commission (Chairman)
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Dr F C Viljoen	æ	Rand Water Board
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Mr P W Weideman	1	Water Research Commission (Committee Secretary)

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Project team

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LIST OF SYMBOLS AND ACRONYMS

1000		
CFU		colony forming units
CLSA		Closed Loop Stripping Analysis
DAF		Dissolved Air Flotation
DMDS		dimethyl disulphide
DMTS	100	dimethyl trisulphide
d.w.		dry weight
FPA		Flavour Profile Analysis
GAC		granular activated carbon
GC/MS		gas chromatography/mass spectrometry
Geosmin		trans-1,10-dimethyl-trans-9-decalol
IBMP		2-isobutyl-3-methoxypyrazine
IPD	1.0	isopropyl disulphide
IPMP		2-isopropyl-3-methoxypyrazine
2-MIB		2-methylisoborneol
NA		sample not available
ND		not detected
PAC/DAF		powdered activated carbon/dissolved air flotation
PAC	-	powdered activated carbon
PACI		polyaluminium chloride
T & O		taste and odour
TCA		trichloranisole
TON		Threshold Odour Number

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1. INTRODUCTION

1.1 Background

Odorous substances are metabolites of numerous microorganisms: bacteria from the order *Actinomycetales*, cyanobacteria, green algae and some diatoms (Hoehn 1988, Lin 1976a). In most cases the unpleasant earthy/muddy tastes and odours (off-flavours) often encountered in surface waters are caused by the presence of the trace amounts (a few ng/l) of certain biogenic sesquiterpenoids - geosmin and 2-methylisoborneol (Gerber 1979). The implications of odorous compounds production can be serious in areas such as aquaculture, limnology, biotechnology and especially in tainting of fish. In water supplies and water treatment processes the presence of odorous substances in the water source may result in unacceptable tastes and odours in potable water. Organoleptic properties of drinking-water are of the utmost importance to a consumer. American surveys (Sigworth 1957, Manwaring *et al.* 1986) revealed that the water quality was the most important feature of respondents' tap water. Nearly all low ratings were caused by bad taste, odour or aftertaste.

Taste and odour incidents in surface waters reported from overseas were frequently traced to the growth of different microorganisms. Among the causative organisms are *Oscillatoria* spp. (Berglind *et al.* 1983b, Izaguirre *et al.* 1982, Izaguirre *et al.* 1983, Leventer and Eren 1969), *Anabaena* sp. (Möhren and Jüttner 1983, Izaguirre *et al.* 1982), *Dinobryon* sp., *Ceratium* sp. and diatoms (Popovska 1983), *Uroglena americana* (Yano 1988), *Anabaena macrospora, Phormidium tenue* and *Oscillatoria tenuis* (Negoro *et al.* 1988; Yagi 1988) and actinomycetes (Silvey and Roach 1953, Lind and Katzif 1988).

South African water bodies experience accelerating eutrophication (Toerien and Grobler 1986). Climatic conditions enabling the prolific growth of water microorganisms for the greatest part of a year and the abundance of nutrients are factors predisposing taste and odour problems in surface waters. A survey undertaken by the University of Cape Town at the request of the Water Research Commission (Reid *et al.* 1988) revealed that there exists a need for research into the causes and methods of control of odours originating from algal and bacterial growth.

This survey indicated that every authority interviewed expected taste and odour problems to increase in the future.

The diversity of odours found in water, the different chemical structures of odorous compounds and the innumerable odour sources make a thorough investigation into the nature and frequency of taste and odour incidents, in a given area, a prerequisite to the application of adequate treatment measures. A survey conducted in 1989 amongst water supply authorities in South Africa (Wnorowski *et al.* 1989) revealed that 30% of the respondents experienced at least intermittent odour problems. The increases in operating costs as a result of the presence of odorous compounds in a water source were estimated at over R3 million annually in this survey. Most of the respondents apply a "trial and error" approach to contain the problem, and proper investigation of causes or preventative strategies were rarely considered.

Nevertheless, systematic scientific investigations into T & O events in conjunction with algae in South Africa have been fragmentary. An incident of taste and odour was reported in Vaal Dam (Bailey 1988). High levels of geosmin were detected, but responsible organisms(s) were not defined. An *Anabaena* bloom producing geosmin in Nagle Dam was a cause of off-flavours in water supplies to Durban; an incident which required several hundred thousands rands per month for additional water treatment (Joubert, Kerdachi *et al.* 1989). The PAC/DAF process was tested for taste and odour removal from water (Le Roux 1988b), a project partially funded by the Water Research Commission.

Health implications form an additional facet of the problem. The odorous organic substances are reckoned not to be toxic (Mackenthun and Keup 1970, McGuire and Gaston 1988b). On the other hand the responsible organisms, especially cyanobacteria, are also known as producers of a range of toxins (Van der Westhuizen *et al.* 1988). The appearance of odour in water may then indirectly indicate a concomitant presence of toxic compounds in water (De Greef *et al.* 1983). Simultaneous production of odorous and toxic organic substances by the same organism needs to be further elucidated.

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1.2 Study objectives

This research project was based on the recommendations of the American Water Works Association Research Foundation and Lyonnaise des Eaux (Mallevialle and Suffet 1987).

The primary objective was to investigate T & O incidents found in the local water bodies, with special attention at identification of microorganisms associated with odour incidents (algae, cyanobacteria, actinomycetes), identification of chemical compounds responsible, confirmation of odour production by a given microorganism in culture and toxicity testing.

The secondary objective was to examine and compare various treatment methods suggested in the literature for the selection of optimal methods suitable for removal of odorous substances frequently implicated in local conditions.

The specific tasks were as follows:

- A literature review.
- Screening of a broad range of water reservoirs for the presence of T & O incidents.
- Identification of organisms associated with odour incidents (algae, cyanobacteria, actinomycetes).
- Adaptation of a gas chromatography/mass spectrometry (GC/MS) method for the detection and quantification of trace concentrations of odorous compounds in water.
- An assessment by means of toxicity tests, of the possibility of concomitant T & O/toxic incidents
- An attempt to isolate and cultivate strains of organisms implicated in odour incidents for the confirmation of odour production in pure culture.

- Preliminary screening of treatment methods.
- Process optimizing for most suitable methods.
- Conclusions and recommendations for investigation, treatment, and prevention of taste and odour incidents in water.

2. LITERATURE REVIEW

2.1 Introduction

Tastes and odours (T & O) appearing in surface waters have received increasing scientific attention over the last thirty years. Public opinion, demanding odourless water supplies and aesthetic recreational waters, has put constant pressure on the relevant authorities to prevent or remove off-flavours. On the other hand, progressive eutrophication and pollution of surface waters aggravates the problem, causing a steady increase in the number of taste and odour incidents.

In spite of the extensive literature on the subject, taste and odour problems continually recur, resulting in considerable costs. Interest shown by government and local authorities is essential regarding legislation on discharge limits and appropriate action towards watershed protection. Expertise, requiring interdisciplinary cooperation between biologists, chemists and engineers, is not always readily available.

A useful practical handbook on the investigation and treatment of various tastes and odours for the water purification industry was compiled by Lyonnaise des Eaux and the American Water Works Association Research Foundation (Mallevialle and Suffet 1987). This book deals with a variety of odours encountered in water sources, methods of identification and the treatment options. There are also several valuable review articles on the subject. Sources of taste and odours in water were discussed by Lin (1976a, 1976b) and Hoehn (1988). The chemistry of aquatic off-flavours was summarised by Skulberg (1988) and Jüttner (1988). Treatment measures were described by Hrubec and De Kruijf (1983) and by Montiel (1983). A comprehensive

study of taste and odour was given by Le Roux (1988a). The state-of-art overviews on aquatic odours were produced by Persson (1983, 1988).

2.2 Taste and odour surveys

World-wide research on T & O problems is evidence of its importance in many countries (Persson 1983). A survey by Sigworth (1957), conducted among water works officials revealed that 82% of a total of 241 respondents had T & O problems originating from algae and 67% from decaying vegetation, which makes biological factors the prime causative agent of T & O problems. In treatment, only activated carbon was applied successfully (82 to 85% of the plants), in contrast to other methods such as free residual chlorination, super-chlorination, chlorine dioxide and aeration, for which success never exceeded 10%. Another survey of this kind, by Mackenthun and Keup (1970) revealed that biological problems in water works were reported by about 25% of the 800 respondents, with algal causes of T & O problems still ranked first. This study reported that chlorine and copper sulphate treatment were employed most often, with activated carbon in the third place (1/4 of the positive respondents). High costs of T&O treatment were also reported. Generally, the results of this survey are comparable with those obtained from the water-related industries in South Africa (Wnorowski *et al.* 1989).

Consumer-orientated surveys (Gallup Poll 1973; Manwaring *et al.* 1986) indicated that organoleptic quality of drinking water was the main concern of consumers, when concern for safety had been eliminated. Although in both surveys only about 10% respondents rated their tap water "poor", as much as 30% complained of bad taste, odour, after-taste or colour. Twenty percent of the respondents indicated that they used alternative sources of water (e.g. bottled water) or tap filtering devices. In California bottled water is used by as much as 75% of the population: in 30% of the cases for health reasons, 30% for tastes and odours in tap water and the rest for both (Pieterse 1992). A recent investigation by the Water Quality Association in the USA (Anonymous 1989c) indicated, that consumers' awareness and expectations of drinking water quality is rising. Although 79% of the respondents considered their tap water to be "acceptable", only 70% regarded it as free from harmful contaminants and about 63% had a positive opinion about its quality.

In South Africa public opinion was also gauged regarding expectations about recreational water quality (Thornton and McMillian 1989). Aesthetic values, mainly the lack of algal blooms and scums, and the lack of tastes and odours, were rated as the most important by respondents to the questionnaire.

2.3 Chemistry of off-flavours

Sources of tastes and odours in water

Water acquires its taste and odour from a variety of sources. Only a few are of truly natural origin, for example, mineral salts which may be leached from the substrate and which may impart a taste to the water. Most tastes and odours are the result of human influence: Indirectly by rapid enhancement of the growth of aquatic organisms under eutrophication or directly by wastewater dumping or accidental spillage as well as by agricultural runoff. The most problematic condition at present appears to be algal production of earthy/muddy odours in eutrophicated sources.

Earthy odours

A range of volatile and semi-volatile organic compounds may cause odours in the water and render it unpalatable. From a practical point of view the most important substances are those with a low Threshold Odour Concentration. Some are offensive to the human senses even if present only in trace amounts of a few ng/*l*. Some microbial metabolites, like geosmin and 2-methylisoborneol, belong to this category. Geosmin was identified, first in actinomycetes and later in cyanobacteria and algae, as the main component imparting an "earthy" odour to soil and water (Gerber and Lechevalier 1965). 2-Methylisoborneol, another earthy-smelling microbial by-product, is also periodically reported in taste and odour incidents (Izaguirre *et al.* 1983; Berglind *et al.* 1983b). Both geosmin and 2-methylisoborneol are tertiary alcohols whose structure makes them resistant to oxidation, a process routinely applied in water purification. This, together with their extremely low odour threshold values, make them the foremost nuisance-substances in taste and odour incidents.

Other earthy/musty-smelling microbial metabolites can periodically be found to be present in water. These are 2,3,6- trichloroanisole, 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine (Gerber 1979, 1983; Krasner *et al.* 1983; Mallevialle and Suffet 1987). The chemical structure of all five odorous compounds is given in Table 1 and their Threshold Odour Concentrations in Table 2.

Biochemical investigation of 2-methylisoborneol and geosmin production by actinomycetes has led to the conclusion that these substances are synthesized by methylation of L-methionine and folic acid (Aoyama 1990). The biosynthesis paths for geosmin in Oscillatoria brevis were studied by Naes and Post (1988) and by Naes et al. (1989). The environmental conditions influencing geosmin production by actinomycetes in water were analyzed by Wood et al. (1983). Conditions found to be relevant to the production of earthy taints by actinomycetes in water were: high nutrient-level, presence of plant debris, structure of the reservoir banks, allowing sediment accumulation and the aerobic conditions in the sediments. Nitrogen was confirmed as an environmental factor influencing geosmin production by actinomycetes by Lind and Katzif (1988). The conditions influencing geosmin production by the cyanobacterium Fischerella muscicola were studied by Wu and Jüttner (1988). Maximum geosmin production was obtained during the lag-phase and decreased with age. Geosmin was produced under both aerobic and anaerobic conditions and was the highest at the minimum and maximum temperatures of the range of the species studied. At optimum growth temperature for the strain geosmin production was at its minimum. Similarly, Miwa and Morizane (1988) found that the deterioration of physiological conditions of Anabaena macrospora in the absence of chelating agents such as EDTA and humic acid resulted in increased geosmin production. Wu and Hsu (1988) established that 98% of geosmin produced by Anabaena was retained within the cells rather than being released to the medium and air. This finding was confirmed for Anabaena macrospora Klebahn, Phormidium tenue Meneghini and Oscillatoria tenuis Agardh from Lake Biwa in Japan (Negoro et al. 1988). The influence of light, nitrogen and phosphorus on geosmin production by Oscillatoria was investigated by Naes et al. (1988). It was concluded that these three factors do not have a direct effect on the geosmin biosynthesis rate. The increase in geosmin concentration in eutrophied waters is the result of increased algal biomass rather than enhanced

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Name	trans-1,10-Dimethyl- trans-9-decalol (geosmin)	2,3,6-Trichloroanisole (TCA)	2-Isopropyl-3- methoxy pyrazine (IPMP)	2-IsobutyI-3- methoxy pyrazine (IBMP)	2-Methylisoborneol (MIB)
Molecular structure	OH ''' OH '' CH3				
Molecular weight	182	212	152	166	168
Molecular formula	C ₁₂ H ₂₂ O	C,HsOCIs	C ₈ H ₁₂ ON ₂	C ₉ H ₁₄ ON ₂	C ₁₁ H ₂₀ O

geosmin production. A laboratory method for high-yield production of geosmin in fermenters followed by extraction and purification was given by Gerber and Lechevalier (1977).

 TABLE 1.
 MOLECULAR CHARACTERISTICS OF ORGANIC COMPOUNDS CAUSING EARTHY ODOURS (Lalezary et al. 1986a) Reprinted from AWWA Journal, Vol. 78, No. 3 (March 1986), by permission. Copyright 1986, American Water Works Association.

Name	trans-1,10-Dimethyl- trans-9-decalol (geosmin)	2,3,6-Trichloroanisole (TCA)	2-isopropyt-3- methoxy pyrazine (IPMP)	2-IsobutyI-3- methoxy pyrazine (IBMP)	2-Methylisoborneo (MIB)
Molecular structure	CH ₅		N N N H C H S C H S C H S C H S		CH-CH-CH-CH-CH-CH-CH-CH-CH-CH-CH-CH-CH-C
Molecular weight	182	212	152	166	168
Molecular	с,,н_о	с,н,осц	C,H,ON,	C,H.,ON,	С.,Н_О

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TABLE 2. CHARACTERISTICS OF EARTHY ODOURANTS (Krasner et al. 1983) Reprinted from Water Science and Technology, Vol. 15, pp. 127-138, (1983), with permission from the publishers, Pergamon Press and the copyright holders, IAWPRC.

