

MINIREVIEW

Biochemical and Ecological Control of Geosmin and 2-Methylisoborneol in Source Waters[∇]

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The majority of all biologically caused taste-and-odor outbreaks in drinking water characterized worldwide are caused by microbial production of (–)-geosmin [(–)-(4*S*,4*aS*,8*aR*)-4,8-dimethyloctahydronaphthalen-4*a*-ol] and (–)-2-methylisoborneol (2-MIB) {(1*R*-*exo*)-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol}. Since they were first identified in the early 1960s, these two earthy-muddy-smelling metabolites have been the focus of considerable research, which has collectively produced over 400 scientific articles, reports, websites, and conference proceedings. Yet despite this substantial body of knowledge, geosmin and 2-MIB remain poorly understood throughout much of the water industry, and misconceptions which impede the prediction, treatment, and control of these volatile organic compounds (VOCs) persist. This paper reviews salient aspects of our current knowledge on the sources and properties of geosmin and 2-MIB which are essential to understanding and managing drinking water malodors. In particular, we highlight some key factors regulating the storage and release of these compounds by cells. These important factors are often overlooked and may contribute to some of the apparent ambiguity of many taste-and-odor outbreaks.

BIOLOGICAL ORIGINS OF GEOSMIN AND 2-MIB

Geosmin and 2-MIB are tertiary alcohols, each of which exists as (+) and (–) enantiomers. Odor outbreaks are caused by biological production of the naturally occurring (–) enantiomers (Fig. 1), which are some 10 times more potent than the (+) molecules (101). It is worth noting here that analytical and sensory (i.e., odor threshold level) standards are based on different chiral purities, i.e., for the commercially available racemic (+/–) mix of geosmin and the (–) enantiomer of 2-MIB, which clearly has implications for detection and control targets in the drinking water industry and for the interpretation of toxicity and bio assays (101).

Geosmin and 2-MIB are produced by members of certain groups of benthic and pelagic aquatic microorganisms found in source waters such as lakes, reservoirs, and running waters. In addition there are several other biological sources that are often overlooked, notably those which originate from terres-

trial ecosystems, industrial waste treatment facilities, and drinking water treatment plants. Many of the known producers are prokaryotes, which include both heterotrophs and photoautotrophs, and most drinking water research to date has focused on these taxa. A number of eukaryotes (various fungi [6], the amoeba *Vannella* [24], and a liverwort [89]) have also been identified as potentially highly prolific producers of geosmin and 2-MIB, and there are numerous related reports of fungal spoilage within the food industry, where this can be a significant issue (81). However, although some are known to generate odors from within water treatment plants (see below), the importance of these eukaryotes as significant sources of drinking water taste and odor has not been systematically investigated, and to date they have been largely disregarded.

Heterotrophic producers. Production of geosmin and 2-MIB has been documented for several different groups of heterotrophic microorganisms (Table 1). In fact, the two compounds were originally identified from isolates of aerobic filamentous actinomycete bacteria (*Streptomyces*) (21, 55, 56), and these organisms for some time were (and often still are) perceived by the water industry as the major sources of these VOCs (86). The genus *Streptomyces* is widely used synonymously with odor-producing actinomycetes, but it is important to note that nonstreptomycete actinomycetes such as *Nocardia* are also potent producers of both geosmin and 2-MIB, while many streptomycetes are nonproducers (112). The two compounds are principal odor components of soil (9, 40), and periods of high terrestrial runoff may introduce actinomycetes and/or their odorous metabolites into surface waters, causing episodic odor outbreaks in rivers, particularly in areas of intensive livestock operations (33, 37, 112, 113).

Early actinomycete studies were highly influential, since they identified the chemical structures and some of the major biological sources of geosmin and 2-MIB. Cyanobacteria (previously termed “blue-green algae”) were also known as producers at that time (55, 80) (see below), but it was not until the important study by Tabachek and Yurkowski (93) that these photoautotrophs were recognized as a more frequent source of geosmin and 2-MIB in water than actinomycetes. Indeed, a careful review of the taste-and-odor literature to date reveals that actinomycetes have been clearly implicated in comparatively few odor episodes. Henatsch and Jüttner (26) found that increased summer epilimnetic geosmin levels in eutrophic Lake Schleinsee (southwest Germany) coincided with a high abundance of the streptomycetes *Nocardia* and *Arthrobacter*,

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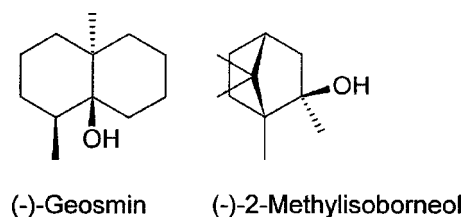


FIG. 1. Structure of geosmin and 2-MIB.

while cyanobacteria were not detected at that time. Sugiura and Nakano (90) concluded that elevated 2-MIB levels in Lake Kasumigaura (Japan) were generated by actinomycete activity in aerobic sediment layers, based on *in vitro* observations of isolates from this habitat. Jensen and coworkers (37) traced the annual spring outbreaks of earthy-musty odor in tap water derived from a major Canadian river to actinomycetes, which were introduced into these surface waters during snowmelt and runoff. Klausen et al. (47) concluded that actinomycetes were responsible for low concentrations of geosmin and 2-MIB in streams flowing past trout breeding aquaculture operations, because isolated strains of *Streptomyces* from these habitats were able to synthesize geosmin and 2-MIB, while cyanobacteria were not present in large numbers. It is important to note, however, that most of these studies did not investigate benthic or littoral biofilms, which can be a major source of these compounds (see below). Furthermore, while persuasive correlative evidence was presented in each of these studies, none provided a direct demonstration that actinomycetes were primarily responsible for the malodor. Overall, however, the contribution of actinomycetes to taste-and-odor outbreaks will continue to be extremely difficult to assess until methods to differentiate *in situ* producing and nonproducing strains are available.

It is well known that odor can originate downstream of water treatment as a result of heterotrophic biological activity in distribution pipes or filtration beds, and posttreatment production of geosmin has also been documented in several studies. For example, heterotrophic eukaryotes such as fungi, which colonize biofilms in activated filters and distribution pipes, can generate potent musty-smelling metabolites such as trichloroanisole (68). In a recent case, geosmin in treated drinking water was traced to the disturbance of thick biofilms that had developed on the pipe surface of a distribution system from a groundwater-supplied treatment plant (F. Jüttner, unpublished data). After a change in water treatment processes in the plant to remove iron from the iron-rich source water, the biofilms degraded and were sloughed off, releasing high levels of geosmin and leading to consumer complaints. In another case, biological activity in poorly maintained filtration media was considered to be the most likely cause of high geosmin levels downstream of filter beds in a small rural treatment plant (S. B. Watson, unpublished data). The biological sources were not identified in these last two cases, and it is not known if they were actinomycetes, fungi, and/or other microorganisms. For example, myxobacteria (83, 111) and molds such as *Penicillium* are known prolific sources of earthy odors in sediments and foodstuffs (51, 78, 81), and the potential role of these organ-

isms in odor outbreaks in raw or posttreatment water is an important issue which has yet to be addressed.

