IDENTIFICATION AND CHARACTERIZATION OF ODOROUS METABOLITES PRODUCED BY SELECTED FRESHWATER ALGAE

by

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(ABSTRACT)

The occurrence of taste-and-odor problems that are caused by algal metabolites in water supplies has been well documented. Several commonly occurring odor-producing algae were selected and cultured for this research. Initial studies involved the algal cells and cell-free media from cultures grown under fairly optimal conditions. Gas chromatography-mass spectrometry (GC-MS) and flavor profile analyses (FPA) were performed to identify the organic compounds produced by the algae and their respective odors.

Three of the algal cultures underwent additional studies that investigated the effects of selected changes in culture conditions on both population growth and compound production. Experimental variables included nitrogen concentration (ammonium, nitrate, and nitrite), phosphorus concentration, light intensity, and temperature. Parametric and nonparametric analyses were performed to identify the environmental factors that had a significant effect on algal production, accumulation, and release of taste-and-odor compounds.

The organic compounds were extracted from both the algal cells and the cell-free media. Continuous liquid-liquid extraction and Kuderna-Danish concentration (CLLE-KD) was an effective and reliable method for the isolation and concentration of a broad range of organic compounds. The plot of flavor profile analysis (FPA) results obtained for odor standards adhered to the Weber-Fechner Law (W-F) over the range of concentrations evaluated. The odor intensities of algal cultures were generally lower than the odor intensities predicted from the W-F plot of the compound standards. Masking of the odor associated with one compound by the odor associated with another was observed. Odors produced by young algal cultures (e.g., low population densities) were

detected in FPA samples at compound concentrations below the limits of detection by GC-MS.

Anabaena laxa retained most of the geosmin it produced within the algal cells. *Phormidium* sp. produced more 2-methylisoborneol (MIB) than geosmin, and the alga retained only a relatively small amount of either compound within the cells. *Synura petersenii* produced more 2t,4c,7c-decatrienal than 2t,6c-nonadienal, and large fractions of the concentrations produced were retained within the algal cells. Various combinations of nutrient reduction, early algal-bloom within-reservoir treatment, and removal of algal cells prior to oxidation were suggested as likely methods by which odor problems may be reduced.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

HISTORY

Mankind has been interested in algae for many centuries, primarily for their various beneficial uses and also the problems they may cause. The literature contains many articles, books, and reviews on algae, but only a few are mentioned here.

The beneficial uses of algae include:

- Food source, food additives, medicines, and vitamins (Ryther 1984; Flament and Ohloff 1984; Becker 1986; Lee 1989; Kay 1991)
- Renewable fuel source (cell lipids), wastewater treatment, and as biofertilizer (McIntosh 1984; Ley 1987; Barclay et al. 1987; Brouers et al. 1987; de la Noue and Proilx 1987)
- Source of fine chemicals (Thepenier et al. 1987; Borowitzka 1988a)

Problems caused by algae include:

- Flocculation and filtration problems during water treatment (Palmer 1980; Poppe et al. 1981)
- Toxins (Carmichael and Gorham 1977; Collins 1978; Carmichael and Mahmood 1984; Berg et al. 1987; Kay 1991; Kenefick et al. 1992)
- Tastes and odors in drinking water supplies

Problems caused by tastes and odors produced by algae can range from water utility personnel dealing with consumer complaints to loss of aquaculture revenue due to tainted fish. Commercial cultures of channel catfish may be severely influenced by musty, muddy tastes and odors in the fish flesh. The algal metabolites 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (β-cyclocitral), (E)-1,10-dimethyl-9-decalol (geosmin), and 2-methylisoborneol (MIB) are, among others, implicated in producing off-flavored fish (Lovell 1983; Martin 1992; Martin and Suffet 1992).