Compound	Chemical Name	Threshold Odour concentration (ng/l)	Odour description
Geosmin	Trans-1,10-dimethyl- trans-9-decalol	10	earthy/musty
2-MIB	2-methylisoborneol	29	earthy/musty/camphorous
PMP	2-isopropylmethoxy- pyrazine	2	earthy/musty/potato-bin
IBMP	2-isobutylmethoxy- pyrazine	2	earthy/musty/bell pepper
TCA	2,3,6-trichloroanisole	7	musty

Other microbial odours

Microbial odorous substances of some importance, other than those described above, are chiefly organic sulphur compounds and hydrogen sulphide. The production of hydrogen sulphide, dimethyl sulphide and methylmercaptan was reported for a range of bacterial and fungal strains by Kadota and Ishida (1972). The role played by bacterial enzymes in the production of volatile sulphur compounds from methionine and cyst(e)ine was studied by Herbert and Shewan (1976). High concentrations of dimethylsulphide (up to 2 500 ng/l) and dimethyldisulphide (up to 1 600 ng/l) were found in algal cultures. The dimethylsulphide concentration in pond water also reached 70 000 ng/l in summer, especially in *Nostoc* and decaying *Spirogyra* and *Oedogonium* communities (Bechard and Rayburn 1979).

There are several papers dealing with the identification of volatile organic compounds produced by algal cultures. Most of these substances are odorous, giving taints to water and may be potential causes of taste and odour incidents. Jüttner *et al.* (1986) identified over sixty volatile compounds in lake waters containing phytoplankton populations dominated by *Dinobryon, Microcystis* and *Aphanizomenon*. Apart from the substances described above, β-cyclocitral (a tobacco-smelling substance of the

nor-carotenoid group) was found to be the dominant volatile product of *Microcystis* cultures. β-cyclocitral was also found in *Microcystis* by Hayes and Burch (1989). They also identified a cucumber-smelling odorous substance, frequently reported from *Synura* blooms, as *trans-2, cis*-6-nonadienal. A study on the odorous compounds produced by *Anabaena* and *Nostoc* was done by Möhren and Jüttner (1983). *Synechococcus* products were investigated by Henatsch and Jüttner (1983). A useful summary of volatile cyanobacterial metabolites was compiled by Slater and Blok (1983a) and, for algae, by Jüttner (1983).

Chlorination by-products

Chlorination is one of the most important stages in conventional water purification schemes. It disinfects the finished water, delivers a free residue for the distribution system and destroys many of the harmful substances by oxidation. It is also relatively cheap and easy to apply. Apart from the advantages, chlorine application may bring about some undesirable effects. Over-chlorination causes consumer complaints because of the "chlorine" smell in the water. In fact, according to a South African survey (Wnorowski et al. 1989), 13% of reported taste and odour problems are caused solely by excessive chlorination. Further, not all organic substances present in the raw water will be easily oxidized. For example, geosmin and 2-methylisoborneol, which are the major T & O substances in surface water, are resistant to chlorine even at high dosages (Lalezary et al. 1986a) and require other methods for their removal. A matter of concern, however, is the creation of additional taste, odour and health problems by the very chlorination procedure. It is true especially when applied in the early phase of a treatment process, as a pre-chlorination of raw water prior to removal of intact algal cells and other organic matter by sedimentation or flotation. Chlorination of algae causes their lysis and release of taste and odour substances and other organic compounds contained within the cells. Their release into the water may drastically increase the concentration of odorous substances, making their removal even more difficult. In addition, chlorine derivatives may also present a health hazard by the production of trihalomethanes (Pieterse 1988), as well as creating an odour nuisance like medicinal-smelling chlorophenols, especially 2-chlorophenol, 2,4-dichlorophenol and 2,6-dichlorophenol (Burttschell et al. 1959). Oxidation products may also be odorous; for example, low molecular weight aldehydes such as methylpropanal, methyl butanal and phenylacetaldehyde (Hrudey *et al.* 1988). Organohalogens may find their way into surface waters through industrial pollution, e.g. during the process of chlorobleaching of pulp (Paasivirta *et al.* 1983).

Taste and odour in water with reference to fisheries and aquaculture

Fish tissue readily absorbs off-flavours from water which can render the fish unpalatable and therefore unmarketable. Serious commercial losses may result from taste and odour in water both in fish husbandry and marine and freshwater fisheries. Aquaculture encounters similar problems to the water treatment industry with fish being tainted mainly by geosmin but also by other natural algal by-products accumulating in fish ponds (Lovell 1983; Martin *et al.* 1988; Wu and Hsu 1988).

Algal growth in commercial ponds is inadvertently enhanced by over-fertilisation with fish feed and faeces. Fish in intensive cage-culture systems (trout and catfish) are particularly susceptible to off-flavours produced by algae, as the fish are unable to move away from the algal blooms. Their removal to clean, odourless water to purge geosmin and 2-methylisoborneol is a means of off-flavour abatement (Wu and Hsu 1988) but this decreases profitability as it leads to a 3 to 10 day delay in the processing of market sized fish until the flavour subsides.

On the other hand, fish occurring in natural waters are often tainted by industrial effluents, apart from acquiring the muddy-musty odours produced by algae. Fresh and brackish water fish in Finland were found to be spoiled mainly by oil and kraft pulp mill odours (Kuusi and Suihko 1983). Terpenes from paper mill effluents were identified as a source of off-flavours in fish in polluted rivers in Norway (Berg 1983), while chlorophenols and anisoles from pulp and paper industries were found to give odours to fish in Finland (Paasivirta *et al.* 1983). Industrial pollution was also the suspected cause of an iodine-like taste in the Baltic Sea herring. The odorous substance was identified as *o*-bromophenol (Bernelmans and Den Braber 1983).

The petrochemical industry also contributes to taste spoilage of aquatic fauna. Oily, petroleum-like off-flavours were investigated by Jardine and Hrudey (1988). Threshold odour concentrations for several hydrocarbons from oil sands wastewaters were established for fish flesh. Effluents from marine petrochemical installations in Japan were found to contaminate fish with mainly toluene and benzene (Ogata 1988). Fish tainted with petroleum chemicals was the subject of a review by Motohiro (1983). Off-flavours in other sea-food were discussed by Whitfield and Freeman (1983) and Whitfield (1988).

2.4 Methods in taste and odour research

The analytical methods available for the determination of odorous compounds in water include sensory evaluation by the human senses and gas chromatographic techniques.

Sensory analysis

Human senses are the most sensitive tools for taste and odour detection and quantification. Their practical utilization, however, encounters several draw-backs. Sensory evaluation is subjective and difficult to standardize. It is unable to discriminate specific odours in the presence of stronger ones and it rapidly suffers from fatigue. Because of differences in olfactory sensitivities (Amoore 1986), a panel consisting of several individuals is usually employed. Sensory analysis has been used for years especially in drinking water assessment. The traditional method used is the Threshold Odour Number (TON). At present the Flavour Profile Analysis (FPA) has been developed for water analysis. Both methods are recommended by *Standard Methods* (1989). Threshold Odour Number is a value reflecting the highest dilution of the odorous sample at which odour is still perceptible. Threshold concentrations are very difficult to assess; they are subjective and vary between individuals. Nevertheless, it is still used in many routine water works laboratories, producing comparable results among panels (Bousquet *et al.* 1983), as well as some research laboratories (Popovska 1983; Tomita *et al.* 1988).

The Flavour Profile Analysis method, using the undiluted sample, has been introduced to overcome drawbacks in Threshold Odour Number assessment. It was first designed for food and beverages flavour analysis and then adapted to drinking water (Krasner *et al.* 1985; Krasner 1988). It was rapidly developed into a standard method for sensory analysis of water (Suffet *et al.* 1988). It is based on the rating of odour intensity in the undiluted sample, together with odour description, given by a group of trained panellists. A library of reference substances and odour descriptors has been established (Bailey *et al.* 1988). The method proved to be precise, reproducible and accurate, provided the panellists have been properly trained (Brady *et al.* 1988). The training methods for Flavour Profile Analysis have been established (Bartels *et al.* 1987; Mallevialle and Suffet 1987).

Instead of a trained panel, a large, untrained consumer panel is sometimes used primarily for assessment of consumer satisfaction with water produced by a given utility (De Greef *et al.* 1983). The trained panel's sensitivity is usually more acute than the consumer's (Sano 1988), but the latter represents more closely the receivers opinion on drinking water quality.

Instrumental analysis

Because of the extremely low threshold odour concentration of many of the odorous organic compounds (a few ng/t), chemical analysis was inadequate for many years. Development by Grob (1973) of the Closed-Loop Stripping Analysis (CLSA) made it possible to concentrate trace organic compounds in water to a degree enabling instrumental methods to achieve the necessary detection limits. Concentrated compounds are then detected by capillary gas chromatography and identified by mass spectrometry (Krasner *et al.* 1983). Some modifications to Grob's method (Savenhed *et al.* 1983) and alternative extraction and concentration techniques (Brownlee *et al.* 1988; Lundgren *et al.* 1988a) have been proposed.

An interesting modification of the gas chromatographic technique has been developed (Veijanen *et al.* 1983, 1988). This combines instrumental and sensory analysis and is called chromatographic sniffing. The capillary column is equipped with an outlet leading to an odour observer. The system is both highly sensitive and accurate for the determination of retention times (Savenhed *et al.* 1985).

2.5 Case studies

Actinomycetes

Proven cases of taste and odour in water that can be attributed to actinomycetes are scarce. Silvey and Roach (1953) demonstrated a persistent earthy odour in Lake Hefner and Lake Overholser near Oklahoma City to be caused by actinomycetes. Bacteria were growing inside the cells of attached green alga *Cladophora* which was forming mats along the concrete or stone banks of both reservoirs. The odour was most pronounced in winter, when disintegrating algae released by-products of actinomycetes into the water. Eradication of algae with copper citrate completely contained the problem. The authors also expressed their conviction that taste and odour incidents involving cyanobacteria are in fact induced by actinomycetes inhabiting algal cells. This opinion proved errant when more evidence on the subject was collected (e.g. Slater and Blok 1983).

Suspected involvement of actinomycetes can be attached to a geosmin incident in the Vaal Dam in South Africa, early in 1988 (Bailey 1988). The dam, filled to only a quarter of its capacity for several years due to severe drought, rapidly filled at the end of 1987 due to extensive rains in the catchment area. Some 32 hectares of previously exposed dam sediments were flooded and shortly afterwards a taste and odour incident occurred. Algae or cyanobacteria could not account for the incident (Scott 1988). One can presume that actinomycetes developed profusely in the dried-out fertile sediment and their odorous metabolites gave rise to taste and odour in the water when they were rapidly leached out by the rising water level. Additionally, decaying vegetation which colonised exposed sediments during drought provided rich nutrient source for odorous actinomycetes.

In spite of the firm views of Silvey (1954) and Roach and Silvey (1958) on taste and odour formation by water actinomycetes, most of the publications monitoring actinomycetes during taste and odour incidents could not find any positive correlation between their numbers and the presence of the odour in water (e.g. Leventer and Eren 1969; Berglind *et al.* 1983b; Yagi *et al.* 1983; Raman and Arbuckle 1989). Finding a relationship is complicated by the fact that most of the actinomycetes may survive in

water in the form of spores (Boon 1989) while only active and metabolizing cells are responsible for the synthesis of odorous by-products (Cross 1981). Indeed, even the mere existence of truly aquatic actinomycetes was questioned (Cross 1981) and any odour production in water disavowed.

On the other hand, actinomycetes are known producers of earthy odours and, even if not directly involved with taste and odour formation in surface waters, they may still contribute to taste and odour incidents, provided the specific conditions occur. The washout of odorous by-products from soil may take place, as soil is the natural habitat for actinomycetes, where they proliferate in high numbers (Romano and Safferman 1963; Cross 1981; Persson 1988). This hypothesis seems logical and feasible but has not been confirmed by reliable scientific evidence. Plant-populated water margins may provide a nutrient rich environment for the growth of actinomycetes (Cross 1981; Wood *et al.* 1983). Enrichment of a water body is also a potential factor, as actinomycetes show a positive correlation between nitrogen concentration and odour production (Lind and Katzif 1988).

Cyanobacteria and algae

There is abundant documentation on taste and odours in surface waters caused by algae and cyanobacteria. Studies have been world-wide with most originating in North America and Japan.

The Metropolitan Water District of Southern California supplies potable water to approximately thirteen million people (Means and McGuire 1986). Water sources of Metropolitan have, since 1967, suffered numerous taste and odour incidents. From Lake Mathews a littoral attached cyanobacterium, *Oscillatoria curviceps*, and from Phoenix Reservoir a benthic strain of *Oscillatoria tenuis* var. *levis* Gardner, were isolated as the main odour producers. A planktonic bloom of *Anabaena scheremetievi* Elenkin was responsible for odours in Tinemaha Reservoir (Izaguirre *et al.* 1982). *O. curviceps* and *O. tenuis* produced 2-methylisoborneol, while *A. scheremetievi* produced geosmin (Izaguirre *et al.* 1982, 1983). The proficient preventive and treatment measures applied in this case will be discussed in session 2.6 of this paper.

for two taste and odour incidents in Schuylkill River in Philadelphia (Burlingame *et al.* 1986). Effective eradication of odour in both cases was a result of an existing taste and odour control programme. From Saskatchewan, Canada, Buffalo Pound Lake was reported to have experienced taste and odour incidents (Slater and Blok 1983b). Blooms involving *Anabaena, Oscillatoria* and *Aphanizomenon* are often present there. Chemical analysis of the lake water revealed the presence of geosmin and the tobacco-smelling β-cyclocitral.

Japan, an overpopulated and highly industrialized country, has suffered severe taste and odour problems during the last twenty years. The Yodo River basin, which include Lake Biwa (the largest lake in Japan), serves a population of some thirteen million people, including the inhabitants of Kyoto and Osaka. Taste and odours of biological origin, first noted in 1969, became recurrent and created considerable social problems (Toui 1989). Initially these incidents were caused by the 2-MIB-producing strain of *Phormidium tenue* Meneghim, but in 1981 the geosmin-producing strain of *Anabaena macrospora* Klebahn was also isolated (Yagi *et al.* 1983). Later, a strain of *Oscillatoria tenuis* Agardh, with significant 2-MIB production was identified (Negoro *et al.* 1988). The highest values for 2-MIB and geosmin were recorded in Lake Biwa water in the autumn of 1985 giving concentrations of 1050 and 860 ng/*t* respectively (Yagi 1988).

The Yodo River originates from Lake Biwa and therefore also experiences musty odour problems. Geosmin and 2-MIB are monitored along the river. Geosmin concentration was found to decrease along the river course while the 2-MIB level was not affected, probably due to its resistance to biodegradation (Hishida *et al.* 1988).