Photoautotrophic producers. As noted above, cyanobacteria are considered to be the major sources of geosmin and 2-MIB in aquatic environments where photosynthetic growth is possible (31, 45, 53, 65, 110). More than 200 studies have made considerable advances in our knowledge of the biochemistry, taxonomy and ecology of some of the cyanobacteria which produce these VOCs. Yet even though cyanobacteria are now considered to be the chief sources of geosmin and 2-MIB, the number of species (here we refer to the phylogenetic species concept, since cyanobacteria are asexual [3]) which are significant producers is unknown, for several reasons. Fewer than 50 of the >2,000 species classified to date (according to the International Code of Botanical Nomenclature) have been directly confirmed as producers, while the majority have yet to be investigated for their production of these and other VOCs (100). Unsightly and highly visible surface blooms are usually considered to be primary sources of source water odor, but in fact many of the known cyanobacterial producers are nonplanktonic (~30%), while the remainder are benthic or epiphytic, with a single isolate from soil (Table 2). Interestingly, geosmin and 2-MIB production appears to be limited to filamentous cyanophytes and to date is unknown among chroococcalean taxa. Furthermore, to our knowledge no marine cyanobacterium has been identified as a producer of either compound to date; in fact, geosmin has been proposed as a chemical land mass homing signal for anadromous fish (94). On the other hand, this may simply reflect the fact that few people rely on marine sources for drinking water rather than any relationship with halotolerance. Persson (64) observed that among four fresh and brackish water isolates of *Oscillatoria aghardhii* (syn. *Planktothrix aghardhii*), only brackish water clones produced geosmin and 2-MIB, indicating that salinity per se does not preclude the biosynthesis of these terpenoids. Along the same lines, geosmin and 2-MIB outbreaks are common problems in many areas of the North American prairies, where the presence of an ancient seabed results in highly saline and productive surface waters (46, 99).

TABLE 1. Actinomycetes and other noncyanobacterial taxa that produce geosmin (GE) and 2-MIB

VOC(s)	Taxon	Reference
2-MIB, GE	<i>Penicillium</i> and <i>Aspergillus</i> species	78
GE	<i>P. expansum</i>	17
GE	<i>Streptomyces albidoflavus</i>	92
GE	<i>S. avermitilis</i>	73
GE	<i>S. citreus</i>	69
GE	<i>S. griseus</i>	105
GE, 2-MIB	<i>S. griseofuscus</i>	1
GE	<i>S. halstedii</i>	82
GE	<i>S. psammoticus</i>	37
GE	<i>S. tendae</i>	17
GE, 2-MIB	<i>S. violaceusniger</i>	78
GE, MIB	<i>Streptomyces</i> spp.	Various
GE	<i>Symphyogyna brongniartii</i> (liverwort)	89
GE	<i>Vannella</i> sp. (heterotrophic amoeba)	24

TABLE 2. Cyanobacteria producing geosmin (GE) and 2-MIB, listed by current taxonomic names, past synonyms, and primary habitat^a

Taxon	Synonym	Habitat ^b	Production (+)		Comments
			of:		
			GE	2-MIB	
<i>Geitlerinema splendidum</i>	<i>Oscillatoria splendida</i>	BEN	+		
<i>Jaaginema geminatum</i>	<i>Oscillatoria geminata</i>	BEN		+	
<i>Leibleinia subtilis</i>	<i>Lyngbya subtilis</i>	BEN	+		
<i>Lyngbya aestuarii</i>		BEN		+	
<i>Oscillatoria curviceps</i>		BEN		+	
<i>Oscillatoria tenuis</i> var. <i>levis</i>		BEN		+	
<i>Oscillatoria variabilis</i>		BEN		+	
<i>Phormidium allorgei</i>	<i>Lyngbya allorgei</i>	BEN	+		
<i>Phormidium amoenum</i>	<i>Oscillatoria amoena</i>	BEN	+		
<i>Phormidium breve</i>	<i>Oscillatoria brevis</i>	BEN	+	+	Strain specific
<i>Phormidium chalybeum</i>	<i>Oscillatoria chalybea</i>	BEN		+	
<i>Phormidium cortianum</i>	<i>Oscillatoria cortiana</i>	BEN	+		
<i>Phormidium favosum</i>		BEN		+	
<i>Phormidium formosum</i>	<i>Oscillatoria formosa</i>	BEN	+		
<i>Phormidium</i> strain LM689		BEN		+	
<i>Phormidium simplissimum</i>	<i>Oscillatoria simplicissima</i>	BEN	+		
<i>Phormidium</i> sp. strain NIVA 51		BEN	+	+	
<i>Phormidium tenue</i>	<i>Oscillatoria tenuis</i>	BEN		+	
<i>Phormidium uncinatum</i>		BEN	+		
<i>Phormidium viscosum</i>		BEN	+		
<i>Planktothrix prolifica</i>	<i>Oscillatoria prolifica</i>	BEN	+		
<i>Porphyrosiphon martensianus</i>	<i>Lyngbya martensiana</i>	BEN		+	
<i>Symplocastrum mülleri</i>	<i>Schizothrix mülleri</i>	BEN	+		Actinomycete contaminant
<i>Tychonema bornetii</i>	<i>Oscillatoria bornetii</i>	BEN	+		Strain specific
<i>Tychonema granulatum</i>	<i>Oscillatoria</i> f. <i>granulata</i>	BEN	+	+	
<i>Hyella</i> sp.		EPI		+	
<i>Microcoleus</i> sp.		EPI	+		
<i>Anabaena circinalis</i>		PL	+		
<i>Anabaena crassa</i>		PL	+		
<i>Anabaena lemmermannii</i>		PL	+		
<i>Anabaena macrospora</i>		PL	+		
<i>Anabaena solitaria</i>		PL	+		
<i>Anabaena viguieri</i>		PL	+		
<i>Aphanizomenon flos-aquae</i>		PL	+		
<i>Aphanizomenon gracile</i>		PL	+		
<i>Oscillatoria limosa</i>		PL		+	
<i>Planktothrix agardhii</i>	<i>Oscillatoria agardhii</i>	PL	+	+	Strain specific
<i>Planktothrix cryptovaginata</i>	<i>Lyngbya cryptovaginata</i>	PL		+	
<i>Planktothrix perornata</i>	<i>Oscillatoria perornata</i>	PL		+	
<i>Planktothrix perornata</i> var. <i>attenuata</i>	<i>Oscillatoria perornata</i> var. <i>attenuata</i>	PL		+	
<i>Pseudanabaena catenata</i>		PL	+	+	
<i>Pseudanabaena limnetica</i>	<i>Oscillatoria limnetica</i>	PL		+	
<i>Symploca muscorum</i>		SL	+		

^a Data from reference 99.