Taste-and-odor episodes at water treatment facilities during algal blooms are well documented (Izaguirre et al. 1982; Jüttner 1983; Sävenhed et al. 1983; Burlingame et al. 1986; Means and McGuire 1986; Hayes and Burch 1989; Hrudey et al. 1992; Wnorowski and Scott 1992). The predominant problems have been earthy and musty odors, usually attributed to geosmin or MIB, respectively. These odors can be produced by either actinomycetes or cyanophycean (blue-green) algae. Geosmin was first isolated from actinomycetes (Gerber and Lechevalier 1965) and was later isolated from blue-green algae by Safferman et al. (1967) and Medsker et al. (1968). Since then, geosmin has been isolated from several blue-green algae, including *Anabaena macrospora* (Miwa and Morizane 1988), *Oscillatoria* sp. and *Anabaena flos-aquae* (Hayes and Burch 1989), and *O. tenuis* (Wu and Jüttner 1988). The musty-smelling compound 2-methylisoborneol was first isolated from actinomycetes by Gerber (1969) and was later isolated from the blue-green alga *Lyngbya* cf. *cryptovaginata* by Tabachek and Yurkowski (1976). It has also been isolated from *Oscillatoria* sp. (Hayes and Burch 1989), *O. tenuis* (Wu and Jüttner 1988; Izaguirre et al. 1982), and *O. curviceps* (Izaguirre et al. 1982).

Algae other than the blue-greens are also associated with taste-and-odor episodes. Odor descriptions include cucumber, grassy, fishy, and tobacco-like, all of which are the result of the extracellular release of algal metabolites. Extracellular products (ECP) include hydrocarbons, fatty acids, ketones, terpenoids, amines, and aldehydes (Jüttner 1987; Slater and Blok 1983a). *Synura* has been implicated in the production of a cucumber odor (Hayes and Burch 1989) and cod liver oil-like odors (Jüttner 1981). Other algae associated with particular odors include green algae that produce grassy, musty, and skunk-like odors; diatoms that produce geranium, spicy, fishy, and musty odors; and flagellates that produce fishy, cucumber, melon, and violet odors (Palmer 1980).

Although research in the area of tastes-and-odors has established that algae do produce numerous odor-causing compounds, little information has been acquired concerning the influence of the algal population growth phase and external factors such as light, temperature, and nutrients on odor production, accumulation, and release. Research that has been conducted includes observing changes in lipid composition (Orcutt and Patterson 1974; Materassi et al. 1980; Shifrin and Chisholm 1980) and in geosmin and MIB production (Naes et al. 1985; Wu and Juttner 1988; Miwa and Morizane 1988; Naes and Post 1988; Naes et al. 1988; Utkilen and Frøshaug 1992, Rosen et al. 1992).

OBJECTIVES

The objectives of this research were to:

1. Identify the organic compounds produced by selected algal cultures and determine whether they were retained within the algal cells or were released into the medium. The accomplishment of this objective required the evaluation of the continuous liquid-liquid extraction and Kuderna Danish concentration method (CLLE-KD) as a procedure for extracting and concentrating the organic compounds produced by the algal cultures.
2. Determine the odors produced by the algal cultures and how those odors were perceived by a flavor profile analysis (FPA) panel; relate, where possible, the odors produced by the algal cultures to specific compounds that were detected by gas chromatography-mass spectrometry (GC-MS); and compare the FPA odor intensities obtained from the concentrations of specific compounds in the algal cultures to those intensities obtained from compound standards.
3. Evaluate the influence of environmental factors on population density and on the production, accumulation, and release of geosmin, MIB, 2t,6c-nonadienal, and 2t,4c,7c-decatrienal, all of which are odorous algal metabolites.

CHAPTER 2
LITERATURE REVIEW

OVERVIEW

The literature contains many sources of information on the subject of algal metabolites, methods of organic compound extraction and identification, odor evaluation, and the influence of environmental factors on algal productivity. Each topic will be discussed in turn.

ALGAL METABOLITES

Investigators have been interested in algal extracellular products (ECP) for many years. The strict definition applies to compounds released by healthy cells, rather than cells that are dying or lysed. The ability to discriminate between methods of cellular release is not always possible, as the same compounds may come from both situations.

Much information about ECP has accumulated, and several reviews have been written (Hellebust 1974; Nalewajko 1977; Jüttner 1983; Slater and Blok 1983a; Becker 1986; Jüttner 1987). Classes of extracellular compounds produced by algae include organic acids, carbohydrates, nitrogenous compounds, lipids, vitamins, growth or inhibition related compounds, nor-carotenoids, toxins, and chelating agents. Several researchers utilized lake water samples collected during bloom conditions, and the compounds detected were not confirmed to be of algal origin (Jüttner 1981; Slater and Blok 1983b; Hayes and Burch 1989).