The Nunobiki Reservoir near Kobe has experienced several blooms of the alga *Uroglena americana* from the family Chrysophyceae. Blooms were accompanied by a strong fishy odour. Two isomers of 2,4-heptadienal were detected and associated with these episodes (Yano *et al.* 1988).

Five water sources in Tokyo were studied by Matsumoto and Tsuchiya (1988). Two 2-MIB-producing cyanobacteria (*Oscillatoria geminata* and *Oscillatoria limnetica*) and three geosmin-producing cyanobacteria (*O. amoena, O. splendida* and Aphanizomenon flos-aquae) were identified as sources of taste and odour in these impoundments.

Taste and odour problems involving cyanobacteria have been reported from Norway. Berglind *et al.* (1983a) investigated southeastern Norwegian water bodies and were able to associate *Oscillatoria bornetti* f. *tenuis* Skuja with geosmin incidents in Lake Mjosa and River Glama, *Oscillatoria agardhii* Gom. with geosmin in Lake Arungen and *Oscillatoria agardhii* var. *isothrix* with muddy odours in Lake Vansjo. Benthic mats of *Oscillatoria brevis* (Kütz). Gom. are abundant in Norwegian inland eutrophic rivers and lakes. A strain of *O. brevis* collected from the River Nitelva was found to produce both 2-MIB and geosmin in culture (Berglind *et al.* 1983b).

Benthic Oscillatoria chalybea was proved to induce earthy smells in the Tsalmon reservoir in Israel (Leventer and Eren 1969).

A Synura bloom was associated with the cucumber-smelling taste and odour incident in Hubenov Reservoir in Czechoslovakia (Popovska 1983).

Odorous algae have also been reported in the Southern Hemisphere. In Australia (Hayes and Burch 1989) *Oscillatoria, Anabaena* and *Microcystis* were found to produce several odorous metabolites, among them geosmin, 2-MIB and β-cyclocitral. The cucumber odour produced by *Synura* was found to be due to 2,6-nonadienal. In South Africa Joubert *et al.* (1989) reported on severe earthy odours which appeared in February 1988 in Nagle Dam, the main water source for Durban. The cause was identified as a geosmin producing *Anabaena* strain. The problem recurred in subsequent years, always associated with a bloom of *Anabaena solitaria* (Wnorowski and Scott 1990). Geosmin-involving incidents were also noted in Vaal Dam, Klipvoor Dam, Bospoort Dam and Wentzel Dam in the northern part of the Republic of South Africa. In all of them *Microcystis aeruginosa* was found to be the most prominent microorganism present at the time of taste and odour formation (Wnorowski and Scott 1990).

2.6 Treatment

Conventional water treatment, with the routine sequence of coagulationsedimentation-filtration-chlorination, does not efficiently remove taste and odour, especially the earthy-type, from water (Montiel 1983). Known techniques deal with tastes and odours either by decomposition of the chemical structure or actual removal of the odorous compounds from the water. A schematic representation of treatment methods for taste and odour removal is presented in Table 3. Some of them will be discussed in more detail below.

Treatment options					
Decomposition			Removal		
Oxidation Radiation		Biodegradation			
chlorine chloramines chlorine dioxide ozone permanganate PEROXONE	 gamma rays UV light high energy electrons 	 bacteria yeasts protozoa 	 aeration powdered activated carbon (PAC) granular activated carbon (GAC) powdered activated carbon/dissolved air flotation (PAC/DAF) 		

TABLE 3. TREATMENT METHODS FOR TASTE AND ODOUR ABATEMENT

Physical-chemical treatment

Oxidation is the major chemical treatment process for taste and odour removal. Oxidizing agents utilized in the water industry include chlorine, chloramines, ozone, chlorine dioxide and potassium permanganate, with chlorine gas (Cl₂) being the most popular oxidant/disinfectant used. Chlorination is cheap, easy to apply, provides a long-lasting free residue in the distribution system and is efficient in destroying certain tastes and odours found in raw water, for example, organic sulphides (Krasner *et al.* 1989). On the other hand, chlorine gas has several serious disadvantages. Firstly, it produces chlorinated by-products with many trace organic compounds present in the water. These organohalogens may be odorous (for example, medicinal-smelling chlorophenols) or present a potential health risk (for example, trihalomethanes), Secondly, chlorine itself, if improperly dosed, gives an unpleasant chlorinous odour to the water. Thirdly, there are a number of odours, particularly from geosmin and 2-MIB, which do not yield to oxidation by chlorination. For these substances chlorine treatment is totally inappropriate (Lalezary et al. 1986a). Replacement of chlorine with chloramines was reported to improve disinfection of drinking water (Cuncliffe et al. 1990) but failed to remove the fishy/swampy odours imparted by methylpolysulphides (Krasner et al. 1989). Oxidation of five earthy/musty odorous compounds was studies by Lalezary et al. (1986a). For all of these chlorine dioxide proved to be the most efficient with potassium permanganate displaying the least efficiency. Ozone was rated second for the recalcitrant 2-MIB and geosmin compounds. A successful chlorine-dioxide application for taste and odour removal, disinfection and prevention of trihalomethane formation was reported from Brussels (Masschelein 1985). Chlorine dioxide was also reported useful for the removal of chlorophenols but not hydrocarbons (Walker et al. 1986). Permanganate may be used for the oxidation of odorous substances, but it should be accompanied by activated carbon addition (McTighe 1971).

Ozone has been studied extensively for taste and odour removal. It was found to be effective for the removal of earthy and fishy odours but not astringent and plastic-like odours, and in addition it generated an intense fruity odour of its own (Anselme *et al.* 1988). Ozone, if applied in low concentrations, improves flocculation, but in concentrations over 1 mg/*t* may cause degradation of algogenic organic matter leading to formation of carboxilic acids which have ability to stabilise colloids and impair flocculation efficiency (Hoyer *et al.* 1987). In comparison with other oxidants the greatest disadvantage of ozone is that it cannot supply an active residual for the reticulation system and so must be supplemented with an additional disinfecting agent. Ozone is however recommended for the treatment of earthy/musty odours, as well as for the oxidation of manganese (Barker and Palmer 1977; Gammie *et al.* 1988). Most recently (Glaze *et al.* 1990), advanced oxidation processes have been introduced. They involve combination of an oxidant with an agent (ozone, UV) facilitating oxidation by forming free hydroxyl radicals from hydrogen peroxide or humic substances. A

hydroxyl radical is a much more powerful oxidant than chlorine or ozone alone, especially towards aliphatic molecules such as geosmin and 2-MIB. The systems employing ozone - H_2O_2 (PEROXONE), O_3 -UV and H_2O_2 -UV have been tested (Glaze *et al.* 1990). The optimization of PEROXONE treatment on a pilot-plant scale was studied by Ferguson *et al.* (1990).

Physical taste and odour treatment involves many diverse technologies. Simple measures comprise the blending of the odorous water with an odour-free counterpart to dilute the odour concentration below its threshold value, or manipulation of the draw-off point to a water level less affected by an odour problem. Air-stripping is satisfactory only for very volatile odorous substances such as hydrogen sulphide (McGuire and Gaston 1988a, 1988b). Radiation technologies (ultra-violet, gamma rays) are efficient but, at present, cost-prohibitive, and have only sporadically found full-scale application (Anonymous 1989b; Comar 1989).

Activated carbon, with its enormous sorption capacity for organic and inorganic molecules, is decidedly the best solution for the removal of odorous substances. Concomitantly, it removes other contaminants from the treated water, some of which being a potential health hazard. The only serious disadvantages of this material are its high prices and lack of selectivity. Both powdered activated carbon (PAC) and granular activated carbon (GAC) have been successfully applied in practice. Le Roux (1988b) listed PAC characteristics making this carbon superior to the granular form, especially for small treatment facilities: more rapid adsorption equilibrium; no major capital costs involved; intermittent application possible; low cost per unit mass. On the other hand GAC, although involving high installation costs, may be used for prolonged periods of time before reaching saturation point and may also be regenerated. Evaluation of performance and optimization of conditions for the different types of carbons were studied by Lalezary et al. (1986b) and Lalezary-Craig et al. (1988). 2-MIB proved to be the substance less adsorbed than geosmin. The presence of chlorine, chloramines and humic acid exerted an adverse effect on geosmin and 2-MIB adsorption on to carbon. Examples of successful application of GAC filters in pilot and full-scale plants have been extensively published (for example Flentje and Hager 1964; Hansen 1972; Gammie et al. 1988; Hattori 1988; Terashima 1988; Vik et al. 1988). The PAC is generally used in conjunction with sedimentation/filtration procedures (Joubert et al. 1989). An interesting alternative seems to be the combination of PAC with the dissolved air flotation technique, namely the PAC/DAF process (Le Roux 1988b). Cyanobacteria, like *Microcystis* spp., possess a system of gas vacuoles, allowing them to maintain the necessary buoyancy in water (Reynolds and Walsby 1975). This phenomenon makes them difficult to sediment. The DAF method concentrates the biomass on the top of the reservoir rather than at the bottom. PAC floats together with algal cells and both materials can then be removed simultaneously. The sludge obtained is dense and easy to handle and does not present odour problems on disposal due to the residual capacity of the PAC which adsorbs any odours arising from decay of the biomass.

An interesting development in water purification seems to be point-of-use treatment. This is an option which is gaining acceptance and popularity in the USA and Europe. In particular, the use of compact units employing activated carbon, ion exchange resin and even reverse osmosis are involved. Such units are typically connected to a tap in the home (EPA Proceedings 1988). Use of these devices indicates the urgency with which consumers view the problem of water quality, to the extent that they treat the water themselves. One must not, however, forget that these filters may eventually become overloaded, allowing contaminants to pass through. They also provide support for bacteria which may contribute new tastes and odours and other by-products to the "filtered" water (Hadeed 1989).

Biological treatment

Biological degradation of odorous organic compounds is an attractive alternative to chemical/physical treatment. Narayan and Nunez (1974) found that *Bacillus cereus* and *Bacillus subtilis* were able to degrade geosmin. The efforts of Danglot *et al.* (1983) to isolate the enzyme, responsible for geosmin decomposition and disappearance of the earthy odour, were unsuccessful. A yeast *Candida* sp., isolated from a slow sand filter, showed a marked ability to degrade 2-MIB, both in a cell-form and as a crude enzyme preparation (Sumitomo 1988; Sumitomo *et al.* 1988). Gel-immobilized biomass or enzyme removed 2-MIB efficiently, but required prolonged (20 to 40 h) contact times. Biodegradation of 2-MIB was also reported by Izaquirre *et al.* (1988a, 1988b)
who found that mixed bacterial populations, consisting mainly of *Pseudomonas* spp., slowly decreased (in 7 to 17 days) the 2-MIB content of water samples. *Thiobaciilus thioparus*, immobilized on porous propylene pellets, was employed for the removal of sulphur-containing odorous substances from air and gases (Tanji *et al.* 1989) and biologically active sand filters were employed for the efficient odour removal from drinking water by Lundgren *et al.* (1988b). Biological processes have also the potential for the removal of total organic carbon, ammonia and nitrate from drinking water (Bouwer and Crowe 1988).

The large number of odorous compounds and their many causal factors indicate the importance of the identification of off-flavours before deciding upon one or more of a variety of treatment methods. Correct and cost efficient treatment needs to be determined for each case individually. A summary of the available water treatment processes to control some of the off-flavours is given in Table 4.

2.7 Reservoir management

Lake and river management, orientated on the improvement of water quality, requires complex activities. It relates to nutrient level, toxic chemicals, microbial life, aquatic macrophytes, zooplankton, macroinvertebrates and fish (McDonnel and Crocker 1989). Preventative treatment by management of the water source is a long-term strategy and, if successful may resolve the taste and odour problem completely. The elements of source management are given in Table 5.

Undoubtedly, the most holistic approach is a proper watershed protection. The efficiency of such a strategy would depend on the cooperation of all those who influence discharge into the water, including industries, municipalities, residents and farmers in the catchment area. A single body carrying sole responsibility for management of a watershed can handle all water matters more easily than if authority is divided (Anonymous 1989a). There are opinions that water from properly protected areas may attain so high a quality that filtration of potable supplies could be discontinued (Kirner and Merry 1988). A restoration policy, applied to a dangerously eutrophied Swedish lake, brought about reversal to its original oligotrophic status (Persson *et al.* 1989).

TABLE 4. SUMMARY OF WATER TREATMENT PROCESSES TO CONTROL OFF-FLAVOURS (McGuire and Gaston, 1988b) Reprinted from Water Science and Technology, Vol. 20, pp. 215-228, 1988, with permission from the publishers, Pergamon Press and the copyright holders, IAWPRC.

Off-flavour		Treatment Processes				
Characteristic	Compound*	Effective	Ineffective			
Earthy/musty	2-MIB, geosmin	O ₂ , O ₃ ./H ₂ O, PAC, GAC, biological	Aeration, Cl ₂ , ClO ₂ KMnO ₄ , NH ₂ Cl			
	IPMP, IBMP TCA	Cl ₂ , ClO ₂ , PAC, GAC ClO ₂ , O ₂ , PAC, GAC, biological	Aeration, KMnO, Aeration, Cl., KMnO,			
Fishy/swampy	DMTS, DMDS, and other compounds	Most oxidants, PAC, GAC, biological	Chloramines			
Grassy	Unknown	Most oxidants (?), PAC, GAC	Chloramines			
Chlorinous	HOCI/OCI-, NHCI ₂ , NCI ₃ , chiorinated organics	PAC, GAC	Biological			
Petroleum	Low-molecular-weight aliphatics & aromatics	Aeration, PAC, GAC, biological	Oxidants			
Medicinal	Phenol, chlorophenols	$\label{eq:clOp} \begin{array}{l} \text{ClO}_p, \mbox{ O}_p, \mbox{ PAC, } \mbox{ GAC, } \\ \mbox{ biological } \end{array}$	Cl _p , NH _J Cl, KMnO,			
Rotten egg	H,S	Aeration, exidants	-			

*Organic compound abbreviations:

2-MIB	=	2-methylisoborneol
IPMP	=	2-isopropyl-3-methoxypyrazine
IBMP	=	2-isobutyl-3-methoxypyrazine
TCA	=	2,3,6-trichloroanisole
DMTS	=	dimethyl trisulphide
DMDS	=	dimethyl disulphide
PAC	=	powdered activated carbon
GAC	=	granular activated carbon

An early warning system has been proposed by McGuire *et al.* (1983) and Means and McGuire (1986). It consists of regular monitoring of nutrient levels in the lake, as well as thorough monitoring of troublesome algae, including benthic inhabitants, when necessary. The odour intensity in the water is also periodically checked. At the early sign of odour development measures are applied, for example adequate dosage with copper sulphate, to prevent bloom formation. Aeration of shallow aquifers as well as destratification and hypolimnetic aeration in deep lakes without destratification, may reverse the undesirable effects of oxygen depletion. Maintenance of adequate oxygen levels prevents the formation of an anaerobic zone. Anaerobic products will not be formed and odour forming cyanobacteria are usually replaced by other algal populations. Aeration also helps to mineralize iron and manganese salts and oxidizes the top sediment layer, thus reducing its demand for oxygen (Geney and Randolph 1988). Long-term destratification of Illinois Lake allowed for return of this reservoir to service as a potable water source (Bartels *et al.* 1987).