^b BEN, benthic; PL, planktonic; EPI, epiphytic; SL, soil.

BIOSYNTHESIS OF GEOSMIN AND 2-MIB

The conclusion that 2-MIB is a monoterpene and geosmin an irregular sesquiterpene (Fig. 1) dates back to early labeling experiments with *Streptomyces* conducted by Bentley and Meganathan (4). These authors found that both compounds were labeled when radioactive acetate was administered to cultures, and they interpreted this as an indication of isoprene synthesis. 2-MIB showed labeling when methionine with a labeled methyl group was added. The authors concluded that 2-MIB is a methylated monoterpene and geosmin a sesquiterpene that has lost an isopropyl group. To our knowledge, no further experiments have been conducted to elucidate the biosynthesis of 2-MIB. Geosmin, however, has received considerable recent interest, and much progress in the knowledge of its biosynthetic pathways has been made.

Initially, successful labeling experiments to trace the biosynthetic pathway(s) of geosmin were difficult to perform with streptomycetes, and they still have not been conducted successfully with cyanobacteria. Farnesyl diphosphate is the immediate precursor of cyclic sesquiterpenes (11), but early attempts showed that farnesol addition to cultures inhibited the growth of bacteria (16, 88) and cyanobacteria (44), and this compound therefore had to be rejected as a tool to study the biosynthesis of terpenoids. More recently, considerable progress has been made using other precursors. Experiments showed that labeled geosmin was produced by *Streptomyces* when labeled 1-deoxy-D-xylulose (88) was added, while labeled mevalolactone and leucine were applied successfully with the myxobacteria *Myxococcus xanthus* and *Stigmatella aurantica* (14). These studies, together with other labeling and genetic work, have provided evidence that in fact several

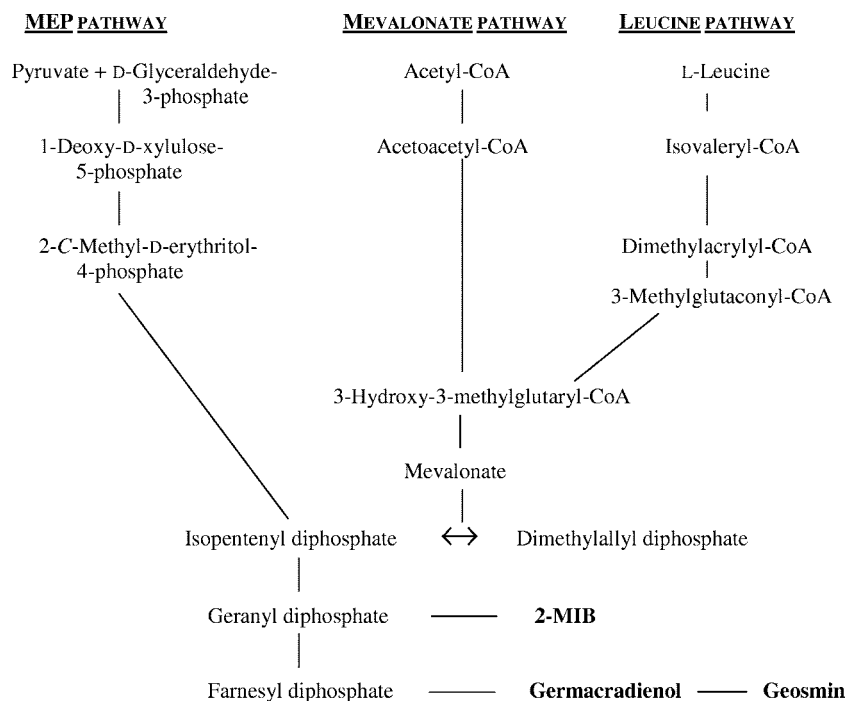


FIG. 2. Simplified biosynthetic scheme (suggested or proven) for the formation of 2-MIB and geosmin in streptomycetes and myxobacteria.

different biosynthetic pathways of isoprenoid synthesis exist in microorganisms, one or more of which may lead to the production of geosmin by different taxa (Fig. 2).

Of particular importance is the 2-methylerythritol-4-phosphate (MEP) pathway. This pathway was only recently discovered and has been now completely elucidated on the genetic and enzymatic levels in higher plants (75). Work with *Streptomyces* showed that labeled geosmin was produced when deuterated [5,4-²H₂]1-deoxy-D-xylulose, but not [4,4,6,6,6-²H₅]mevalolactone, was administered, demonstrating the predominance of the MEP isoprenoid pathway over the mevalonate (MVA) route in these organisms (88). The genes coding for the MEP pathway have been found in the cyanobacterium *Synechocystis* sp. strain PCC 6803 (48), although members of this genus (and other chroococcales) do not produce geosmin. However, this suggests that the same isoprenoid pathway may also function in the geosmin-producing cyanobacterial taxa.

The above discussion suggests that the MEP pathway is the major biosynthetic isoprenoid route in many bacterial groups; nevertheless, there is some evidence that the MVA pathway is also used. The latter pathway may function exclusively in the synthesis of geosmin and other isoprenoids in some groups such as myxobacteria (14) and contribute to geosmin production in the stationary growth phase of streptomycetes (84, 85). Archaea use the MVA pathway exclusively for isoprenoid synthesis (50), but geosmin producers have yet to be found among these taxa. Myxobacteria also use the MVA pathway as a major route to synthesize a range of isoprenoid compounds, including geosmin. In these microorganisms a minor pathway starts with L-leucine and feeds label via dimethylacrylyl coenzyme A (CoA) into 3-hydroxy-3-methylglutaryl-CoA, a precursor of MVA (14). The original results reported by Bentley and Meganathan (4) favored the MVA pathway for streptomycetes,

based on the production of labeled geosmin and 2-MIB from labeled acetate. However, more recent studies failed to find similar labeling using this precursor (88), and a plausible explanation for this apparent inconsistency is that streptomycetes are capable of gluconeogenesis and can use acetate and ethanol as sole C sources (35). When no exogenous sugar is supplied, acetate would be metabolized in long-term experiments to sugar compounds, and labeling can then be introduced into the MEP pathway. Nevertheless, there is some evidence that some streptomycetes may use both pathways but at different growth stages, with the MEP pathway providing the predominant route during active growth and the MVA pathway providing the predominant route in the stationary phase (84, 85). However, rigorous proof that the MVA pathway is a widespread and significant route in geosmin biosynthesis is still lacking, and there is a need for the development of more specific tracing techniques. For example, 3-hydroxy-3-methylglutaryl-CoA reductase is often used as a key enzyme indicating the presence of the MVA pathway (22), but this enzyme can also function in a catabolic pathway, the breakdown of MVA. Its presence is therefore not necessarily indicative of an MVA pathway to geosmin.