Carbohydrates, Organic Acids, and Amino Acids

A large fraction of the material released by algae consists of carbohydrates (polysaccharides), organic acids (glycolate), and amino acids. These compounds may influence the growth of both algae and bacteria present in the water. Several odoriferous volatile organic acids, including isovaleric acid and butyric acid, have been detected in algal cultures (Aaronson et al. 1980). Odor descriptors for these organic acids include cheesy, rancid, putrid, dirty socks (linen), sweaty, sour milk, vinegary, musty, sickening, and fecal (Dravnieks 1985; Hsieh et al. 1989; Aldrich 1994).

Researchers observed algal excretion of 1 to 12 percent of total carbon fixed in radioactive carbon uptake experiments involving *Cyanidium caldarium* and *Synechococcus lividus* in both laboratory and field experiments (Belly et al. 1973; Bauld and Brock 1974). The total mass of the available fixed carbon that was released was greatest during optimal conditions for photosynthesis (Belly et al. 1973), but the percentage of the available fixed carbon that was released was greater in the dark (Bauld and Brock 1974).

Nalewajko and Marin (1968) conducted experiments with both algal cultures (*Asterionella formosa* Hass., *Melosira binderana* Kütz., *Stephanodiscus tenuis* Hust., and *Chlorella pyrenoidosa* Chick) and lakewater samples brought to the laboratory. They observed that the percentage of the fixed carbon excreted by the lakewater phytoplankton, which were predominately *Asterionella formosa* and *Stephanodiscus tenuis* Hust., was much higher than that observed in the algal cultures (i.e., from 23.1 to 76.1 percent and from 1 to 8 percent, respectively). In a later investigation, Nalewajko et al. (1976) studied both axenic and mixed algal-bacterial cultures of *Chlorella pyrenoidosa* Chick and *Anabaena flos-aquae*. It was determined that values obtained for ECP release from primary productivity studies may be seriously underestimated. Bacterial uptake of excreted compounds resulted in lower primary productivity values from a seven to eight hour incubation period than from a one hour incubation period.

Investigators have observed the response of bacteria to algal excretion products in several different studies. The algae involved were *Asterionella formosa* Hass., *Melosira binderana* Kütz., *Staphanodiscus tenuis* Hust., and *Chlorella pyrenoidosa* Chick (Nalewajko and Marin, 1968), *Skeletonema costatum*, *Cyclotella nana*, *Dunaliella tertiolecta*, and *Isochrysis galbana* (Bell and Mitchell 1972), *Chlorella pyrenoidosa*, *Anabaena flos-aquae*, *Asterionella formosa* and *Navicula pelliculosa* (Nalewajko and Lean 1972), *Cyanidium caldarium* (Belly et al. 1973), *Synechococcus lividus* (Bauld and Brock 1974), *Chlorella pyrenoidosa* Chick and *Anabaena flos-aquae* (Nalewajko et al. 1976), *Oscillatoria redekei* (Herbst and Overbeck 1978), *Skeletonema costatum* and *Chaetoceros affinis* (Bell 1980; Bell and Sakshaug 1980). The filtrate from axenic *Chlorella* cultures contained from 1.5 to 4.3 mg/L glycolic acid (Bell and Mitchell 1972; McFeters et al. 1978), and from 10^{-8} to 10^{-6} M of both amino acids, which may be reutilized, and sugars (Bell and Mitchell 1972). The presence of amino acids, peptides, and proteins in culture media and natural waters has been well documented (Hellebust 1974). Palenik and Morel (1990) found that L-amino acid oxidase was used at the cell

surface of some marine algae to break down extracellular amino acids and resulted in the production of free NH_4^+, which could then be utilized by the algae as a nitrogen source.

Hellebust (1974) stated that glycolate "is the organic acid most commonly liberated by algae." Nalewajko and Lean (1972) found that *Chlorella pyrenoidosa*, *Anabaena flos-aquae*, *Asterionella formosa*, and *Navicula pelliculosa* cultures excreted glycolate when the photosynthetic rate was highest and excreted polysaccharides at later stages. They observed also that rates of excretion in axenic cultures decreased with age and that smaller molecular weight compounds were taken up most readily, while large molecular weight compounds accumulated in the media. In axenic algal cultures inoculated with bacteria, it was unclear as to whether the accumulation of large compounds resulted from their inability to be assimilated or because bacteria assimilated the smaller compounds and then excreted larger compounds. The interaction between algae and bacteria may be quite marked. Herbst and Overbeck (1978) noted that only 20 to 50 percent of net primary production reached the hypolimnion.