TABLE 5. SOURCE MANAGEMENT TOOLS



The simpler measures of source treatment are manipulation of the reservoir's level to expose and dehydrate benthic cyanobacteria (McGuire and Gaston 1988a, 1988b), application of algicides, especially for blue-green algae (Barnhard 1980; Scott *et al.* 1989) and prevention of light (Montiel 1983). Montiel (1983) mentions popular

algicides in use: amongst the mineral algicides are copper sulphate, potassium permanganate, chlorine, chlorine dioxide and silver preparations, while the organic algicides include aramzide, 2,3-dichloronaphtoquinone and polymyxime. It must be remembered, however, that algicide treatment of the algal bloom may bring about the disastrous effect of a sudden kill, with the release of odorous and toxic metabolites and the subsequent deterioration of the water (Wu and Jüttner 1988; Montiel 1983).

Prevention of light in the aquifer can be attained by maintaining water turbidity, e.g. with silt or activated carbon or by covering the reservoir (Montiel 1983). Cutting off the light prevents photosynthesis and reduces production of odorous metabolites. Krasner and Means (1986) achieved a marked improvement in microbial quality when the finished water reservoir was covered with floating covers.

Biomanipulation techniques are only in the early stages of development. Some biomanipulation successes were unplanned, such as the reduction of turbidity by cyanobacteriovorous *Daphnia* population which suddenly increased in number in Lake Washington (Edmonson and Abella 1988). Biomanipulation involves introducing useful species, from a lake management point of view, or promoting the success of indigenous desirable organisms. This leads to reduction in the rate of reproduction and survival of undesirable organisms. Natural competition and antagonism are employed, predation or parasitism commonly being the most practical (Sudo *et al.* 1989). Control of nutrient input and removal of excess nutrients may also aid in biomanipulation procedures (McDonnel and Crocker 1989).

Biodegradation of odorous organic compounds, mentioned earlier with biological water purification (Chapter 2.6), is the naturally occurring phenomenon. The concentration of odorous compounds in water is a function of the speed at which the bacterial population degrade it (Means and McGuire 1986). Some microbial populations, able to degrade 2-MIB, were found by Izaguirre *et al.* (1988a, 1988b).

Lake management strategies aimed at maintaining the overall balance of the water body bring advantages to nature conservation, wildlife, tourism, health and water purification industries. The analysis of costs, brought about by the adverse effects of algal growth on drinking water supplies, was performed by Magara and Kunikane (1986). The cost of conventional treatment increases five times and, together with the additional costs of taste and odour and organohalide removal, results in a twenty-fold increase when algae are present. Intensive lake management activities result in improved water quality and that brings both aesthetic and economic advantages for the whole community.

3. MATERIALS AND METHODS

3.1 Sampling

Samples were collected from 1989 to 1992 in the summer season. Fifty-four reservoirs, mostly from the Transvaal and Orange Free State were included in this study. Both odour-experiencing and odour-free water bodies were studied. Wherever possible the following samples were taken:

- (a) Surface water (150 mit) in a sterile glass bottle for bacteriological examination, chlorophyll a estimation and toxicity testing.
- (b) Top sediment (50-100 g) a grab sample collected in a sterile glass container for bacteriological examination.
- (c) Surface plankton scum (50-100 m/), when present, or alternatively a concentrated plankton sample by means of a 60 μm mesh plankton net for initial examination of the algal composition and bacteriological assessment.
- (d) Plankton sample (10-20 m/) preserved with an equal volume of 6:3:1 fixative (Standard Methods 1989) for further algal species identification and to keep for the record.
- (e) Surface water (4,5 litre) in a screw-cap jar, which had previously been baked overnight at 160°C, for sensory odour evaluation and instrumental analysis of odorous substances.

3.2 Bacteriological examination

Bacteriological tests were aimed at enumeration of total heterotrophic flora, fungi and actinomycetes. Samples were refrigerated if they could not be processed immediately upon arrival at the laboratory. Ten-fold dilutions were prepared in sterile saline; algal colonies were first disrupted by crushing with a sterile mortar and pestle.

Spread-plates were inoculated with 0,1 m/ of the following dilutions: 10° to 10° for water, 10° to 10° for sediments and 10° to 10° for plankton. Each sample was plated onto a range of solid media: Plate Count Agar (Difco); Sabouraud Agar (Oxoid); Nutrient Agar (Difco); Actinomycetes Isolation Agar (Difco); Columbia Agar + Selectatab (Mast Laboratories); Chitin Agar (Williams 1989); Starch-casein Agar (Waksman 1961).

All media, with the exception of the Plate Count and Sabouraud Agars, were supplemented with cycloheximide (100 mg/l) (Calbiochem) to prevent contamination by moulds. Sabouraud Agar was supplemented with chloramphenicol (50 mg/l) (Fluka) to inhibit bacterial growth.

Incubation of all plates took place at 28°C. Enumeration of total heterotrophic flora was performed after 48h, fungi after 72h and actinomycetes after 7,14 and 21 days.

Because performance of the actinomycete media was not uniform, enumeration was performed on plates where the highest counts were obtained.

3.3 Estimation of chlorophyll a content

The analysis was performed according to Sartory (1982). The chlorophyll data reflect algal concentrations at the sampling site - usually selected for its high algal density. They do not present the average values for a given water body.

3.4 Toxicity testing

- (a) Daphnia test: Toxicity assessments were conducted using the water flea lethality test with Daphnia pulex, according to EPA recommendations (1985).
- (b) Cyanobacterial toxin analyses: Extraction and analyses for microcystin toxins followed the procedure described in Scott (1991).

3.5 Investigation of odours

- Sensory analysis: An FPA (Flavour Profile Analysis) method was used (Mallevialle and Suffet 1987). Each sample was assessed by three to four trained panellists. Odour description was given and a rating was agreed upon, using a 5-point scale (See 3.7.2).
 Only odour of a sample was tested; no tasting was performed.
- (b) Instrumental analysis: If the results of the sensory investigation indicated that the sample contained odorous substances, detection and identification of these substances was attempted. A Closed-Loop Stripping Analysis (CLSA), followed by Gas Chromatography/Mass Spectrometry analysis was used, according to Krasner *et al.* (1983). The samples were analyzed at the Institute for Chromatography, University of Pretoria.

3.6 Cultivation of algal strains from odour incidents

Various dilutions of plankton samples were incubated under a 16 hours light; 8 hours darkness cycle at 25°C in Volk and Phinney's mineral medium (1968) modified as follows: EDDHA-Fe was replaced by EDTA-Fe to give the concentration of 1,2 mg Fe/*t* and the A5 trace element mixture (Stanier *et al.* 1971) replaced the Hoagland's A-Z trace element mixture.

3.7 Preliminary evaluation of treatment methods

3.7.1 Chemical compounds investigated

Tap water aliquots were odourised with geosmin, 2-MIB or isopropyl disulphide (IPD). Geosmin and IPD were chosen as substances known from local water sources and 2-MIB as a common nuisance in other countries, being very difficult to remove from water. The concentrations used in the screening experiments were 1 μ g/ℓ for geosmin and 2-MIB and 500 μ g/ℓ for IPD. Geosmin (98% pure) and 2-MIB (99,6% pure) were purchased from Wako Pure Chemicals Industries and IPD (96% pure) from the Aldrich Chemical Company.

3.7.2 Sensory analysis

The efficiency of a given treatment was tested by Flavour Profile Analysis, the olfactory method using three or more trained panellists for each set of samples. There are individual differences in odour perceptibilities, so the results produced by only one panellist would be insufficient for the proper assessment of samples. The Flavour Profile Method is subjective and panellists are prone to so called "odour fatigue" - a phenomenon of decreased susceptibility to a given odour after sniffing at several samples. This happens most often with 2-MIB which has a strong ability to block the nose. Sometimes the results for samples treated with lower concentrations of the agent tested were higher than those with higher concentrations. Despite these problems, the sensory method was chosen for the screening experiments because of rapidness, the relative ease of operation and the prohibitive cost of instrumental analysis for the large number of samples. The results reported here are the averages of all results obtained from individual panellists. Only odour was measured; no taste analyses were performed. According to Flavour Profile Analysis the intensity of smell was rated according to the following scale:

0 . no odour just perceptible 1 . 2 faint . 3 moderate 4 . strong 5 . very strong

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After preparing the relevant dilution of the odorous compound, a sample was transferred to a glass bottle and sealed. It was immediately analyzed by a sensory panel. This represented the untreated base-line against which the treated water was compared. After treatment samples were collected in glass bottles, sealed, and immediately analyzed by the panel.

3.7.3 Conventional water treatment

Water samples spiked with 2-MIB, geosmin and IPD, respectively, were flocculated with conventional coagulants and the floc was allowed to settle or was treated by Dissolved Air Flotation (DAF). After each treatment the samples were analyzed for residual odour. The following primary coagulants were used to flocculate the water:

- aluminium sulphate (alum)
- polyaluminium chloride (PACI)
- ferric chloride (FeCl₃)
- Catfloc T2 (a cationic polymeric flocculant)
- bentonite clay

<u>Settling</u>: These tests were conducted using a six paddle laboratory stirrer and 1 litre beakers. The beakers were filled with 500 m/ of the odourized water at pH 7 and whilst being stirred at 100 rpm the coagulant was added to the beakers. After 2 minutes of this rapid mixing the mixing speed was reduced to 40 rpm for a further 8 minutes to enable floc growth. A period of 30 minutes of settling was allowed and a sample of the supernatant was drawn off for analysis by the odour panel. After a further 30 minutes another sample of the supernatant was taken and analyzed. No pH measurements were performed at the termination of the experiment. A control experiment was run with each experimental batch. It had no coagulant added but was otherwise treated in exactly the same manner as those with coagulant.

<u>Flotation</u>: These tests were conducted using a bench scale DAF apparatus consisting of a stirrer and six flotation cells of 2 litre capacity each and a saturator with its related equipment. The configuration is illustrated in Figure 1.



Figure 1. Configuration of a bench scale DAF unit

The cells were filled with 1 litre of the odourised water and whilst being stirred at 100 rpm the coagulant was added to the cells. After 2 minutes of this rapid mixing the mixing speed was reduced to 40 rpm for a further 8 minutes to enable floc growth. Tap water saturated with air at 400 kPa was then introduced into the bottom of the cells. After the last of the air bubbles had reached the surface (approximately 10 minutes) a sample of the subnatant was drawn off and analyzed by the odour panel.

A recycle ratio of 40% was used for all the experiments. It has been empirically found that this equates to 20% recycle on full scale applications. Control experiments without coagulants were run in an identical fashion.

3.7.4 Activated carbon (screening experiments)

The experimental setup for activated carbon and oxidation treatment is shown in Figure 2.



Figure 2. The experimental setup used for activated carbon treatment and oxidation by chlorine, chlorine dioxide and hydrogen peroxide.

An activated carbon Norit SA-42, frequently used in water treatment, was utilized in this investigation. The carbon was used in powdered form (PAC) to increase adsorption rate. Different dosages of PAC were added to the beakers containing water spiked with geosmin, 2-MIB or IPD. The contents of beakers was stirred at 200 rpm to ensure sufficient contact between the activated carbon and the compound in water. As a control, the pollutant in water was subjected to stirring, but no PAC was added to the sample. The solutions were stirred for 60 minutes to ensure that equilibrium is reached between the concentration of the pollutant in water and the amount of pollutant adsorbed onto carbon. Finally, the activated carbon was removed by filtration through a 0,45 µm filter and the filtrate was analyzed by a sensory panel for residual odour.

3.7.5 Oxidation (screening experiments)

The oxidants used in this study were chlorine (Cl₂), chlorine dioxide (ClO₂), hydrogen peroxide (H₂O₂) and ozone (O₃). The experimental set-up for first three compounds is shown in Figure 2. Different doses of chlorine, chlorine dioxide or hydrogen peroxide were added to water spiked with 1 μ g/ℓ geosmin, 1 μ g/ℓ 2-MIB or 500 μ g/ℓ IPD. The contact time in the experiment was 60 minutes to ensure sufficient time for oxidation to take place. The analysis of the treated water was done by a sensory panel. Control samples did not receive oxidants.

Ozone treatment: Different ozone dosages were fed to the water contaminated with odorous substances in an ozone contact reactor (Figure 3). The contact time was 4 minutes. After treatment samples were immediately tested by a sensory panel.

3.8 Optimisation of selected treatment options

3.8.1 Activated carbon

Three model substances were tested: geosmin, 2-MIB and IPD. A range of concentrations for each compound (50 ng - 1000 ng/ ℓ for geosmin and 2-MIB; 250 - 1000 μ g/ ℓ for IPD) was exposed for 30 minutes with continuous stirring to the following carbons:

- Norit SA 2 powdered, steam activated carbon, with internal surface of 800 m²/g.
- Norit CA 3 powdered, chemically activated carbon, manufactured by the phosphoric acid process. Internal surface = 1000 m²/g.
- Norit SA 42 granular, steam activated carbon, internal surface area = 800 m²/g.
- Norit RB 3 granular, steam activated carbon, internal surface area = 1000 m²/g.

All types of carbon are manufactured and available locally.



Figure 3. Ozone contact reactor

To each concentration of odorous substance tested different dosages of a given carbon were added, ranging from 5 to 60 mg/ ℓ . After incubation, carbon was removed by filtration through a 0.45 μ m filter and the filtrate tested for any residual odour by the FPA method using a trained sensory panel. The first carbon

concentration at which complete T&O removal was achieved was regarded as a minimum effective dosage for a given concentration of the odorous compound tested.

3.8.2 Oxidation studies

Ozone (Ferguson *et al.* 1991) and PEROXONE (Ferguson *et al.* 1990) treatments were studied, using the same experimental setup. The three model substances: geosmin, 2-MIB and IPD, were prepared in concentration ranging from 20 to 1000 ng/ ℓ for geosmin and 2-MIB and from 100 to 1000 µg/ ℓ for IPD. Approximately 500 m ℓ of each dilution was used for every run. An ozone generator using oxygen as feed gas was used. Contact time of at least 4 minutes was applied. The experiment was terminated when ozone discoloured a KI solution which indicated that the ozone demand of the solution has been satisfied. An ozone dosage required to remove a given concentration of odorous compound was calculated from the known amount of ozone dosed before KI discolouration.

For PEROXONE experiments, a hydrogen peroxide to ozone ratio of 0,4 was used. The dosage of hydrogen peroxide was applied to the experimental solution prior to ozonation and the ozone dose necessary for the complete removal of each of the model substances was estimated as before.

RESULTS

4.1 Description of the reservoirs tested

Over the three year period of investigation fifty-four reservoirs, impoundments or pans, situated mostly in the Transvaal and Orange Free State, were sampled. Apart from irrigation most of the water bodies serve as a drinking water source and/or recreational site. The water bodies included in this study are listed in Table 6. On the day of sampling twenty-two of them (37%) displayed algal blooms of different intensities as manifested by a visible green coloration of the water or by scum formation. Of these, seventeen (31,5% of the dams tested and 77% of the dams with a bloom) experienced a T&O incident.