For both geosmin pathways, there has been much recent interest in the elucidation the final steps: the cyclization of farnesyl diphosphate to a mono- or dicyclic sesquiterpene. Pollack and Berger (69) found that a strong geosmin-producing strain of *Streptomyces citreus* also produced measurable quantities of the sesquiterpene alcohol (4*S*,7*R*)-germacra-1(10)*E*,5*E*-diene-11-ol, and they postulated that farnesyl diphosphate cyclization yields germacradienol as the immediate precursor of geosmin. More recently germacradienol/germacrene D synthases of *Streptomyces coelicolor* (23, 38) and *Streptomyces avermitilis* (11) were cloned, and the recombinant

enzyme was studied in more detail. The enzyme was shown to catalyze the formation of (4*S*,7*R*)-germacra-1(10)*E*,5*E*-diene-11-ol, (–)-(7*S*)-germacrene D, and geosmin from farnesyl diphosphate. Magnesium ions supported the reaction; other cofactors were not necessary (11, 38). However, in these experiments, germacradienol was the major product and geosmin production was minor, while under natural conditions, the cellular concentrations of geosmin are much higher than those of germacradienol. This suggests that the enzymatic environment may strongly affect the formation of these two products. Our understanding of the genetic coding and of the sesquiterpene synthase involved in the Mg-dependent conversion of farnesyl diphosphate to germacradienol was further advanced by two recent independent studies. Gust and coworkers (23) used PCR-targeted gene replacement to identify the gene (Sco6073) (*cyc2*), which codes for a synthase with two domains, with one required for geosmin biosynthesis. In a parallel study, Cane and Watt (10) used PCR amplification of this gene to determine the identity of this synthase as a protein encoded by 2,181 bp (designated SC9B1.20), and they concluded that production of germacradienol represents the committed step in the geosmin biosynthetic pathway.

Several studies used precursors with deuterium labeling in different positions to investigate the cyclization process leading to the formation of geosmin. A number of different mechanisms and intermediates were postulated by the authors to explain the labeling patterns observed (10, 11, 14, 25, 38, 88). However, some key questions remain unresolved, notably, which of the postulated intermediates occur in which taxa and under which conditions they are released and contribute to the odor bouquet of a particular microorganism. Importantly, for cyanobacteria it has not been resolved which pathway is used, while for streptomycetes, it has yet to be established whether all strains use the same pathway or whether both pathways are used for geosmin under different growth conditions. This insight is essential to explain why geosmin producers and non-producers, and low and high producers, are found among different species and cospecific strains of both cyanobacteria and streptomycetes. A second, molecular level of understanding is also missing, which is fundamental to these questions: the complete knowledge of the genes and enzymes responsible for the synthesis of geosmin and other sesquiterpenes and their regulating mechanisms.

TRACING GEOSMIN AND 2-MIB PRODUCERS

In the following section, we elucidate several key factors which may explain why many efforts to trace the biological sources of geosmin and 2-MIB have been unsuccessful. Above all, a reliable method to distinguish between the contributions of cyanobacteria and actinomycetes (or other potential producers) to geosmin and 2-MIB in surface waters has not been developed, and a considerable number of taste-and-odor outbreaks caused by geosmin and/or 2-MIB remain unsolved (7). Importantly, there is a general lack of standardized units among papers reporting production and production rates, which should be expressed per volume/weight of cells or per unit chlorophyll (or any other cell-specific parameter). Frequently, however, these measures are given as geosmin and 2-MIB concentrations per culture volume, which does not pro-

vide insight into the relative capacities of different taxa to produce these VOCs or allow comparison between different studies. Few studies have differentiated between total and cell-bound production capacities, which can differ considerably and are imprecisely estimated by most current extraction and analytical protocols (see below). Environmental factors (such as light intensity, temperature, ion concentrations, etc.) have been shown to modulate the production rate of odor compounds for both cyanobacteria (57, 58, 59, 76, 79, 95, 102) and actinomycetes (1, 15, 17, 82, 92, 105), but these alone cannot explain the substantial differences in concentrations often observed in surface waters under natural conditions (Table 3 gives an overview of typical environmental levels).

Actinomycetes. In general, techniques currently used to measure actinomycete abundance typically yield poor correlations between these estimates and geosmin and/or 2-MIB concentrations in surface waters, for several reasons. First, actinomycetes are difficult to enumerate. They are filamentous (hyphal), spore-producing organisms which fragment when plated, with each fragment or spore yielding a CFU. Highly selective media are needed to identify and enumerate actinomycetes; different studies use different media, and these media vary in the biomass they will generate (18). Estimates using these traditional methods suggest that the abundance of actinomycetes in open waters is generally low, ranging from below detection to $\sim 1.4 \times 10^6$ cells · liter⁻¹ (64, 113, 114). Immunofluorescence techniques have been employed extensively in medical and soil sciences to investigate these and other mixed microbial assemblages but are often limited by their nonspecific nature, and without appropriate controls, these methods can yield estimates that include many (other) bacterial taxa and which differ significantly from CFU. Recent work, however, has made considerable advances in sensitive enumeration techniques using fluorescence in situ hybridization (FISH), FISH with catalyzed reporter deposition, and combined microautoradiography. These techniques indicate that abundances of active streptomycetes may be considerably higher in some waters, reported at levels of 0.4×10^8 to 3.7×10^8 cells · liter⁻¹ in oligotrophic and eutrophic streams and fish ponds (60). In essence, because of the large discrepancies among these methods, our knowledge of the actual abundances of active cells in different environments is almost completely lacking.

A further key consideration is that not all streptomycetes produce geosmin or 2-MIB (13), yet plate counts and other enumeration methods estimate a total count, i.e., both odor producers and non-odor producers. Studies using FISH and FISH with catalyzed reporter deposition have demonstrated that active actinomycetes (which metabolize and/or incorporate labeled thymidine) are ubiquitous (60) and can be important components of the plankton even during periods when there is little or no odor (47, 49). Authors have reported a wide range in the proportion of potentially odorous actinomycetes in different environments: between approximately 20% and 70% of isolates from river and lake samples have been found to be active (in vitro) producers of geosmin and/or 2-MIB (37, 47, 113, 114).

In addition to the above points, it is important to note that there is considerable variation in the cell-specific capacity to produce geosmin or 2-MIB among active individual isolates.