Reports have indicated that although algae excrete glycolate, they may not be capable of reutilizing it. Hess and Tolber (1967) studied cultures of *Chlorella pyrenoidosa* Chick, *Chlamydomonas reinhardtii*, *Ankistrodesmus braunii*, *Scenedesmus obliquus*, and *Chlorella* (Warburg), and did not detect any glycolate oxidase activity. The investigators, however, did detect NADH:glyoxylate reductase, phosphoglycolate phosphatase, and isocitrate dehydrogenase. Lee (1989) stated that glycolate was metabolized within algae by either glycolate dehydrogenase or glycolate oxidase. Nalewajko (1977) stated that active transport systems for glycolate were either rare in algae or not very effective.

Vitamins: B_{12}, thiamin, and biotin

The proper growth of algal cultures may require that supplements of the vitamins B_{12}, thiamin, and biotin be added to their media. Some algae, however, are able to produce one or more of these compounds. Examples of axenic algal cultures that produced and excreted vitamins include *Coccolithus huxleyi* (produced biotin and B_{12}), *Gonyaluax polyedra* (produced thiamin), *Phaeodactylum tricornutum* (produced biotin), and *Dunaliella tertiolecta*, *Skeletonema costatum*, and *Stephanophxus turris* (produced thiamin and biotin) (Carlucci and Bowes 1970a; Carlucci and Bowes 1970b). Carlucci and Bowes (1970b) determined from mixed culture experiments that the vitamins excreted

by one alga were then utilized by other algae. Vitamin uptake in these studies was greatest during the first few days of culture growth.

Lipids

Lipids

Much information about lipids is available; particularly in the food-industry literature. The lipids category is composed of a variety of compounds, including total fatty acids, specific classes of fatty acids, unsaponifiable lipids (e.g., hydrocarbons, alcohols, and sterols), and unsaturated fatty acids. Lipids are significant in that they may influence both odor and flavor in several ways: (1) they may have their own odor or flavor, (2) they may serve as precursors of odor and flavor compounds, and (3) they may act as modifiers of the odors and flavors that are caused by other compounds present (Forss 1973). Several articles, books, and reviews of lipids, their synthesis and metabolism, breakdown products and mechanisms, and odors associated with both them and their breakdown products have been published. These publications will not be discussed here, but they are cited for future reference by interested readers. The publications include: Hoffmann 1962; Seals and Hammond 1970; Selke et al. 1970; Hitchcock and Nichols 1971; Ke et al. 1975; Hatanaka et al. 1975; Galliard and Phillips 1976; Chan 1977; Terao and Matsushita 1977; Frankel et al. 1979; Gurr and James 1980; Galliard and Chan 1980; Stumpf 1980; Frankel 1980; Seifert and Buttery 1980; Tressl et al. 1981; Frankel et al. 1981; Frankel 1982; Frankel et al. 1983; Josephson et al. 1985; Grosch 1987; Hamilton 1989; Hsieh and Kinsella 1989; Hsieh et al. 1989; Karahadian and Lindsay 1989; Josephson 1991).

Fatty Acids

The formation of 14-carbon, 16-carbon, and 18-carbon fatty acids by algae is well documented (Kenyon et al. 1972; Orcutt and Patterson 1974; Wood 1974; Orcutt and Patterson 1975; Fulco 1977; Rezanka et al. 1983; Cranwell et al. 1988). The saturated fatty acids in algae are synthesized from acetate by β-addition (Wood 1974). Unsaturated fatty acids are formed from the saturated fatty acids through a strictly aerobic pathway (Fulco 1977).

Orcutt and Patterson (1974) observed that the intracellular total fatty acid content in axenic *Nitzschia closterium* cultures increased at high light intensity compared to the amount of total fatty acid within cells grown at low light intensity, but the relative percentage of unsaturated fatty acids decreased. They observed also that a large portion of the lipids were cellular pigments whose concentration appeared to decrease at increased light intensity.