TABLE 6. LIST OF WATER BODIES SAMPLED, IN CHRONOLOGICAL ORDER

Water body	Sampling date	Locality	Drinking water source	Recreation	Algal bloom present
Rietviei Dam	7.8.1989	Pretoria	+	+	
* Vaal Dam	17.1.1990	Northern OFS	+	+	+
* Wentzel Dam	1.2.1990	Schweizer Reneke	+	+	+
• Nagle Dam	8.2.1990	Pietermaritzburg	+		+
Witbank Dam	8.5.1990	Witbank	+	+	
Kruger Dam	8.5.1990	Middelburg	-	+	4
Rietkuil	8.5.1990	Hendrina	-	-	
Lake Chrissie	8.5.1990	Chrissiesmeer	-		-
Tweelingpan	8.5,1990	Chrisslesmeer			+
Douglas Dam	8.5.1990	Ermelo	+	+	
Mahawane Dam	8.5.1990	Volksrust	+	+	
 Grootdraai Dam 	8.5.1990	Standerton		+	+
Hartbeespoort Dam	9.5.1990	Brits	+	+	
Klipvoor Dam	9.5.1990	Borakalalo NP Bephuthatswana	4	+	+
Vaalkop Dam	9.5.1990	Boestekraal	+	+	
Roodekopjes Dam	9.5.1990	Beestekraal			
* Bospoort Dam	9.5.1990	Rustenburg	+	+	+
Potchefstroom Dam	9.5.1990	Potchefstroom	+	+	
Boskop Dam	9.5.1990	Potchefstroom	+	+	18
Roodeplaat Dam	10.5.1990	Pretoria		+	
Pretoria Power Station	10.5.1990	Pretoria	4		+
* Jan Smuts	10.5.1990	Brakpan	-	+	4
Boksburg Lake	10.5.1990	Boksburg	.+	+	+
Victoria Lake	10.5.1990	Germiston	-	+:	-
 Zoo Lake 	10.5.1990	Parktown, Johannesburg	-	+	+
Florida Lake	10.5,1990	Florida, Roodepoort		+	
Albasini Dam	24.4.1991	Venda	+		
Hans Merensky Dam	24.4.1991	Tzaneen			-
Fanie Botha Dam	24.4.1991	Tzaneen	+	+	
Magoebaskloof Dam	24.4.1991	Tzaneen			
Dap Naude Dam	24.4.1991	Tzaneen		+	4
	-		-		

T&O incident present on sampling day.

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+

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Tzaneen

24.4.1991

Ebenezer Dam

Water body	Sampling date	Locality	Drinking water source	Recreation	Algai bloom present
PTC du Plessis Dam	24.4.1991	Lydenburg	+	+	- 22
Braam Raubenheimer Dam	24.4.1991	Lydenburg		+	- X.
* Vaal Dem	29.4.1991	Northern OFS	+	+	+
Uniefees Dam	29.4.1991	Heilbron	+	+	
Koppies Dam	29.4.1991	Koppies	+	+	1.1
Bloemhoek Dam	29.4.1991	Kroonstad	+	+	
* Allemanskraal Dam	29.4.1991	Winburg	+	+	+
Erfenis Dam	30.4.1991	Winburg		+	+
Flamingo Pan	30.4.1991	Welkom		+	
* Toronto Pan	30.4.1991	Welkom			+
* Bloemhof Dam	30.4.1991	Bloemhof	+	+	+
* Wentzel Dam	30.4.1991	Schweizer Reneke	+	+	+
Barberspan	30.4.1991	Sannieshof		+	+
Rustfontein Dam	17.3.1992	Thaba Nchu	+	+	
Knellpoort Dam	17.3.1992	Wepener	+	÷	
Welbedacht Dam	17.3.1992	Wepener		+	
Van Stadensrus Dam	17.3.1992	Van Stadensrus	+	+	
* Hendrik Verwoerd Dam	17.3.1992	Hendrik Verwoerd Dam	+	+	+
Krugersdrift Dam	18.3.1992	Bloemfontein	+	+	
Klerksdorp Dam	18.3.1992	Klerksdorp	+	+	1.1
* Hartbeespoort Dam	26.3.1992	Brits	+	+	+
* Bridledrift Dam	10.4.1992	East London	+	+	+
* Umzoniana Dam	10.4.1992	East London	+		+

4.2 Enumeration of bacteria, fungi and actinomycetes

The results for each year of bacteriological examination of water, plankton and sediments are given in Tables 7 - 9. In cases of where the sampling site had a rocky bottom, sediment samples could not be collected and no bacteriological results are reported for these reservoirs. The same applies for phytoplankton analyses for dams not experiencing algal blooms. Overall, total heterotrophic bacteria count in surface water samples ranged from 10³ to 10⁹ CFU/m². Fungi counts, comprised mainly of moulds, ranged from <10 to 7·10⁴ CFU/m². Actinomycetes were present in detectable numbers in 13 non-odorous lakes (from 10 to 6·10³ CFU/m²) and in 3 odour-experiencing reservoirs (Vaal Dam 2,5·10², Grootdraai Dam 10³ and Bloemhof Dam 3·10³ CFU/m²).

No correlation between bacterial, fungal or actinomycete counts in water and the presence of odour in the reservoir was found.

Bacterial counts in the plankton samples were generally very high (up to 10¹³ CFU/m/), which may be an indicator of the decay of scums. Actinomycetes were found in seven plankton samples tested. In three samples the count was below 10³ CFU/m/, but in other four samples significant numbers were present. For these localities the contribution of actinomycetes to the odour of water could not be excluded. The four samples were Wentzel Dam in 1990 (7,5·10⁵ CFU/m/), again in Wentzel Dam in 1991 (5·10⁶ CFU/m/), Allemanskraal Dam (10⁵ CFU/m/) and Bloemhof Dam (8,5·10⁵ CFU/m/).

Counts of bacteria, fungi and actinomycetes in sediments were uniformly high both in odorous and non-odorous sources. No correlation between their number and the presence of odour in water could be found.

TABLE 7. BACTERIOLOGICAL EXAMINATION OF WATER, PLANKTON AND SEDIMENTS, 1990

NA - sample not available * - T&O incident present

Neg. - negative result: < 10 CFU/mℓ for water and algae, <100 CFU/g for sediments

			Microbial cou	Microbial counts (CFU/g d.m.)						
Water body		Water			Plankton			Sedimenta		
	Total count	Fungi	Actinomycetes	Total count	Fungi	Actinomycetes	Total count	Fungi	Actinomyorian	
Hetriel Dam	1,4-10*	100	3.10	NA	NA	NA	1,5-104	5.10*	101	
* Vesi Dam	7.60*	4.102	2,540°	6,810*	3.402	3.610	240'	1,6.10*	3,110*	
* Wentzei Dem	2,140*	2:10 ²	Neg	1,1.10*	4.104	7,5-60°	74A	NA	NA	
* Nage Dam	3.5-10*	3402	Neg	8-10*	2.404	3·10 ²	3,410'	7,8 10	1,1-10*	
Witbank Dam	107	5-10'	Neg	4.604	5.40*	Neg	144	NA	NA	
Kruger Dem	B.E-10 ⁸	100	Neg.	NA	NA	ŇA	1,6-10*	4,8-10 ²	6.1.10*	
Retivul	3,7:10*	5.40*	Neg	NA	NA	NA	8.9-10*	4,610*	3.2.10*	
Lake Christee	3-10*	101	101	NA	NA	NA	5-10'	4,610	1.2.10*	
Tweelingsan	2.10*	410	Neg	10+	104	Nep	1,3-10*	9.107	2,6-10 ³	
Dougles Gem	440*	10	2101	10*	4-10 ²	3-10 ⁶	4,1-10*	2,7:109	1,4-104	
Mahawahe Dan	4,1 107	0.10	210	NA	NA	NA.	1,3.10*	6,9.407	8.4.10*	
* Grootpiaai Dan	1,3.40*	2.40	10 ^p	a.10'	1,1-102	10*	2,4.40	1,2:10*	1,2:10*	
Hartbeespoort Dam	4.40*	10'	Neg	764	NA	NĂ	5,2-104	8.6-10*	3,9-10*	
 Klipvoor Dam 	1,2:10*	10'	Neg.	2,5:10*	1,1-10*	Neg	8.8-10*	1,1-10*	3,7:10 ²	
Vashop Dam	8.10	ti\$ ⁴	90 ¹	NA	NA	NA	4,1:104	2.110*	4.10*	
Roodekopjes Dam	4.10*	4.10	90'	NA	NA	NA	8,2-10*	2.3.10*	2,5:10 ³	
* Blospoort Dam	1,2:10*	107	Neg.	10%	3:10	Neg	8.4-10*	2,6-10*	3,8-10*	
Poluhefutroom Dam	1.5-10*	10 ⁴	Neg	NA.	NA	NA	2,6.10*	1,3:10*	5,2:40*	
Boskop Dam	10*	4.10'	Neg.	NA.	NA	NA	2,6-10*	1,6-10*	2,540*	
Pretoria P. Station	104	10'	Neg.	2,5:10*	Neg	Neg.	NA	NA	NA	
Jan Smuts	10*	2:10'	Neg	10.0	5-10*	Neg	1,5 004	8,7.10*	2,9-10*	
Bokaburg Lake	2.7.104	4.401	Ning	NA	NA	NA	NA	NA	NA	
Victoria Lake	10*	Neg.	heg.	NUA	NA	NA	6.9101	8,2:40°	810*	
* Zoo Lake	50'	Neg.	Neg	10%	3:10'	Neg	3.1.10*	1,1-10 ⁸	7.7.40'	
Florida Loke	2.10*	310	Ned	NA	NA	NA	8.2-10	2.6 10	2.5 40*	

TABLE 8. BACTERIOLOGICAL EXAMINATION OF WATER, PLANKTON AND SEDIMENTS, 1991

NA .

Sample not available negative result: <10 CFU/mℓ Neg. -

* T&O incident present .

			Microbial court	ts (CFU/mę			Mic	robiel counte (CFU)	g d.w.)	
Water body		Water			Flankton			Sediment		
	Total count	Fungi	Actinomycetee	Total rount	Fungi	Actinomycetes	Total count	Fungi	Actinomycele	
Albasini Dam	610*	102	Neg	NA	NA	nin.	3,7 104	7.310*	a.7 10 ⁴	
Hans Mersnaky Dem	7.10*	7.10*	Neg.	NA	NA	NA	2,8:402	2,1.10*	2.1.10*	
Farie Botha Dem	3-10*	8.107	Neg.	NA	NA	NA	4,3-10*	1,8-10*	1,51/10*	
Magoebaskloof Dam	3.10*	3105	Neg.	NA	NA	NA	4,8.10*	3.2 10*	1,6:10*	
Dep Naude Dam	3.5-10*	4.10 ⁴	Neg	NA	NA	NA	1.6-10*	1,1 10*	8.2-10*	
Ebenezer Dan	# 10 ⁴	104	Neg.	NA.	NA	NA	1,4.10 [#]	4,1.10*	6.610*	
PTC du Plesais Dan	10*	101	Nep	NA	NA	NA	1,6:10*	1,1.40*	3,1.10*	
Braam Raubenheimer Dam	1,4-10*	5-10 ⁰	2.101	NA	NA	NA	3,2 10'	2,5 10'	3.8.10*	
Uniefees Dare	10*	6.10	Neg	NA	NA	NA	4,1.10*	2, ¥ ±0*	10°	
Roppies Dam	1.410*	5-10	5.107	NA	NA	NA	6.7 ×0 ⁴	1,7.404	2.7 104	
Bicemhoek Dan	5-10*	hing	3-10'	NA	NA	NA	1010	104	1.3.10/	
Erfenie Dan	8 10 ⁴	4.10*	8-10*	NA	NA	NA	2.9.10*	7,1:10*	7.1.10*	
Flamingo Pan	4.50°	Neg	3.10	NA	NA	NA	3,8-10*	1,9-10*	2,5.10*	
Barberspan	10%	3.10	5.10 ^p	NA	NA	N/A	7,7.10'	4.5.10%	1,8-107	
• Vaai Dan	1.2 10 ⁴	4.102	Neg.	1,7-10*	1,1/10	Neg	3,6 104	1.2.10*	9,5.10'	
Alemanikraal Dam	2.10'	2,4-10 ²	Neg	B-10 [#]	5-10 ⁴	104	6,5-10*	3,9.10*	10#	
Taronto Pan	10'	2.10°	Neg.	NA	NA	NA	4.5:10*	3,8 00*	5.1.10*	
Bioemitol Dam	5,410*	810*	340	B-10 ^e	3.10*	0.10 ^p	4,9.10*	1,2:50*	7.3 10*	
Wentuck Dam	10*	10'	Neg	3,2.10'*	4.10*	5.10*	8,1.107	3.7.50*	2.5.10*	

TABLE 9. BACTERIOLOGICAL EXAMINATION OF WATER, PLANKTON AND SEDIMENTS, 1992

Sample not available NA

negative result: <10 CFU/mℓ, <100 CFU/g T&O incident present Neg. . *

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		Microbial counts (CFU/m.)						Microbial counts (CFU/g d.w.)		
Water body		Water			Plankton		Sediment			
	Total count	Fungi	Actinomyoetee	Total count	Fungi	Actinomycetes	Total sount	Fungi	Actinomycete	
Rustontein Dem	10*	2.10 ⁰	Neg	N/A	NA	NA	NA	NA	NA .	
Knelpoort Dam	10*	5.40 ⁴	Neg	NA	NA	NA	NA	NA	NA	
Weibedacht Dam	19-10 [#]	Neg.	Neg.	NA:	NA	NA	6,3-10 ²	1,5-10*	3,140*	
Van Standensrus Diem	1,2:10*	101	Neg	NA	NA	NA	3.5-10*	1,4.10*	7.609	
Krugenschift Dam	1,5-10'	8.10 ¹	Neg	NA	NA	NA	1,6-1011	4,8-10*	Nig	
Klerkadorp Dam	3.10*	100	3.40'	NA.	NA	NA	8,410*	3.8.101	8-101	
* Hendrik Veneslend Dam	6,5-10*	201	Neg	1010	2,1:10 ⁶	Neg	4.6.10*	100	Nerg.	
* Hartbeespoort Dain	1,3-10*	5/10	Neg	1010	6-10 ²	Neg.	1.6-10*	1.3.10 ²	4,4:10	
* Bricledrift Dam	104	Neg	Neg	4.10*	Neg	Neg	30*	Neg.	1,7-104	
· Linzonena Dan	1,5-102	Neg	Neg	6-10*	Neg.	Neg	NA	NA	NA	

4.3 Examination of plankton

Lists of the more important algal species recorded from water bodies without or with odour formation are presented in Tables 10 and 11. A total of 41 species from 5 algal groups was found.