TABLE 3. Typical concentrations of geosmin (GE) and 2-MIB in different habitats, microorganisms, and drinking water

Source	Details	Concn ^a	Reference
Cultures			
Cyanobacteria	<i>Oscillatoria brevis</i> , light limited	0.35 $\mu\text{g GE mg (dry wt)}^{-1}$ = 58 $\mu\text{g GE mg Chl } a^{-1}$	57
	<i>Anabaena lemmermannii</i> , P limited, total GE	0.15 $\mu\text{g GE mg (dry wt)}^{-1}$ = 670 $\mu\text{g GE mg Chl } a^{-1}$	Wa ^b
	<i>A. lemmermannii</i> , P limited, cell-bound GE	0.14 $\mu\text{g GE mg (dry wt)}^{-1}$ = 500 $\mu\text{g GE mg Chl } a^{-1}$	Wa
	<i>A. lemmermannii</i> , P replete, total GE	0.5 $\mu\text{g GE mg (dry wt)}^{-1}$ = 850 $\mu\text{g GE mg Chl } a^{-1}$	Wa
	<i>A. lemmermannii</i> , P replete, cell-bound GE	0.41 $\mu\text{g GE mg (dry wt)}^{-1}$ = 700 $\mu\text{g GE mg Chl } a^{-1}$	Wa
Streptomycetes	<i>Streptomyces tendae</i> , grown on different agars	0.25–33 (52) ^c ng GE mg (dry wt) ⁻¹	18
Lake water			
Temperate/subpolar	Oligotrophic (various; Canada)	0–5 ng GE liter ⁻¹	Wa
	Mesotrophic; Lake Zürich (depth and seasonal differences)	2.7–23 ng GE liter ⁻¹	19
Subtropical	Eutrophic; Central Europe, shallow lake	600 ng GE liter ⁻¹	45
	Eutrophic; Lake Kasumigaura, Japan	Up to 900 ng liter ⁻¹ 2-MIB and 700 ng liter ⁻¹ GE	110
	Various reservoirs in South Africa	150–3,170 ng GE liter ⁻¹	106
Spatial/temporal	Central Europe (eutrophic)		
	Oxic epilimnion	50 ng GE liter ⁻¹	26
	Anoxic hypolimnion	950 ng GE liter ⁻¹	26
Winter turnover	3 ng GE liter ⁻¹	26	
Running water			
Running water	Australia (Carcoar Dam)	4,000 ng GE liter ⁻¹	39
	Canada/United States (St. Lawrence River)	2–40 ng 2-MIB liter ⁻¹ ; 5–40 ng GE liter ⁻¹	102
	Central Europe (streams; Zürich, Switzerland)	3–7 ng GE liter ⁻¹ (mostly dissolved)	43
	Japan (Sakagawa River, polluted; Tokyo)	3,600 ng MIB liter ⁻¹ ; 30 ng GE liter ⁻¹	53
	Mediterranean area (Llobregat River)	15–20 (200) ng GE liter ⁻¹	97
	Tropical Africa (Senegal River)	47 ng GE liter ⁻¹	7
	Dammed river (Ruhr River, Germany)	2-MIB (<MDL); 35 ng GE liter ⁻¹	42
Biofilms			
Biofilms	Benthic cyanobacteria, aqueduct water (California)	Up to 78 ng 2-MIB liter ⁻¹ ; 48 ng GE liter ⁻¹	36
	Attached biofilms (Tokyo, Japan)	100 ng GE mg (dry wt) ⁻¹ = 6,300 ng GE cm ⁻²	53
	Canada (oligotrophic; N. Alberta streams)	<MDL up to 18 ng GE cm ⁻²	Wa
	Floating biofilms (Spain)	1,000 ng GE mg (dry wt) ⁻¹ = 36,500 ng GE cm ⁻²	97
	Aquatic GE concn from floating biofilms	10–100 ng GE liter ⁻¹	8
Drinking water			
Customer complaints	United States	46–88 ng GE liter ⁻¹	8
	Canada	(5) 10–120 ng GE liter ⁻¹	Wa
Odor episodes	France	2–10 ng GE or 2-MIB liter ⁻¹	7

^a Chl *a*, chlorophyll *a*; MDL, minimum detection level.

^b Wa, S. B. Watson, unpublished data.

^c Values in parentheses represent single minimum or maximum values observed which were not included in the more typical range shown.

For example, up to 200-fold differences have been reported for strains of *Streptomyces* (46). Zaitlin et al. (114) found that ~60% of 41 Lake Ontario isolates were producers, with ca. 100- and 300-fold variation in geosmin and 2-MIB production, respectively; these included both *Streptomyces* and nonstreptomycete (i.e., nonhyphal) genera, but high production was seen only among the former taxonomic group.

Not surprisingly, the growth and VOC production of an individual strain also varies with the environment and, in many cases, is not well supported in the pelagic zone. As early as 1985, Wood and coworkers (107) demonstrated that natural reservoir water did not support geosmin production by *Streptomyces albidoflavus* and other isolates but required a source of

enrichment such as found in sediment material and plant debris. In a later study, Sugiura and coworkers (91) reported that sedimented cyanobacteria and diatom cells (*Microcystis aeruginosa*, *Anabaena spiroides*, and *Synedra acus*) also provided good substrates for VOC production by benthic streptomycete isolates (we note here that in cases where this involved the mineralization of geosmin/2-MIB-producing cyanobacteria, odor could originate from active streptomycete metabolism and/or the release of cell-bound VOCs from the decaying cyanobacteria cells [see below]). As a further significant complication, optimal conditions vary among taxa, and in some cases even nutrient-poor environments can support VOC production by some actinomycetes. For example, Zaitlin and coworkers

(113, 114) observed considerable differences in the capacity to produce geosmin and 2-MIB among three streptomycete and nonstreptomycete actinomycetes grown with enriched media or sterile/nonsterile (unenriched) river water, with one isolate showing equivalent production on agar and river water. Geosmin production has been induced in cultures of *Streptomyces tendae* under stationary-phase conditions (as frequently found when antibiotics are applied) (18). However, it has not yet been clarified whether growing or nongrowing streptomycetes are more active geosmin producers under natural conditions.

Cyanobacteria. Because of their link with poor water quality, the seasonal dynamics of cyanobacteria are monitored more frequently than those of actinomycetes in freshwaters, allowing more opportunity for correlation analyses between these taxa and volatile compounds such as geosmin. In contrast to the case for actinomycetes, there is often a strong relationship between these measures. For example, the seasonal concentrations of geosmin were well correlated with the abundance of *Aphanizomenon gracile* in a eutrophic freshwater lake (45) and, in another study of the Australian Hay Weir Dam and Carcoar Dam, with *Anabaena* (39).