Orcutt and Patterson (1975) investigated the diatoms *Amphora exigua*, *Amphora* sp., *Biddulphia aurita*, *Fragilaria* sp., *Nitzschia frustulum*, *N. longissima*, *N. ovalis*, *N. pelliculosa*, *Phaeodactylum tricornutum*, and *Thallasiosira pseudonana* for intracellular lipid, sterol, and fatty acid composition. They determined that the 18-carbon fatty acids were rare; but palmitoleate, palmitate, eicosapentaenoate, and myristate were prevalent. Other researchers have reported that the 18-carbon fatty acids represented a large fraction of the total intracellular fatty acid content in several cultures of *Chrysophyceae* (golden-brown algae), *Chlorophyceae* (green algae), and *Cyanophyceae* (blue-green algae) (Kenyon et al. 1972; Wood 1974; Rezanka et al. 1983; Becker 1986; Cohen 1986; Cranwell et al. 1988). Fatty acid distribution among algae appears to be quite diverse, and Kenyon et al. (1972) suggested that it be included as a criterion for taxonomic comparison.

Although some fatty acids do have an odor, both the wide range of breakdown products that may be formed and their associated impacts upon tastes and odors are of greater interest to food and water industry researchers. The breakdown products are formed from fatty acids through oxidative, photo-oxidative, and lipoxygenase activity. The interest in breakdown products is particularly true for the 18-carbon unsaturated fatty acids oleate (18:1, representing 18 carbons and 1 double bond), linoleate (18:2), and linolenate (18:3) (Forss 1973; Grosch 1987). Autoxidation occurs in these three acids at different rates, with linolenate being oxidized the fastest. The rate of reaction ratio among them is 1:64:100, respectively (Hamilton 1989).

Hellebust (1974) stated that "little is known about the possible release of lipids by healthy algal cells." Her review cited a study where 2.8 to 10.3 percent of the total extracellular material from the media of marine phytoplankton were lipid compounds. *Synura*, which is a chrysophyte, produces lipids whose breakdown products may be responsible for cod liver oil and cucumber odors in surface water supplies (Jüttner 1981; Hayes and Burch 1989).

Toxins

Toxins are produced by a wide variety of algae, including the fresh water algal classes *Chlorophyceae*, *Chrysophyceae*, and *Cyanophyceae* (Collins, 1978). Toxin production by blue-green algae, particularly by *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, and *Microcystis aeruginosa*, has been well-documented (Carmichael and Gorham 1977; Collins 1978; Carmichael and Mahmood 1984; Berg et al. 1987; Kenefick et al. 1992). Other blue-green algae that may produce toxins include *Oscillatoria agardhii* and *O. rebescens* (Carmichael and Mahmood 1984) and *Coelosphaerium* sp., *Gloeotrichia* sp., *Lyngbya* sp., *Nodularia* sp., and *Nostoc* sp. (Collins 1978).

The toxins produced by blue-green algae include cyclic peptides (e.g., microcystin LR and RR), anatoxins, alkaloids, and aphantoxin (Carmichael and Mahmood 1984; Berg et al. 1987; Lee 1989; Kenefick et al. 1992). Aphantoxin is similar to the paralytic shellfish poison saxitoxin, and anatoxin-a is an alkaloid neurotoxin (Carmichael and Gorham 1980). The cyclic peptides are heptatotoxins (Berg et al. 1987).

Algal toxins are of concern because they can kill livestock and waterfowl (Carmichael and Gorham 1977; Carmichael and Mahmood 1984; Lee 1989). Reports of human illness caused by algal toxins are rare (Collins 1978; Carmichael and Mahmood 1984); however there is an increased potential danger posed to humans as blue-green algae are considered for consumption as a protein source (Carmichael and Gorham 1980; Kay 1991).

Chelating Agents

Algal populations, succession patterns, and surface water management programs may be influenced by the presence of metal-chelating compounds in impoundments. Lange (1974) speculated that natural metal chelators that originated from either algal extracellular compounds or humic substances may promote algal growth in surface waters and influence succession patterns by enabling the growth of algae that could not otherwise survive. Control of algal populations in surface waters with copper sulfate is impeded by the presence of increased organic matter concentrations (Raman and Cook 1988; Hering and Morel 1988). Copper sulfate toxicity may be reduced by the release of algal extracellular copper-chelating compounds (McKnight and Morel 1979).

Lange (1974) determined that the production of natural, extracellular, metal-chelating compounds by axenic blue-green algae cultures (e.g., *Anabaena cylindrica*, *Anacystis nidulans*, *Lyngbya* sp., *Microcystis aeruginosa*, *Nostoc muscorum*, and *Phormidium foveolarum*) enabled them to grow at high pH when artificial chelators (e.g., EDTA plus citrate) were absent from the culture media. Some of the blue-green algae Lange investigated did not produce a chelator (e.g., *Anabaena circinalis*, *Gloeotrichia echinulata*, *Oscillatoria rubescens*, and *Aphanizomenon flos-aquae*), but they were able to grow in media that was supplemented with either EDTA plus citrate or the filtrate from axenic chelator-forming cultures. The natural chelators produced by the algae were presumed to be either peptides or polysaccharides. Lange cited a prior study where fulvic acids demonstrated significant chelating properties.