Reservoir	Abundance (as chlorophyll a) (µg/ð)	Algae present
Witbank Dam	<5	Cyanophyceae: * Microcystis aeruginosa Dinophyceae: Gymnodinium sp. Bacillariophyceae: Melosira granulata var. angustissima
Kruger Dam	<5	Cyanophyceae: * Microcystis aeruginosa Spirulina sp. Chlorophyceae: Pediastrum simplex Bacillariophyceae: Melosira granulata var. angustissima Melosira granulata Nitzschia sp.
Rietkul	11,5	Chlorophyceae: * Monoraphidium sp. Scenedesmus sp. Crucigenia sp.
Tweelingpan	149.0	Cyanophyceae: * Microcystis aeruginosa Microcystis aeruginosa t. flos-aquae Pseudanabaena sp. Chlorophyceae: Staurastrum sp. Scenedesmus sp. Monoraphidium sp. Pediastrum simplex Bacillariophyceae: Meiosira granulata Meiosira granulata Meiosira granulata var angustasima
Douglas Dam	27.5	Cyanophyceae: Microcystis aeruginosa Chlorophyceae: Staurastrum sp. Bacillariophyceae: * Melosira granulata var. angustissima Melosira granulata Melosira varians

TABLE 10. ALGAL INVENTORY FOR ODOUR-FREE LAKES * - dominant species

Reservoir	Abundance (as chlorophyll a) (µg/ð)	Algae present
Mahawane Dam	<5	Cyanophyceae: Anabaena circinalis Microcystis aeruginosa Bacillariophyceae: * Melosira granulata var. angustissima Cocconeis ap. Nitzschia sp.
Hartbeespoort Dam	<5	Cyanophyceae: Anabaena sp. Chlorophyceae: Pediastrum simplex Bacillariophyceae: * Melosira granulata var. angustissima Melosira granulata Cyclotella sp.
Vaalkop Dam	<5	Cyanophyceae: * Microcystis aeruginosa I. flos-aquae Microcystis viridis Chlorophyceae: Pediastrum simplex Scenedesmus sp.
Roodekopjes	20,6	Cyanophyceae: * Microcystis aeruginosa Microcystis aeruginosa t. flos-aquae Anabaena solitaria Lyngbya sp. Chlorophyceae: Pediastrum simplex Bacillariophyceae: Melosira granulata var. angustissima Nitzschia sp.
Potchefstroom Dam <5		Cyanophyceae: Microcystis aeruginosa Pectodictyon sp. Chlorophyceae: Pediastrum simplex Bacillariophyceae: Melosira granulata var. angustissima
Boskop Dam	<5	Cyanophyceae: Microcystis aeruginosa Merismopedia sp. Chlorophyceae: Pediastrum simplex Scenedesmus sp. Euglenophyceae: Euglena sp. Bacillariophyceae: * Navicula sp. Melosira granulata var. angustissima Melosira granulata Cymbella sp.
Pretoria Power Station	1485,7	Cyanophyceae: * Microcystis wesenbergii Bacillariophyceae: Melosira granulata var. angustissima

Reservoir	Abundance (as chlorophyll a) (µg/ð	Algae present
Boksburg Lake	316,4	Cyanophyceae: Microcystis wesenbergii Monoraphidium sp. Chiorophyceae: * Chiorella sp.
Victoria Lake	6,9	Cyanophyceae: Spirulina sp. Aphanothece sp. Chlorophyceae: Pediastrum simplex Pediastrum duplex Scenedesmus sp. Bacillariophyceae: * Melosira granulata var. angustissima
Albasini Dam	7,17	Bacillariophyceae: * Melosira granulata var. angustissima
Hans Merensky Dam	8.6	Bacillariophyceae: * Melosira granulata var. angustissima
Fanie Botha Dam	. 15,76	Cyanophyceae: * Microcystis aeruginosa I. flos-aquae Chlorophyceae: Botryococcus braunii
Magoebaskloof Dam	21,5	Bacillariophyceae. * Melosira granulata var. angustissima Nitzschia sp. Cyanophyceae: Microcystis aeruginosa
Dap Naude Dam	8.6	Cyanophyceae * Microcystis aeruginosa Bacillariophyceae: Cocconeis sp. Nitzschia sp. Euglenophyceae: Euglena sp.
Ebenezer Dam	1,0	Bacillariophyceae: * Melosira granulata var. angustissima Nitzschia sp. Cyanophyceae: Staurastrum sp.
PTC du Plessis Dam	1.0	Bacillariophyceae: * Melosira granulata var. angustissima Melosira granulata Chlorophyceae: Pandorina sp.
Braam Raubenheimer Dam	4,3	Cyanophyceae: * Microcystis aeruginosa f. flos-aquae Bacillariophyceae: Melosira granulata var. angustissima Nitzschia sp. Chiorophyceae: Pediastrum sp. Staurastrum sp.

Reservoir	Abundance (as chlorophyll a) (µg/ð)	Algae present
Jniefees Dam	1,0	Cyanophyceae * Microcystis aeruginosa Bacillariophyceae: Melosira granulata var. angustissima Chlorophyceae: Pandorina sp. Coelastrum sp.
Koppies Dam	17.2	Cyanophyceae: * Microcystis aeruginosa t. flos-aquae Anabaena circinalis Bacillariophyceae: Melosira granulata var. angustissima Nitzschia sp.
Bloemhoek Dam	7,48	Cyanophyceae * Microcystis aeruginosa Aphanothece sp.
Erfenis Dam	103,2	Cyanophyceae: * Microcystis aeruginosa f. fios-aquae Anabaena circinalis Bacillariophyceae: Nitzschia sp.
Flamingo Pan	17,2	Cyanophyceae: * Nodularia sp. Bacillariophyceae: Navicula sp. Cocconeis sp. Nitzschia sp.
Barberspan	75,95	Cyanophyceae: * Microcystis aeruginosa Microcystis aeruginosa t. fios-aquae Rivularia sp.
Rusfontein Dam 10,0		Cyanophyceae: * Aphanothece sp. Euglenophyceae: Trachelomonas sp. Bacillariophyceae: Melosira granulata var. angustissima
Knellpoort Dam	1,5	Bacillariophyceae: * Melosira granulata var. angustissima Cyclotella nana Amphora sp. Cyanophyceae: Phormidium sp. Nodularia sp. Chlorophyceae: Botryococcus braunii Staurastrum sp. Cosmarium sp.
Welbedacht Dam	0,10	Euglenophyceae: Trachelomonas sp.

.

Reservoir	Abundance (as chlorophyll a) (µg/#)	Algae present
Krugersdrift Dam	0,83	Cyanophyceae: * Morocysts aeruginosa t. flos-aquae Merismopedia sp. Bacillariophyceae: Melosira granulata var. angustissima Chlorophyceae: Pandorina sp. Chlamydomonas sp. Euglenophyceae: Euglena sp.

TABLE 11. INVENTORY OF THE DOMINANT PLANKTON SPECIES IN LAKES EXPERIENCING ODOUR INCIDENTS

1

	* -		most	abundant	species		
_	_	-				_	

Reservoir	Abundance (as chlorophyll a) (µg/n)	Algae present		
Vaal Dam 1990	>100	Cyanophyceae: Morocystis aeruginosa Pseudanabaena sp. Bacillariophyceae: Nitzschia sp.		
Wentzel Dam	>100	Cyanophyceae: * Microcystis aeruginosa Microcystis aeruginosa t. flos-aquae Microcystis wesenbergii Anabaena sp.		
Nagle Dam	>100	Cyanophyceae: * Anabaena solitaria		
Klipvoor Dam	573,2	Cyanophyceae: Microcystis aeruginosa Anabaena circinalis Microcystis viridis Pseudanabaena sp. Chlorophyceae: Pediastrum simplex Staurastrum sp. Bacillariophyceae: Melosira granulate var. angustissima Nitzschia sp.		
Bospoort Dam 187.56		Cyanophyceae: * Microcystis aeruginosa Anabaena circinalis Pseudanabaena sp.		
Jan Smuts	57,3	Cyanophyceae * Morocystis aeruginosa Bacillariophyceae: Melosira granulata var. angustissima Nitzschia sp.		

Reservoir	Abundance (as chlorophyll #) (µg/#)	Algae present		
Grootdraal Dam	369,1	Cyanophyceae: * Microcystis aeruginosa Anabaena circinalis Pseudanabaena sp. Bacillarlophyceae: Nitzschia sp.		
Zoo Lake	165,1	Cyanophyceae: * Microcystis seruginosa Anabaena circinalis Chlorophyceae: Oocystis sp. Pediastrum boryanum var. boryanum Euglenophyceae: Phacus sp. Bacillariophyceae: Melosira granulata Melosira granulata var. angustissima		
Vaal Dam 1991	5488,4	Cyanophyceae: * Microcystis aeruginosa Anabaena circinalis Pseudanabaena sp.		
Allemanskraal Dam	120,1	Cyanophyceae: * Microcystis aeruginosa		
Toronto Pan	77,2	Cyanophyceae: * Nodularia sp. Microcystis aeruginosa		
Bloemhof Dam	318,1	Cyanophyceae: * Microcystis aeruginosa t. flos-aquae		
Wentzel Dam	682,1	Cyanophyceae: * Microcystis aeruginosa Pseudanabaena sp. Bacillariophyceae: Melosira granulata var. angustissima		
Hendrik Verwoerd Dam	32.65	Cyanophyceae: * Microcystis aeruginosa 1. flos-aquae		
Hartbeespoort Dam	49,85	Cyanophyceae: * Microcystis aeruginosa t. fios-aquae Microcystis viridis Anabaena circinalis Pseudanabaena sp. Bacillariophyceae: Melosira granulata var. angustisaima Nitzschia sp. Chlorophyceae: Pediastrum sp.		
Bridlednitt Dam	442.20	Cyanophyceae: * Microcystis aeruginosa Bacillariophyceae Melosira granulata var. angustissima		
Umzoniana Dam	834,10	Cyanophyceae: * Microcystis aeruginosa Microcystis aeruginosa t. flos-aquae Pseudanabaena sp. Bacillariophyceae: Melosika granulata var. angustissima		

Abundance of the total phytoplankton population has been expressed in terms of the chlorophyll *a* content. It is noticeable that the chlorophyll levels in water bodies with odours were generally substantially higher than those without odours.

All odour experiencing lakes were dominated by cyanobacteria, with two species of *Microcystis, M. aeruginosa* and *M. aeruginosa* 1. *Ilos-aquae*, dominant in most of the cases. Odour-free reservoirs were dominated by diatoms (11 cases, mostly *Melosira granulata* var. *angustissima*) and cyanobacteria (17 cases, mostly *Microcystis aeruginosa*).

Amongst the odour-free water bodies only 5 had an algal content reaching bloom proportions (Tweelingpan, Pretoria Power Station, Boksburg Lake, Erfenis Dam and Barberspan), all other localities had low amounts of algae.

4.4 Investigation of odour incidents

The data relating to odour-experiencing lakes and dams are compiled in Table 12. Taste and odour incidents were noted 17 times (Vaal and Wentzel Dams were inspected twice and T&O incidents were recorded on both occasions for each of the dams). All odour-experiencing lakes serve as drinking water supplies and/or recreational sites. At all of them a smell, most often described as earthy/muddy, was noticeable in the air. Flavour Profile Analysis of water samples gave them intensity scores from 1 (just perceptible) to 5 (very strong). Water samples were further analyzed by means of gas chromatography/mass spectrometry. At the beginning of the project, chromatograms were examined only for the presence of the following geosmin, 2-MIB, isopropyl disulphide, isopropyl selected T&O substances: methoxypyrazine, isobutyl methoxypyrazine and trichloroanisole. From the second year, full chromatographic analyses were performed. Examples of chromatograms are given in Figures 4 to 8. The quantification of odorous substances could only be done if standards were available, with other compounds assessment of their concentrations in water samples was not possible. The mass spectra of the compounds identified without the confirmation by comparison with a standard are presented in Figure 9a-d.

Geosmin was the most common T&O factor detected in South African water reservoirs during the course of this study. It was found in eight cases ranging from 7,5 to 3 170 ng/ℓ. Various sulphur compounds were found at 9 localities, hydroxyanisole derivatives in three reservoirs and isopropyl-2-methoxypyrazine in one case. 2-MIB, commonly associated with odours in water overseas, was not detected during this survey.

Cyanobacteria dominated all odour-experiencing lakes. The biomass of planktonic algae varied from 32 to 5500 μ g/l, as chlorophyll a. *Microcystis aeruginosa* was a predominant organism in 13 cases, *M. aeruginosa* f. *flos-aquae* in 2 cases, and there was one bloom of *Anabaena solitaria* and one of *Nodularia* sp. Sediments were not evaluated for algae. During sampling dam edges and sediments at close range to the banks were visually inspected for the presence of attached algae. No significant benthic algal development was found. Most South African reservoirs have fluctuating water levels and high silt content resulting in high turbidity which discourages growth of benthic autotrophs.

TABLE 12. TASTE AND ODOUR INCIDENTS

Odour intensity (FPA method): 0 - none, 1 - just perceptible, 2 - faint, 3 - moderate, 4 - strong, 5 very strong ND - not determined Negative toxicity = < 10%

Reservoir	Chlorophyll a (µg/ð	Dominant organism	Odour in water (FPA rating)	Odorous compound detected	Concentration	Toxicity
Vaal Dam 1990	> 100	Microcystis aeruginosa	4	geosmin	1630 ng/2	Neg.
Wentzel Dam 1990	> 100	Microcystis aeruginosa	5	geosmin	150 ng//	Neg.
Nagle Dam	> 100	Anabaena solitaria	2	geosmin	220 ng/I	Neg
Kipvoor Dam	573,2	Microcystis aeruginosa	5	geosmin	470 ng/I	Neg.
Bospoort Dam	137,6	Microcystis aeruginosa	4	geosmin	3170 ng/i	Neg.
Jan Smuts	57,3	Microcystis aeruginosa	2	geosmin	390 ng/ł	Neg.
Grootdraai Dam	369,1	Microcystis aeruginosa	4	dimethyl trisulphide	ND	Neg.
Zoo Lake	165,1	Microcystis aeruginosa	3	dimethyl trisulphide	ND	Neg.
Vaal Dam 1991	5 488,4	Merocystis aeruginosa	з	geosmin	7,5 ng/2	45%
Allemanskraal Dam	120,1	Microcystis aeruginosa	2	isopropyl disulphide	2 µg/l	25%
Toronto Pan	77,2	Nodularia sp.	4	geosmin	105 ng//	Neg.
Bloemhof Dem	318,1	M aeruginosa t. flos-aquae	1	isopropyl disulphide isopropyl-2-methoxypyrazine	0,2 µg/i 0,5 µg/i	Neg.
Wentzel Dam 1991	682,1	M aeruginosa	1	isopropyl disulphide	0,1 µg/2	Neg.
Hendrik Verwoerd Dam	32,6	M aeruginosa t. Bos-aquae	2	sulphur polymer molecular sulphur tertiary butylhydroxyanisole	ND ND ND	Neg.
Hartbeespoort Dam 1992	49,9	Microcystis aeruginosa	3	sulphur polymer molecular sulphur tertiary butylhydroxyanisole	ND ND ND	Neg.
Bridledrift Dam	442,2	Microcystis aeruginosa	2	dimethyl trisulphide molecular sulphur tertiary butylhydroxyanisole	ND ND ND	Neg.
Umzoniana Dam	834,0	Microcystis aeruginosa	2	molecular sulphur tertiary butylhydroxyanisole	ND ND	Nog.