Nevertheless, cyanobacteria can be as challenging and enigmatic as actinomycetes. For example, between 1999 and 2006, annual late summer peaks of geosmin in western Lake Ontario (ranging between 5 and 200 ng · liter⁻¹) have shown little consistent relationship with the abundance of the large bundle-forming cyanobacterium *Anabaena lemmermannii* in the surface waters, although this organism has been the only likely candidate source identified to date (101). Similarly, substantial geosmin peaks (up to 2,000 ng · liter⁻¹) in raw water in a reservoir supplying the city of Tulsa, OK, have been poorly related to the abundance of the dominant planktonic cyanobacterium *Anabaena circinalis* (62) even though this particular taxon is known to be a major cause of taste and odor in other source waters (87, 96).

Even though they are often more visible, considerable expertise is required to identify cyanobacteria microscopically, a factor that is often overlooked. Their taxonomy is continually evolving, as the traditional emphasis on morphological traits as key criteria for positive classification is increasingly integrated with biochemical and molecular data. As a result, many species (again we refer to the phylogenetic species concept, since cyanobacteria are asexual [3]) identified as geosmin or 2-MIB producers by early studies have since been renamed, leading to confusion among many workers (Table 2), particularly since there is considerable plasticity in the morphology of many odor-producing (and non-odor-producing) cyanobacteria (27). Similar to the case for actinomycetes, there are low- and high-VOC-producing strains of (apparently) the same cyanobacterial species, for example, as seen for *Calothrix parietina*, *Oscillatoria limosa*, *Anabaena lemmermannii*, and *Fischerella* (29; Jüttner and Watson, unpublished data), which contributes to the overall ambiguity, together with the fact that the morphotypes and a large portion of the genotypes of producers and nonproducers may be the same. A further and very important consideration is that some cyanobacterial species identified as geosmin and/or 2-MIB producers may in fact not be the source of these compounds. One of the essential steps in the identification of a producer is the verification of VOC production by isolated strains of the study organism. A positive feature of all

cyanobacteria, however, is that geosmin is constitutively produced and its induction (as observed for streptomycetes) has yet to be conclusively shown.

From the above discussion, it is clear that the capacity for geosmin and 2-MIB production is a complex phenomenon which varies considerably among and within different taxa. Thus, a simplistic approach to source tracking (for example by measuring total cyanobacteria or actinomycetes in a water body) is unlikely to succeed (see below). Both taxonomic groups (and other potential VOC producers) show considerable diversity in their biochemistry, morphology, and habitat, and this should be carefully considered in any study. Above all, in order to understand the fundamental drivers behind the variability in geosmin and 2-MIB production (as with any other secondary metabolite) at the different organizational levels (i.e., cell, species, population, and environment), the intrinsic molecular control mechanisms need to be fully characterized in future work.

INTRA- AND EXTRACELLULAR VOC FRACTIONS

As elucidated below, it is essential to recognize that geosmin and 2-MIB occur in surface waters as cellular (cell-bound) and dissolved fractions and that the differentiation between these two fractions is key to the effective management of water quality control and treatment. Apart from the treatment-related issues, the dynamics of cell-bound and dissolved fractions also have an important effect on the sensory assessment of odor (and hence any related drinking water monitoring techniques) (71), because the bound VOC fraction does not follow Henry's law.

Unambiguous evidence for any organism can be obtained only by comparing cell counts to cell-bound VOC concentrations. If the dissolved component, which can sometimes represent the majority, is not differentiated from the particulate fraction, the correlation with biomass may be obscured. Furthermore, the proportions of intra- and extracellular production cannot be assumed to be constant, as these vary among taxa and with physiological state (growth phase and environmentally induced stress) (76) (Table 3).

Intracellular dissolved and protein-bound geosmin and 2-MIB. Work by Wu and Jüttner (108, 109) demonstrated clearly that particulate geosmin occurs in cyanobacterial cells as two distinct intracellular fractions, one which is dissolved in the aqueous cytosol and a second which is bound to proteins. Using polar solvents, these authors showed that this second geosmin fraction is bound to membrane proteins and not dissolved in the aqueous cytosol, as is also seen with chlorophylls and carotenoids, which are integral to the macromolecular protein-pigment photosystem units. In fact, the phycobilin proteins are attached to the surface of the thylakoids and may be the macromolecules to which geosmin and 2-MIB are bound by hydrogen bonds and van der Waals forces.

The presence of bound and dissolved intracellular geosmin fractions has important implications for protocols commonly employed for extraction and analysis, because these fractions behave differently. Stripping analysis using freeze-thaw, sonication, grinding, or nearly saturated NaCl (20%, wt/vol) to disrupt cells (e.g., as with the increasingly popular headspace solid-phase microextraction technique) primarily determines

cytosol-solubilized geosmin. The recovery of intracellular fractions may improve where these methods include heating, but this needs to be carefully verified. In many cases it is likely that the proportion of protein-bound geosmin is underestimated by such techniques, since a suitable polar solvent is required to cleave the bonding forces and access this fraction, which often represents the predominant geosmin moiety in a healthy cyanobacterial cell. In *Fischerella muscicola*, for example, separate water- and solvent-based extractions and analyses showed that 82% of the geosmin was protein bound (109). In a typical procedure, cells are filtered onto a glass fiber filter and treated briefly with methanol. Once separated, geosmin is not readily again sorbed by the protein when water is added and can be easily stripped by a conventional method. As a nonpolar compound, geosmin is lipophilic. The fact that treatment with water does not lead to the rebinding of this compound to cellular constituents supports the premise that the lipid membranes (thylakoids) themselves are not the primary binding sites for geosmin. Since the thylakoids are the most ubiquitous and abundant components in the cell lipid phase, if they were the primary sites, much of the geosmin should again be bound to these structures after dilution of the aqueous phase.

On the other hand, considerable research is still required to elucidate 2-MIB fractions in cyanobacteria. In a study of pigments and intra- and extracellular 2-MIB synthesis rates in *Oscillatoria perornata* (syn. *Planktothrix perornata*) and *Pseudanabaena articulata*, Zimba and coworkers (115) found that the size of the intracellular 2-MIB pool was independent of lipophilic and phycobilin pigment production. However, since it is still unknown if, similar to the case for geosmin, there are two intracellular pools of 2-MIB, we recommend that the two extraction methods described above also be applied for analysis of this terpenoid.