Simpson and Neilands (1976) studied a culture of *Anabaena cylindrica* that released a large, pigmented, peptide-containing material that complexed iron. Miwa and Morizane (1988) observed that cultures of *Anabaena microspora* grown without EDTA reached cell densities one-tenth of those grown in either EDTA or humic acid; however, the extracellular geosmin in the EDTA-free medium was greater than that in the medium with a chelator.

Inhibitors of Carotenogenesis: nor-carotenoids

The inhibition of blue-green algal growth by nor-carotenoids is likely achieved by the inhibition of carotene synthesis. *Cyanidium caldarum* excretes the nor-carotenoids 6-methylhept-5-en-2-one, geranylacetone, β-ionone, dihydrotrimethylnaphthalene, and butenylidenetrimethylcyclohexene. Two of the compounds, 6-methylhept-5-en-2-one and geranylacetone, may be either degradation products of open-chain carotenes formed by oxygenase activity (Jüttner 1979a; Jüttner 1987) or otherwise connected to lipid and carotenoid metabolism (Henatsch and Jüttner 1983). The growth of the blue-green algae *Anabaena variabilis*, *Synechococcus* 6911, *Nannochloris coccoides*, and *Cyanidium caldarium* was inhibited when nor-carotenoid concentrations were approximately 50 mg/L. Chlorophyll synthesis was only slightly affected. Geranylacetone inhibited carotene synthesis in *Synechococcus* 6911, apparently by preventing the conversion of phytofluene into ζ-carotene (Jüttner 1979b).

A later study (Jüttner and Bogenschütz 1983) demonstrated that low concentrations of the geranyl derivatives geranylacetate, geraniol, pseudoionone, and

farnesol were capable of retarding growth in *Synechococcus* 6911 cultures . Higher concentrations of these compounds caused culture bleaching and cell death. Total carotenoid synthesis decreased, with neither phytofluene nor ζ-carotene being metabolized; however, both the β-carotene concentration and chlorophyll-a synthesis were apparently normal.

Synechococcus 6911 produced 6-methylhept-5-en-2-one, methylheptenol, nerol, geraniol, tetrahydrogeranylacetone, and dihydroactinidiolide; whereas *Synechococcus* 6301 produced 6-methylhept-5-en-2-one, 2,2,6-trimethylcyclohex-2-en-1,4-dione, epoxy-β-ionone, and dihydroactinidiolide (Henatsch and Jüttner 1983). The authors suggested that these excretion products are connected to the metabolism of both carotenoids and lipids. Their *in vitro* experiments produced β-ionone, epoxy-β-ionone, and dihydroactinidiolide when they treated linoleic acid and β-carotene with soybean lipoxygenase.

The nor-carotenoids can also inhibit the growth of bacteria. Geranylacetone, 6-methylhept-5-en-2-one, β-ionone, and dihydrotrimethylnaphthalene were evaluated for inhibition of growth, pigment production, and glucose utilization of the bacteria *Cytophaga johnsonae*, *Chromobacterium lividum*, *Arthrobacter* sp., *Pseudomonas fluorescens*, and *Bacillus mycoides* (Reichardt 1981). The 6-methylhept-5-en-2-one exhibited the most widespread inhibition, with bacterial growth, pigment production, and glucose utilization inhibited.

Some Volatile Algal Metabolites

β-cyclocitral

β-cyclocitral is the common name for the nor-carotenoid 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde. The odor of this compound is typically described as either "tobacco-like" (Jüttner 1976) or "minty-fruity-green" (Aldrich 1994). This compound, like the nor-carotenoids previously discussed, may be a degradation product of carotenes and has been observed in *Microcystis wesenbergii*, *M. viridis*, and *M. aeruginosa* (Jüttner 1976; Jüttner 1983; Jüttner 1984a; Jüttner 1987).

When cleaved, β-carotene and zeaxanthin form β-cyclocitral and hydroxy-β-cyclocitral, respectively (Jüttner 1987). The enzyme that cleaves the carotenes may be a form of β-carotene oxygenase. *Microcystis* can produce large amounts of β-cyclocitral.