Fig. 4. Chromatogram of a water sample taken from Vaal Dam, 1990. 51



Fig. 5. Chromatogram of a water sample taken from Bospoort Dam, 1990.



Fig. 6. Chromatogram of a water sample taken from Bridledrift Dam, 1992.



Fig. 7. Chromatogram of a water sample taken from Hartbeespoort Dam, 1992.



Fig. 8. Chromatogram of a water sample taken from Hendrik Verwoerd Dam, 1992.





- a/ dimethyl trisulphide,
 c/ molecular sulphur,
- b/ tertiary butylhydroxyanisole,
- econer sorprior,

d/ sulphur polymer

Toxicity tests

Odorous algal by-products are generally not toxic. On the other hand, organisms which produce them are known producers of toxins (Scott, 1991, Himberg *et al.* 1989). There could be the danger of one strain producing both types of by-products simultaneously. For this reason all odorous surface water samples were subjected to the *Daphnia* toxicity test. In two samples out of 17 some toxicity to *Daphnia* was detected (Table 12). At Vaal Dam toxicity was 35% after 24h and 45% after 48h (values of <10% lethality are interpreted as not toxic). At Allemanskraal toxicity was 15% after 24h and 25% after 48h. Unfortunately no odorous algae were isolated from these reservoirs so examination of toxin production by these strains could not be performed.

Microcystis toxins

Analyses of the *Microcystis* toxin content in a limited number of freeze-dried algal samples collected from the blooms are summarised in Table 13. The *Microcystis* toxins are known as microcystins and the different microcystins are identified by the presence of L-amino acids in the heptapeptide molecule. Microcystin-LA therefore indicate the toxin containing L-leucine and L-alanine.

	Microcystin			
	YR'	LA'	LR*	YA'
Grootdraai Dam	2100	455	0	0
Klipvoor Dam	2350	20	90	270
Pretoria Power Station	800	0	470	0
Vaal Dam 1991	3500	0	375	0
Jan Smuts	trace	0	0	0
Bospoort Dam	0	0	0	0

TABLE 13. MICROCYSTIN CONTENT (MG/G DRY WEIGHT) FROM SURFACE SCUMS OF MICROCYSTIS AERUGINOSA

 Standard amino acid abbreviations A = alanine, L = leucine, R = arginine, Y = tyrosine
Several microcystin toxins were positively identified by HPLC in the *Microcystis* samples from Vaal Dam, Klipvoor Dam, Grootdraai Dam and Jan Smuts Lake. All these dams as well as Bospoort Dam had T&O incidents. The Bospoort Dam *Microcystis*, however, did not produce toxins, while the odour-free Pretoria Power Station *Microcystis* did. There is then certain, though not a universal, correlation between odour production from *Microcystis* blooms and the presence of microcystins.

Cultivation of algal strains from T&O incidents

Algae from all incidents were cultivated in Volk's mineral medium. Inoculation of algae into the medium was done immediately after collection, at the sampling site. In spite of this precaution against early die-off cells, no algal species which were dominant in the samples were isolated. This is in line with the well known difficulty to establish laboratory cultures of *Microcystis* and other planktonic cyanobacteria. Those algal strains which were cultivated successfully were with one exception (see below) all non-odorous.

Because of the failure of algal cultivation, it was impossible to establish unequivocally which algal strains were responsible for odour formation. We may suspect, that a species in dominance was a producer of odorous metabolites, but hard evidence by confirmation of odour production in culture is lacking (Persson, 1988).

In one case (Grootdraai Dam) an unialgal culture of the attached cyanobacterium *Phormidium* sp. was isolated. According to the GC/MS analysis this strain produced geosmin (conc: 120 ng/l). However, at the time of sampling this strain formed only an insignificant fraction of the algal content, which was dominated by *M. aeruginosa*. The low count of this organism possibly can explain why no geosmin could be detected by the instrumental analysis (in this case only dimethyl trisulphide was identified in the chromatogram).

Interestingly, *Daphnia* toxicity tests conducted on the filtrate of the *Phormidium* culture demonstrated the presence of toxic metabolites. Thus we appear to be dealing with a toxin-producing and a T&O-producing strain. This fact indicated the possibility of a potential threat of toxins in drinking water contaminated with taste and odour. The earthy smell of geosmin, which itself is harmless, might be indicative of the presence of other, more toxic metabolites. However, it must borne in mind that the *Daphnia* test does not necessarily indicate the presence of microcystin toxins (Nizan *et al.* 1986).

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4.5 Preliminary evaluation of treatment options

4.5.1 Conventional water treatment

The results of treatment of geosmin with five coagulants are presented in Tables 14-18. Values in the tables are averages of odour assessments by Flavour Profile Analyses done by three or more panellists. The most effective removal was obtained with bentonite clay after flotation (Table 17) where 57% of the geosmin odour intensity was removed with 50 mg/(bentonite.

TABLE 14. TREATMENT OF GEOSMIN WITH ALUM

Dosage mg/(Control	10	15	30	50	100
Settling 30 min	2,0	2.0	2,0	2,0	1,3	1,0
Settling 60 min	2,0	2,0	1,3	1,3	1,0	1,0
Flotation	2,0	2,0	1,7	2,0	2,0	1,0

Odour value for untreated sample: 1,8

TABLE 15. TREATMENT OF GEOSMIN WITH POLYALUMINIUM CHLORIDE Odour value for untreated sample: 2,0

Dosage mg/e	Control	10	15	30	50	100
Settling 30 min	2,0	2,0	2,0	1,2	1,7	1,7
Settling 60 min	2,0	2,0	2,0	1,7	1,7	1,7
Flotation	1,7	2,0	1,7	1,5	1,5	1,8

TABLE 16. TREATMENT OF GEOSMIN WITH FERRIC CHLORIDE Odour value for untreated sample: 3,0

Dosage mg/e	Control	10	15	30	50	100
Settling 30 min	3,0	3,0	3,0	3,0	3,0	2,7
Settling 60 min	3,0	2,7	2,7	2,7	2,7	2,3
Flotation	3,0	3,0	3,0	3,0	2,7	2,3

TABLE 17. TREATMENT OF GEOSMIN WITH BENTONITE CLAY Odour value for untreated sample: 2,3

Dosage mg/t	Control	10	15	30	50	100
Settling 30 min	2,0	2,0	1,8	2,0	1,8	1,3
Settling 60 min	2,0	2,0	2,0	1,7	1,7	1,7
Flotation	2,0	1,8	1,8	1,8	1,0	1,0

TABLE 18. TREATMENT OF GEOSMIN WITH CATFLOC T2

Odour value for untreated sample: 3,0

Dosage mg/e	Control	0,5	1,0	2,0	4,0	10
Settling 30 min	3,0	3,0	3,0	3,0	3,0	2,7
Settling 60 min	2,7	2,7	2,3	2,3	2,3	2,0
Flotation	3,0	3,0	3,0	3,0	2,7	2,7

Treatment of 2-MIB with five coagulants is summarized in Tables 19-23. The best removal of odour intensity (33%) was achieved with alum, after settling for 60 minutes (Table 19) with 15 mg/t of coagulant added.

TABLE 19. TREATMENT OF 2-MIB WITH ALUM

Odour value for untreated sample: 2,7

Dosage mg/l	Control	10	15	30	50	100
Settling 30 min	2,3	2,0	2,3	2,0	2,5	2,5
Settling 60 min	1,8	2,0	1,8	1,8	2,0	2,0
Flotation	2,7	2,7	2,7	2,7	2,7	2,7

TABLE 20. TREATMENT OF 2-MIB WITH POLYALUMINIUM CHLORIDE Odour value for untreated sample: 2,7

Dosage mg/t	Control	10	15	30	50	100
Settling 30 min	3,0	3,0	2,3	2,3	2,0	2,0
Settling 60 min	2,7	2,7	2,7	2,7	2,3	2,3
Flotation	3,0	3,0	3,0	2,7	2,3	2,3

TABLE 21. TREATMENT OF 2-MIB WITH FERRIC CHLORIDE

Odour value	for	untreated	samp	le:	2,5	
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Dosage mg/l	Control	10	15	30	50	100
Settling 30 min	2,3	2,3	2,3	2,5	2,3	2,3
Settling 60 min	2,5	2,3	2,3	2,3	2,3	2,5
Flotation	2,7	3,0	3,0	2,7	3,0	3,0

TABLE 22. TREATMENT OF 2-MIB WITH BENTONITE CLAY

Odour value for untreated sample: 2,7

Dosage mg/l	Control	0.5	1,0	2,0	4,0	10
Settling 30 min	2,7	2,7	2,7	2,7	2,7	2,7
Settling 60 min	2,0	2,0	2,0	2,0	2,0	2,0
Flotation	3,0	3,0	3,0	2,8	2,7	2,7

TABLE 23. TREATMENT OF 2-MIB WITH CATFLOC T2

Odour value for untreated sample: 2,8

Dosage mg/t	Control	0,5	1	2	4	10
Settling 30 min	2,7	2,7	2,7	3,0	2,7	2,7
Settling 60 min	2,7	2,7	2,7	2,7	2,7	2,7
Flotation	3,0	3,0	3,0	3,0	3,0	2,7

The summary results of IPD treatments are presented in Tables 24-28. The highest odour intensity removal of 50% was achieved with ferric chloride sedimentation for 60 minutes, with 4 mg/t of coagulant added (Table 26).

TABLE 24. TREATMENT OF IPD WITH ALUM

Odour value for untreated sample: 3,7

Dosage mg/t	Control	10	15	30	50	100
Settling 30 min	3,5	3,2	3,0	3,0	2,7	2,3
Settling 60 min	3,7	3,7	3,0	3,0	2,7	2,7
Flotation	3,7	3,3	3,3	3,0	2,7	2,3

TABLE 25. TREATMENT OF IPD WITH POLYALUMINIUM CHLORIDE Odour value for untreated sample: 4,0

Dosage mg/t	Control	10	15	30	50	100
Settling 30 min	4,0	4,0	4,0	4,0	4,0	4,0
Settling 60 min	4,0	4,0	3,7	3,7	3,7	3,7
Flotation	4,0	4,0	4,0	4,0	4,0	4,0

TABLE 26. TREATMENT OF IPD WITH FERRIC CHLORIDE

Odour value for untreated sample: 4,0

Dosage mg/t	Control	0,5	1	2	4	10
Settling 30 min	3,7	3,3	3,0	3,0	3,0	3,0
Settling 60 min	4,0	3,3	2,3	2,3	2,0	2,0
Flotation	4,0	4,0	4,0	4,0	3,7	3,7

TABLE 27. TREATMENT OF IPD WITH BENTONITE CLAY

Odour value for untreated sample: 3,7

Dosage mg/l	Control	10	15	30	50	100
Settling 30 min	3,7	3,7	3,7	3,7	3,3	3,0
Settling 60 min	3,7	3,7	3,7	3,7	3,0	2,6
Flotation	3,7	3,7	3,7	3,7	3,7	3,7

TABLE 28. TREATMENT OF IPD WITH CATFLOC T2

Odour value for untreated sample: 3,7

Dosage mg/t	Control	10	15	30	50	100
Settling 30 min	3,7	3,7	3,7	3,7	3,7	3,7
Settling 60 min	3,7	3,7	3,7	3,7	3,3	3,3
Flotation	3,7	3,7	3,3	3,3	3,3	3,3

4.5.2 Preliminary assessment of activated carbon

Results of PAC treatment for geosmin, 2-MIB and IPD are presented in Table 29. The odour was removed completely (beyond sensory detection limit) by 50 mg/ℓ of carbon for geosmin, 60 mg/ℓ for 2-MIB and 10 mg/ℓ for IPD.

TABLE 29. REMOVAL OF TASTE AND ODOUR COMPOUNDS BY POWDERED ACTIVATED CARBON (NORIT SA 42)

Compound	Activated Carbon Dosage (mg/()	Odour value (FPA units)	
Geosmin (1 µg/ℓ)	0 (0 time) 0 (control) 10 50 150	2,5 2,6 1,0 0	
2-MIB (1 μg/l)	0 (0 time) 0 (control) 10 25 40 60	2,7 2,7 1,3 0,7 0,3 0	
IPD (500 µg/()	0 (0 time) 0 (control) 10 25 40 60	2,75 2,75 0 0 0	

4.5.3 Preliminary assessment of the oxidation of taste and odour compounds

The results of treatment of odorous substances by chlorine, hydrogen peroxide, chlorine dioxide and ozone are summarized in Tables 30-33. The percentages of removal were calculated, as in all other treatment experiments, on the basis of sensory panel assessments. Chlorine was unable to remove geosmin and 2-MIB completely. The maximum dose tested for geosmin, 16 mg/l, removed only 53% of the odour. 87% removal was achieved with 2-MIB, but at much higher dosage of oxidant, 64 mg/t. IPD was more easily treated; the removal was complete at 4 mg/t chlorine (Table 30). Hydrogen peroxide was ineffective for removal of geosmin and 2-MIB: treatment seemed to intensify odour rather than to destroy it. Removal of IPD reached 42% with 32 mg/t of oxidant (Table 31). Removal of geosmin and 2-MIB with chlorine dioxide (Table 32) reached 43 and 30%, respectively. Chlorine dioxide was more effective (82,5%) in treating IPD, although removal here was also not complete. Ozone at a dosage of 25 mg/t removed 63 and 43% of geosmin and 2-MIB, respectively, and at 12 mg/t it removed 72% of IPD (Table 33). Treatments with chlorine and chlorine dioxide, apart from being inefficient in the removal of the odorous substances tested, imparted additional strong and offensive smell of chlorine to the water.