The cellular pools of geosmin and 2-MIB in actinomycetes, which lack the extensive thylakoid membrane complex, have similarly not been examined. Combined evidence points to the important effects of growth conditions on total VOC production and its allocation to intra- versus extracellular fractions. In a study of the growth, sporulation and geosmin production by *Streptomyces tendae*, Diogini et al. (18) observed that a large fraction of geosmin was stored in the cells relative to that released into the environment. This is consistent with what is exclusively observed with cyanobacteria. However, those authors also found that growth, sporulation, and geosmin production varied significantly among different agar growth media and that VOC yield was highest in sporulating cultures. This suggests that geosmin production is intensified either in these propagules or in both these and the accompanying somatic cells. In contrast, Pollack and Berger grew *S. citreus* in a liquid broth media and showed that the majority of this compound was extracellular over much of the growth cycle (69). Those authors did not examine the cultures for spore production. Both studies applied suitable extraction methods to recover cell-bound fractions, and taken together, their results indicate that extra- versus intracellular production may vary according to whether these organisms are suspended or on a substrate (and that differences in sporulation may be the reason), which has implications for suspended versus sediment-associated cells and treatment efficacy. The existence of major differences in streptomycete abundances estimated by different techniques

means that reported cell densities of these bacteria in open-water environments vary considerably among studies and likely misrepresent the true levels of active cells (see above). However, it appears that most cells are largely bound to suspended sediment particles (114). The contribution of their intracellular fractions to particle-bound geosmin (and likely also 2-MIB) may therefore be generally rather small but seasonally variable, for example, during storm events or periods of high runoff (37).

The occurrence of these different VOC fractions has important implications for the interpretation of published data. Most workers continue to overlook the fundamental differences among cell-bound and dissolved geosmin and 2-MIB fractions, even though much of this knowledge was available almost 20 years ago, and a simple filtration step easily allows their separation and analysis (19). Thus, since there are a number of different analytical procedures for geosmin and 2-MIB, in each case it is essential to know which method has been employed.

Conversion of cell-bound to dissolved geosmin fractions. The presence of separate particulate and dissolved VOC fractions in source waters has other important implications, both for the interpretation of field data and for the choice of optimal water treatment processes (discussed below). As previously noted, the relative amounts of these fractions can vary among and within species, while cell-bound VOCs can be transferred rapidly into the dissolved form. One major mechanism for this is via cell degradation by heterotrophic microorganisms (which could include both producers and nonproducers, e.g., fungi and streptomycetes [90, 91]). This process, for example, liberates geosmin from the cyanobacterial cell protein matrix. Much of the cell-bound material can be transferred into the dissolved form by this process because geosmin itself is much more slowly degraded by most bacteria than other cell components.

This phenomenon is observed frequently in water layers near the sediment, and the resulting elevated concentrations of dissolved geosmin are readily visible in depth profiles (19). The VOC release takes place when geosmin-containing microorganisms sediment into deep oxic or anoxic waters and are mineralized by one or more of several processes: the catalytic enzymes of both the producers themselves and other microbial flora and, in some cases, benthic grazers (26, 29). In the pelagic zones of lakes, particularly the epilimnion, grazing may be a more important liberating mechanism. Experiments with the geosmin-producing cyanobacterium *Aphanizomenon gracile* showed that when grazed by the crustacean *Simocephalus* or *Daphnia magna*, the cell-bound geosmin was almost completely transferred into the dissolved form (19). This process is typically overlooked by most taste-odor studies and yet is likely to be a significant modifier in many water bodies. Crustaceans can turn over a large portion of the phytoplankton in short time periods, and thus it is likely that relative proportions of dissolved and cell-bound geosmin in source waters are often related to grazing activity. 2-MIB may show similar patterns, although this is yet to be examined. Similarly, as noted below, the role of this VOC release mechanism in biofilms is unknown but is also likely to be significant.

IN SITU AND ALLOCHTHONOUS PRODUCTION OF GEOSMIN AND 2-MIB

Many running waters investigated to date have contained geosmin (8, 41, 43), 2-MIB (31), or both odor compounds (36,

97, 102) (Table 3). VOC concentrations can range considerably, and in these lotic systems the major fractions of both compounds are often in the dissolved form. For example, Jüttner (43) found 4 to 7 ng · liter⁻¹ geosmin in some minor streams near Zürich (Switzerland), with the particle-bound fraction being between 3.5 and 22.2% of the total. Hoson and coworkers also found that the dissolved 2-MIB fraction was larger than the cell-bound fraction (32). In some cases, allochthonous soil runoff may be important (33, 113). Biofilms can also be very significant sources, even in large, fast-flowing rivers. For example, Watson and Ridal (102) concluded that most of the fairly significant levels of geosmin and 2-MIB (up to ~70 ng · liter⁻¹) sustained during late summer every year in the Saint Lawrence River are produced by shoreline biofilms (cyanobacteria, actinomycetes, and others) on rocks, macrophytes, and mussels. With a flow of ~8,000 m³ · s⁻¹ of the river, this represents substantial in situ production. Vilalta and Sabater (98) found a very strong correlation between geosmin levels in the open water and those in attached and floating cyanobacterial mats in the Llobregat River (Spain). Baker and coworkers (2) traced high 2-MIB levels in the Murray River to the proliferation of a toxic benthic mat of *Phormidium*. Under normal growth conditions, cyanobacteria (5, 109) and streptomycetes (18) excrete very little of these compounds into the medium, and in many flowing waters, their liberation into open water from these attached or floating producers is likely caused by a combination of grazing and environmental stressors, such as desiccation and photooxidation, that can lead to the disintegration of these cells and release of cell contents during sloughing off or water level changes. In addition, the concerted interaction of actinomycetes and cyanobacteria in biofilms could contribute to VOC release in a different way than when these organisms act independently (90, 91).

Alternatively, plankton may be primary sources of geosmin and 2-MIB in some slow-moving turbid rivers. For example, this was observed in the Murrumbidgee River (Australia), where high geosmin levels were caused by the buoyancy-regulating cyanobacterium *Anabaena circinalis* (5), and in the Yodo River (Japan), where the stagnation of dammed river water promoted the growth of geosmin-producing *Anabaena macrospora* (28). Investigations of several other dammed rivers have shown elevated geosmin concentrations near to the dam (42), for example, in the Ruhr River (Westfalia, Germany), where this was most likely produced by the liberation of geosmin from microorganisms undergoing accelerated sedimentation in the stagnant waters beside the dam.

Spatial distribution of geosmin and 2-MIB in lakes. In both lotic and lentic systems, three zones should be investigated as possible sources of geosmin and 2-MIB: the epilimnetic water and associated plankton; the hypolimnetic water (oxic or anoxic), which is often below the light compensation point for oxygenic photoautotrophic microorganisms; and the littoral/benthic zone. Efficient transport of geosmin and 2-MIB among these compartments is mediated primarily by particles (cyanobacteria and other geosmin-producing microorganisms) that carry these odor compounds. As long as these VOCs remain cell-bound, sedimentation can introduce a rapid and efficient displacement. Henatsch and Jüttner (26) measured epilimnetic geosmin concentrations of up to 190 ng · liter⁻¹ in Lake Schleinsee (Germany) in July. By September, surface water

concentrations were reduced to ~50 ng · liter⁻¹, but at the same time a substantial increase from ~190 to 950 ng geosmin · liter⁻¹ was observed in the hypolimnion. Maximum levels were found at the 10-m depth just above the sediment. The total geosmin amount in the lake was nearly constant over that same 1-month observation period. Although cell-bound geosmin was not determined at that time, the most likely cause of these temporal and spatial patterns was the sedimentation of particle-bound geosmin. The organic seston fraction was readily digestible under anoxic conditions, but geosmin degradation was very low under these same conditions, and most was therefore transferred from the cell-bound into the water-soluble fraction. It is also important to note here that to date, geosmin (or 2-MIB) production has not been described for anoxic environments.