A culture of *M. aeruginosa* contained 1.1 μg β-cyclocitral per gram cells, dry weight (Jüttner, 1976) and a surface water contained up to 14,000 ng β-cyclocitral per liter of sample (Jüttner 1984a).

2-Methylisoborneol (MIB)

The compound MIB is a methylated monoterpene alcohol that is produced by both actinomycetes and blue-green algae. Gerber (1969) was the first researcher to isolate MIB from actinomycete cultures, and Tabachek and Yurkowski (1976) were the first to isolate MIB from a blue-green algae culture. Blue-green algae that are known to produce MIB include *Oscillatoria tenuis* and *Oscillatoria* sp. (Wu and Juttner 1988; Hayes and Burch 1989) and *Phormidium* sp. (Izaguirre 1992). The compound has a musty odor that is detectable in aqueous samples at low ng/L levels (Krasner et al. 1983).

Geosmin

Geosmin is the common name for (E)-1,10-dimethyl-9-decalol. As previously mentioned, this compound is produced by both actinomycetes and a variety of blue-green algae. Gerber and Lechavalier (1965) were the first researchers to isolate geosmin from actinomycetes, and Safferman et al. (1967) and Medsker et al. (1968) were among the first researchers to isolate geosmin from blue-green algae. Blue-green algae known to produce geosmin include *Anabaena flos-aquae* (Hayes and Burch 1989), *Anabaena macrospora* (Miwa and Morizane 1988), *Anabaena circinalis* (Rosen et al. 1992), *Aphanizomenon gracile* (Jüttner 1984b), *Oscillatoria bornetii* (Utkilen and Frøshaug 1992), *Oscillatoria brevis* (Naes et al. 1985; Naes et al. 1988, Naes and Post 1988; Utkilen and Frøshaug 1992), and *Oscillatoria tenuis* (Wu and Jüttner 1988). Geosmin has an earthy odor that is detectable in aqueous samples at low ng/L concentrations (Krasner et al. 1983).

In algae, geosmin has been considered to be both an overflow product of the isoprenoid pathway (Naes et al. 1988) and a byproduct (Utkilen and Frøshaug 1992). Support for the byproduct hypothesis comes from the response of chlorophyll and carotenoid production paralleling geosmin production. Both geosmin and carotenoid concentrations in *Oscillatoria bornetii* were greater at 10 $\mu Em^{-2}s^{-1}$ than at 25 $\mu Em^{-2}s^{-1}$, indicating the influence of light intensity. Similar experiments were performed with *Oscillatoria brevis* at 20 °C (Naes et al. 1985). As light intensity increased,

concentrations of both geosmin and chlorophyll-a decreased. Other experiments were less definitive in that "neither during light or nutrient limited growth nor during transient states can the production of geosmin be enhanced to a marked extent. Even short term studies using specific inhibitors of intermediate synthesis in the isoprenoid pathway could not invoke an excessive production of geosmin" (Naes and Post 1988).

Distribution of MIB and Geosmin

Wu and Jüttner (1988) studied the intra- and extracellular distributions of both MIB and geosmin produced by a strain of *Oscillatoria tenuis*. The thylakoid membranes, cytoplasmic membranes, and soluble proteins contained tightly bound geosmin; whereas MIB was located in a colorless protein fraction. Rosen et al. (1992) studied the relationship between geosmin release and algal population growth phase. The authors determined that once cells reached stationary phase, the concentration of geosmin in the media increased rapidly.

ORGANIC COMPOUND EXTRACTION AND IDENTIFICATION

The literature contains a variety of methods for extracting volatile and semi-volatile compounds from aqueous samples; including steam distillation, batch liquid-liquid extraction (LLE), continuous liquid-liquid extraction (CLLE), closed-loop stripping (CLSA) and the open-loop stripping (OLSA) variant, and simultaneous distillation extraction (SDE). Closed-loop stripping has been utilized since the mid-1970's to concentrate strippable organic compounds. The original method of Grob and Zurcher (1976) underwent modifications that made the method more applicable to the analysis of drinking water. The addition of sodium sulfate was a modification that resulted in both increased extraction efficiency and reduced stripping times (Hwang et al. 1984; Means and McGuire 1986; Krasner et al. 1989). Sävenhed et al. (1983) modified the closed-loop system to the open-loop system by replacing the recirculating pump with a nitrogen gas cylinder, followed by an activated carbon filter that removed gas impurities. Higher stripping temperatures were used with the open system.