TABLE 30. REMOVAL OF TASTE AND ODOUR COMPOUNDS BY CHLORINE

Compound	Chlorine dosage (mg/f)	Odour value (FPA units)	
Geosmin (1 µg/()	0 (0 time) 0 (control) 2 4 8 16	3.0 2.5 2.0 2.0 1.8 1.4	
2-MIB (1 µg/l)	0 (0 time) 0 (control) 8 16 32 64	2,3 2,3 1,7 0,3 0,6 0,3	
IPD (500 μg/t)	0 (0 time) 0 (control) 4 8 16 32	3.0 2.7 0 0 0	

Compound	H ₂ 0 ₂ dosage (mg/ <i>t</i>)	Odour value (FPA units)	
Geosmin (1 µg/≬)	0 (0 time) 0 (control) 4 8 16 32	3.0 3.3 3.3 3.3 3.7 3.7	
2-MIB (1 µg/t)	0 (0 time) 0 (control) 4 8 16 32	3,3 3,7 3,7 3,7 3,7 3,7 3,7	
IPD (500 µg/t)	0 (0 time) 0 (control) 4 8 16 32	4,0 4,0 3,0 3,2 2,7 2,3	

TABLE 31. REMOVAL OF TASTE AND ODOUR COMPOUNDS BY HYDROGEN PEROXIDE

TABLE 32. REMOVAL OF TASTE AND ODOUR COMPOUNDS BY CHLORINE DIOXIDE

Compound	CIO ₂ dosage (mg/f)	Odour value (FPA units)
Geosmin (1 µg/l)	0	2,3
or a set of the set of the set	5	1,7
	10	1,3
	15	1,3
	20	1,3
2-2-MIB (1 µg/()	0	3.3
	5	3,0
	10	3,0
	15	2.7
	20	2.3
IPD (500 µg/()	0	4.0
	5	0.7
	10	0.7
	15	0.7
	20	0,7

66

Compound	O3 dosage (mg/f)	Odour value (FPA units)	
Geosmin (1 µg/ℓ)	0 1 3 5 8 25	2.7 2.7 2.7 2.7 2.3 1.0	
2-2-MIB (1 µg/ℓ)	0 3 5 8 12 25	3 3 2.7 2.0 1.7	
IPD (500 µg/l)	0 6 12 25	3,7 1,3 1,0 1,0	

TABLE 33. REMOVAL OF TASTE AND ODOUR COMPOUNDS BY OZONE

4.6 Process optimizing

4.6.1 Activated carbon adsorption

The performance of four types of carbon tested are shown in Figures 9-11. Minimum dosages necessary for the removal of a given concentration of odorous compound below sensory detection limit are presented. For all three odorous compounds the most effective treatment was obtained with powdered carbon Norit CA 3 followed by powdered SA 2. Complete (e.g. below sensory detection limits) removal of geosmin and 2-MIB at the highest concentration tested was achieved at a level of 30 mg/ ℓ PAC. For 1000 µg/ ℓ IPD carbon requirements were in the range of 10-20 mg/ ℓ . Efficiencies of granular carbons were lower at a given pollutant concentration. This lower performance may have to a certain extent been influenced by chosen experimental conditions (batch mode), which favoured powdered carbons designed to work in suspension. No column filtration was investigated.

4.6.2 Oxidation studies

Figures 12-14 present the ozone consumption required for the complete removal of geosmin, 2-MIB and IPD. Concentrations up to 750 mg/ ℓ geosmin and 2-MIB required less than 8 mg $0_{y}/\ell$. For 1000 ng/ ℓ geosmin the ozone requirement increased to 13 mg/ ℓ , while that for 2-MIB increased to about 10 mg/ ℓ . IPD was easily removed at a concentration level of 1000 µg/ ℓ with 8 mg $0_{y}/\ell$.

Figures 15-17 present PEROXONE requirements. PEROXONE utilizes the effect of the decomposition of H₂0₂ by ozone to produce hydroxyl radicals. These OH- radicals have been observed to be a less selective and more powerful oxidant than ozone (Ferguson *et al.* 1991).

With the addition of hydrogen peroxide ozone requirements decrease. The method can be cheaper and more effective than ozonation done.

Fig 10. REMOVAL OF GEOSMIN BY ACTIVATED CARBON (below sensory detection limit)



Fig 11. REMOVAL OF 2-MIB BY ACTIVATED CARBON (below sensory detection limit)



Fig 12. REMOVAL OF IPD BY ACTIVATED CARBON (below sensory detection limit)



Fig 13. REMOVAL OF GEOSMIN WITH OZONE



Fig 14. REMOVAL OF 2-MIB WITH OZONE



Fig 15. REMOVAL OF IPD WITH OZONE



Fig 16. PEROXONE REQUIREMENTS FOR GEOSMIN REMOVAL



Fig 17. PEROXONE REQUIREMENTS FOR 2-MIB REMOVAL

Ozone

Hydr. per.



Fig 18. PEROXONE REQUIREMENTS FOR IPD REMOVAL





5. DISCUSSION

Investigation of odour incidents

Taste and odour research in South Africa is not particularly advanced, especially when compared with the amount of manpower and money invested in this field elsewhere in the world. Local literature on the subject is scarce. A short communication on an geosminincident (probably caused by sediment-bound actinomycetes), was published by Bailey (1988). Another case, described by Joubert *et al.* (1989), related to an incident at Nagle Dam, an important water source for the Durban municipal area, where geosmin was produced by an *Anabaena* sp. Bailey *et al.* (1989) reported on the Rand Water Board contribution to the development and standardization of Flavour Profile Analysis. Some treatment methods were discussed by Le Roux (1988a, b). Incidence of taste and odour in surface waters were studied by Wnorowski *et al.* (1989), by means of a questionnaire. Some early findings resulting from the present project were reported by Wnorowski and Scott (1992). A literature review on tastes and odours was published by Wnorowski (1992).

The results of the present study, establishing a 30% incidence of taste and odour, are a clear indication of the magnitude of the problem in this country. Odour experiencing water sources displayed blooms of cyanobacteria which were probably responsible for synthesis of odorous metabolites. However, criteria for evaluation of algae for their odour-forming properties include isolation of the odorous organism and confirmation of odour production in pure culture (Mallevialle and Suffet, 1987). Mere common occurrence of an odour and organism is not sufficient evidence of odour production by this organism (Persson, 1988). Unfortunately, cultures of the dominant organisms in plankton blooms were not obtained in this study, so it is impossible to unequivocally state at this point that they contributed to odour formation. Moreover, M. aeruginosa which was a dominating species in 90% of cases, is known to produce 8-cyclocitral (Jüttner 1976; Hayes and Burch, 1989) but there is no other evidence so far in the literature about this species producing geosmin. Similarly, the role of actinomycetes in odour formation in water is very difficult to assess (Cross 1981, Persson, 1988). In this study they were found in relatively high numbers of 105 - 105 CFU/m∉ in four phytoplankton samples. However, whether they existed in these samples as mere spores or in the form of vegetative cells capable of odour production is an open question. It is therefore possible that they were responsible for the odour incidents rather than the phytoplankton which could serve only as a habitat for them.

The choice of *Daphnia pulex* for toxicity tests was based on availability of resources. However, although *Daphnia* is sensitive to certain toxic substances produced by *Microcystis*, daphnids are unsuitable as test organisms where toxicity to mammals is concerned (Nizan *et al.* 1986). Consequently, the results with *Daphnia* may not indicate the presence of microcystins which preferably should be measured directly by HPLC or other techniques.

Microcystins were measured directly in four dams that had T&O incidents. One dam with a T&O incident contained no microcystins despite dominance by *M. aeruginosa* and another dam with no odour did contain microcystins. The microcystins levels in three dams (Grootdraai, Klipvoor and Vaal) were of a sufficiently high concentration to cause concern.

There was one case, where a geosmin producing strain of *Phormidium* sp. was isolated in culture. This strain also produced toxic metabolites. This fact together with the observations above indicated that there is a potential threat of the presence of cyanobacterial toxins in drinking water contaminated with odorous substances produced by algae. The earthy smell of geosmin, a compound which itself is harmless, might be indicative of the presence of algal toxins. More evidence is necessary before any well-founded conclusions can be drawn.

Treatment options

Geosmin, the predominant odorous compound detected in this study, is a substance not yielding to conventional water treatment procedures (Lalezary *et al.* 1986a, b). Another option may be the use of activated carbon adsorption, but this is costly and inadequate dosage will not contain the problem (Joubert *et al.* 1989). In every case of taste and odour an investigation into the problem is recommended before an optimal treatment method is selected. Hasty action does not guarantee success and may substantially increase removal costs. To optimize treatment options for specific South African conditions, several methods were screened. Those most suitable were then studied in greater detail in order to establish the dosages required for the effective removal of odorous contaminants.

Geosmin and 2-MIB were chosen as model compounds being the most common T&O substances all over the world and IPD was selected as representative of the sulphurcontaining compounds found in South African waters. Out of these three, IPD proved to be easiest to remove, while geosmin and 2-MIB were persistent in water even after treatment with various treatment methods.

Very little odour removal was recorded in any of the experiments with coagulation/ sedimentation/flotation. This confirms that the standard potable water treatment methods are ineffective in reducing odorous substances present in the raw water. Odour removal employing alum varied between 33 and 44%. It was most successful in the removal of geosmin and least successful in the removal of 2-MIB. There was no difference between sedimentation and flotation. Polyaluminium chloride was also most successful in reducing the geosmin odour (40% removal), but only achieved a 7% reduction of the IPD odour. Ferric chloride removed 50% of odour caused by IPD, but was ineffective towards 2-MIB (8% reduction). The bentonite treatment had somewhat more promising results: geosmin odour was reduced by 57%. 2-MIB and IPD were reduced by 26 and 30%, respectively. The 57% odour reduction of geosmin was achieved with a dosage of 50 mg/ℓ of bentonite, followed by flotation. The use of Catfloc T2 as a flocculant produced the lowest odour reduction. Those were a mere 3% for 2-MIB, 11% for IPD and 23% for geosmin. In general it was found that the sedimentation experiments produced a treated water of slightly better quality than the water treated by flotation. As there were a few exceptions to this observation and as the actual differences were insignificant, no firm conclusions can be made as to whether sedimentation is more effective than flotation to reduce odours in water.

Geosmin and 2-MIB are very difficult to oxidize. It can be seen from the results obtained from the oxidation studies where chlorine dosages as high as 60 mg/ℓ could not remove these substances from water. In contrast, IPD was removed to below detection levels at a chlorine concentration of 4 mg/ℓ. Hydrogen peroxide as an oxidizing agent failed in all cases even at high dosages to remove T&O compounds

from water. The use of ozone and chlorine dioxide were more successful than hydrogen peroxide. However, neither of them could succeed in removing odour below detectable levels in the screening experiments.

Activated carbon adsorption as a treatment option for the removal of T&O from water gave satisfactory results. In preliminary tests at a carbon dosage of 50 mg/ ℓ both geosmin and 2-MIB were removed completely. This high dosage was necessary, as selected concentration of the odorous compounds of 1 μ g/ ℓ was relatively high. Often in natural condition the T&O problems are caused by concentrations in the range of nannograms per litre. IPD removal with activated carbon was excellent at a dosage of 10 mg/ ℓ . Summarising, activated carbon adsorption proved to be the best method for T&O removal.

This method was studied further to establish optimal dosages for effective treatment. Powdered carbons Norit CA 3 and Norit SA 2 proved to be most efficient sorbents. Their requirement was not higher than 30 mg/ ℓ , even at extremely high pollutant concentrations of 1000 ng/ ℓ .

The other treatment method studied was ozonation versus the PEROXONE method. With both methods complete removal of odorous compounds was achieved at ozone dosages between 5-13 mg/*i*, depending on the concentration of substance tested. With the addition of hydrogen peroxide (PEROXONE method) the ozone requirements decreased, but not too spectacularly. This result differs from the one reported by Ferguson *et al.* (1990), where for the same 2-MIB removal efficiency the ozone dose at the ozonation process was twice as high as the dose required with the PEROXONE method.

It is trusted that curves describing the minimal dosages needed for the removal of odorous substances can serve as guidelines for treatment schedules in water purification works. It is however stressed again that an initial investigation into the causes of a taste and odour incident and an assessment of the chemical nature and concentration of the offending substance is necessary for the most cost-effective and satisfactory treatment.

Water source management

Water source management, aimed at the prevention of taste and odour incidents, appears to be a better alternative than costly treatments. An early warning system, involving the monitoring of algal status, followed by the early application of an algicide or herbicide to prevent the development of blooms, was recommended by Means and McGuire (1986). A well planned and consistently monitored pollution control programme involving nutrient load reduction can remedy eutrophication and revert a reservoir to its previous trophic status (Persson *et al.* 1989). Proper watershed control brings significant benefits, not only on environmental and health issues, but it also improves water quality to the extent that purification costs can be drastically reduced (Kirner and Merry 1988). Other control measures include destratification with aeration (Raman and Arbuckle 1989) and reservoir level manipulation to expose and dehydrate attached algae (McGuire and Gaston 1988a, b).

6. CONCLUSIONS

- The literature survey indicated that taste and odour problems are common world-wide. They are increasing in developed countries due to progressing eutrophication and degradation of natural habits.
- During a 3-year study a 30% taste and odour incidence in South African surface waters was found, based on an investigation of 54 water bodies.
- Seventy five percent of plankton blooms encountered were accompanied by an easily perceptible odour, in most cases described as earthy/muddy.
- All odour experiencing lakes encountered in this project were dominated by cyanobacteria, with *Microcystis* dominant in 90% of the cases.
- Geosmin was the most common odour identified in nearly 50% of incidents. Other important odorous substances in water were numerous sulphurcontaining compounds.

- Several microcystins were detected in algal cells collected from odourexperiencing water sources.
- Activated carbon adsorption, ozonation and PEROXONE were selected as most suitable methods for the removal of common T&O substances.
- Minimum dosages necessary for the effective removal of selected odorous pollutants were established experimentally.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

Sensory analysis, although standardised to some extend by introduction of FPA method, is subjective and difficult to reproduce. The results obtained at different centres will undoubtedly vary, making comparisons difficult. Standard reference odour kits, developed lately will certainly help, but they are not popular yet and can not address every odour. Assessment of odours by instrumental analysis, although much more efficient and reproducible, is cost-prohibitive at a larger scale. There is still also many types of off-flavours which have not yet been sufficiently characterized in chemical terms.

In South Africa there is a great need to establish conclusive evidence on organisms involved in production of odorous metabolites. The present study failed in this respect as odour-producing cyanobacteria were not successfully cultured axenically in the laboratory. Circumstantial evidence suggests that *Microcystis aeruginosa* is implicated in odour incidents locally and that it produces geosmin, but further studies to provide proof in scientific terms are urgently required. In order to establish whether an organism produces odours or not, the conditions defined in Koch's postulates should be fulfilled. These postulates were formulated for clinical microbiology; adapted to environmental sciences make use of the following criteria (Persson 1983):

- ecological evidence: concurrence of odour and organism in the field
- isolation of the organism and proof of odour production in the laboratory
- chemical identification and sensory characterization of odorous compounds

The biochemical process of odour production by microorganisms is known only fragmentarily. Why some strains of a given species are odorous while other are not is uncertain. Environmental conditions which stimulate production of odorous metabolites are recognised only partially. The biosynthesis path of geosmin was studied to a certain degree, but the biochemistry and physiology of odour production is still open to further research. Advances in this field are especially important from both scientific and practical point of view. More detailed knowledge of the conditions influencing odour production by certain bacterial and algal strains would facilitate strategies for the abatement and prevention of tastes and odours in the aquatic environment.

In the field of odour removal a broad range of chemical/physical methods has been proposed. A new and interesting developing area is an biological approach to odour abatement. Various biotechnologies for biodegradation of odorous compounds are presently under investigation in many leading T&O centres. Single species and mixtures of odour-metabolizing microorganisms have been isolated in laboratory conditions. Cellular extracts and purified enzymes are also being tested. A promising data have been collected from slow sand filtration systems. Conditions there allow for the development of natural flora adapted to biodegradation of odorous trace organic compounds in the treated water. Slow filters could then be a potential addition to a range of odour treatment methods.

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