Vertical transport caused by water or particle movements can be an effective vector of VOCs. For example, the extensive transport of offshore particle-bound geosmin to deep-water treatment plant intakes occurs each summer in Lake Ontario as a result of large-scale wind-induced downwelling (70, 101). Detachment of benthic and epipelagic VOC-producing cyanobacterial populations (e.g., *Gloeotrichia echinulata* and *Oscillatoria limosa*) and their subsequent flotation can introduce these compounds into the pelagic zone (8, 98, 99). Gases that accumulate in small bubbles in the sediment below cyanobacterial mats can increase their buoyancy sufficiently to loosen large biofilm patches, which then rise and transport cell-bound geosmin to the water surface (87). Such a mechanism would explain, for example, the observed surface maxima of bound geosmin in Lake Zürich (19).

PROACTIVE CONTROL OF GEOSMIN AND 2-MIB IN WATER SOURCES: TREATMENT REMOVAL AND LONG-TERM MANAGEMENT

Geosmin and 2-MIB are relatively stable to chemical (66, 104) and biological degradation and can persist in the open water in the dissolved form for some time. This is an important factor to consider when attempting to understand and trace the distribution, transport, and fate of these VOCs in aquatic systems and their response to water treatment. Dissolved geosmin is slowly degraded by microorganisms in oxic freshwater (19), but little is known about the fate of this compound under anoxic conditions. Similarly, to our knowledge, little research has been carried out on natural 2-MIB degradation. Grazing by crustaceans, the major herbivores in lakes and reservoirs, does not appear to change the total amount of geosmin significantly, as shown by Durrer and coworkers (19), who measured this process in closed vessels to prevent volatilization (which can be a major source of losses).

Most taste-and-odor outbreaks are unanticipated, and thus there is a heavy reliance on water treatment plants to control their impairment of drinking water. An extensive review of the treatment removal of geosmin and 2-MIB from raw water is beyond the scope of this paper, which focuses on microbial processes. For overviews of the numerous chemical and photooxidative techniques widely in use, the reader is referred to the numerous studies dealing with these processes and their effectiveness for different source waters (52, 54, 61, 77). These techniques are generally combined with various filtration

media such as sand, activated carbon, and, more recently, membrane systems (12, 34, 72).

Because of their stability, both compounds are difficult to oxidize with the conventional processes applied during water purification (54). More advanced treatments, such as granular or particulate activated carbon, ozonation, and membrane filtration, can be applied with variable success; their effectiveness is modified by factors such as age of filter beds, type of carbon used, levels of source water dissolved organic material, and proportion of dissolved/particulate geosmin or MIB (12, 34). Because the bulk of the two compounds is often present in the particulate form, treatments such as the application of chemical oxidants or algicides that disrupt cells and liberate the cell-bound material prior to their removal should be avoided (67). Ideally, removal of intact particulate material should be achieved (e.g., by dissolved air floatation, filtration, and sedimentation) prior to disinfection and other oxidative processes (103).

In comparison, the microbial treatment methods applied by the water industry (river bank filtration and activated filter beds) are few in number (63), but some salient considerations are relevant to our discussion. In fact, microbial removal offers a potentially highly effective means to treat geosmin and 2-MIB in source water, particularly when integrated with other treatment processes. For example, recent bench experiments with granular carbon (20) and sand columns (30) have demonstrated active microbial degradation activity for these odor compounds under defined conditions. River bank filtration and slow sand filtration have been used in treatment facilities throughout Europe for some time and offer a very effective means to obtain high elimination rates. Jüttner (42), for example, reported that a slow sand filtration unit (flow rate of 420 liters m^{-2} day $^{-1}$) achieved excellent rates of elimination of geosmin and other terpenoid alcohols. That study found that geosmin was not detectable in the upper layers of the sand filter when this material was removed and chemically extracted, indicating the efficient degradation of this compound by the immobilized microorganisms. This technology has only recently been recognized in North America for its potential application.

We conclude this section by emphasizing that although many of these treatment processes are quite effective at geosmin and 2-MIB removal, they are often extremely expensive to install, maintain, and operate, particularly where source waters are of poor quality or high in dissolved organics (12). More long-term proactive management needs to address the source(s) of the problem, by identifying the environmental and biological agents and their controls (99, 101). A significant proportion of odor events are linked to intense runoff from highly developed watersheds (37, 47, 113) and/or source water degradation and eutrophication (39, 45, 46, 100) and the associated increase in odor-producing biota such as cyanobacteria. Clearly, therefore, ecologically sound watershed and source water remediation and management is essential to the long-term effectiveness and sustainability of a successful mitigation program.

CONCLUSIONS

Despite considerable research, the treatment and control of geosmin and 2-MIB are still hindered by common misconcep-

tions about their biological and spatial origins and their storage and release by cells. In particular, (i) it is often assumed that planktonic or benthic aquatic cyanobacteria and streptomycetes are the major sources of these VOCs, while eukaryotes (e.g., fungi), and terrestrial and distribution system sources are disregarded; (ii) discrete cell-bound and dissolved VOC fractions respond very differently to treatment, and their relative proportions have major implications for data interpretation, yet these fractions are rarely considered and, furthermore, are incompletely measured by most current analytical methods; (iii) key physical and biological processes (e.g., treatment, grazing, and bacterial digestion) may interconvert these fractions and thus affect source water quality and treatment, and yet the importance of these processes is rarely addressed; and (iv) problems associated with many of the methods currently used to identify and enumerate source biota may obscure any correlations with VOC levels and thus confound tracking efforts.

Clearly, many intriguing questions still need to be addressed. These include the spatial and temporal analysis of cell-bound and dissolved geosmin and 2-MIB; the molecular regulation of their production; the major physiological, biochemical, and molecular traits which determine which species/strains are low and high odor producers; the physiological and ecological roles (i.e., chemical ecology) of these compounds; and the mechanisms which trigger their extracellular release (i.e., whether this occurs primarily as a result of cell disruption and decay or via a transporter that is activated/induced under certain conditions). Future research into these and other related issues will likely provide insight into much of the current ambiguity surrounding many outbreaks of these major odor compounds.

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