Liquid-liquid extraction can be used in conjunction with CLSA to extract semi-volatile odor compounds, which are not extracted well by CLSA. The LLE and SDE methods supplement the CLSA method, because they extract compounds of higher

molecular weight and relatively higher polarity than those extracted through CLSA alone (Slater and Blok 1983b; Anselme et al. 1988; Krasner et al. 1989). Potential problems associated with LLE include the foaming of cultures and emulsion formation. Drawbacks to CLSA include the possibility of foaming, especially with algal cultures, and contamination of the recirulating pump with oils and low molecular weight compounds.

Surface water analyses typically require the extraction of 1-1.5 L of water. When algal cultures are studied, the high concentration of organic compounds in the cultures permits the extraction of smaller aliquots (e.g., Krasner et al. (1983) extracted 4-25 mL aliquots by the CLSA method).

Samples obtained by the various extraction methods are typically analyzed by gas chromatography-flame ionization detection (GC-FID) or GC-MS (Sävenhed et al. 1983; Krasner et al. 1983; Anselme 1988). Sensory evaluation (SE) is another technique that may be used. Two versions of SE are described as follows: (1) the post-extraction water samples are evaluated to determine extraction completeness (Sävenhed et al. 1983), and (2) the exit port of the GC is sniffed to determine the odors as they elute (Jüttner 1976; Preti et al. 1993). The latter method; which is referred to as sniff-, smell-, or sensory-chromatography, is useful in that the odors within a sample may be directly matched with the responsible compounds (Khiari et al. 1992; Preti et al. 1993).

ODOR EVALUATION

Odor Quality

The mechanisms by which an odorous substance evokes a sensory response in humans are still largely unknown; however, several theories have been developed (Jurs et al. 1981; Wright 1982; Anholt 1992). There are instances where very dissimilar compounds produce similar odors, as well as where stereoisomers of the same compound produce different odors (Punter et al. 1981; Engen 1982). Methods of determining perceived odor quality, or classification, have evolved with time. One classification method involves combining smell sensations into specific groups such as spicy, fragrant, fruity, resinous, ethereal, and putrid (Harper et al. 1968; Wright 1982; Engen 1982).

Problems arise when two compounds are assigned similar odor classifications. For example, trying to describe apples and pears, without using their names, may result in the description of fruity-pleasant for both. Further verbal discrimination is often necessary.

Methods have been developed that utilize verbal descriptors that compare the odor in question to common odor names; however, these methods are arbitrary and descriptors will differ among localities because of familiarity with local descriptors (Harper et al. 1968; Wright 1982; Amoore 1986). References are available that describe the compounds, their odor characteristics, and general usage or availability; two examples are Arctander (1969) and Aldrich (1994).

Several studies have been directed toward the determination of both odors and groups of odorous compounds based upon structural configuration (Harper et al. 1968; Dravnieks et al. 1981; Engen 1982). Researchers have studied homologous series of compounds to understand better the influence of chain-length on odor perception and, ideally, to gain information on the mechanisms of olfactory response. Punter et al. (1981) stated that "the efficacy of the n-alcohols and n-fatty acids is a linear function of chain-length: to obtain the same effect, a lower concentration is needed as the chain-length increases."

Odor Mixtures

Mixtures of odorous compounds complicate further the analysis and characterization of individual compounds. Engen (1982) established that "the perceived strength of a mixture of odors is generally less than that predicted from the sum of the individual intensities of the components." Masking is an extension of Engen's concept; whereby the perception of one odor is totally blocked by the presence of another, more readily perceived, compound. Additionally, two compounds can be at sub-odor-threshold concentrations when alone, but, when they are combined together, their odor can be readily perceived. Several publications present detailed discussions on the various aspects of odor perception, classification, and interaction (Harper et al. 1968; Moskowitz 1981; Engen 1982; Wright 1982).

Odor Intensity

Several methods for relating odor intensity to the concentrations of compounds are available; the better known ones are the Weber-Fechner (W-F) Law and the Stevens Ratio Estimation Method. Additional information on the Stevens method is available in Stevens

(1970). Water industry researchers often apply the W-F Law to odorant intensities of compound standards. The W-F Law may be written as:

$$S = aLogC + b$$

where "S" is the odor intensity, "C" is the concentration, and "a" and "b" are the constants for slope and y-axis intercept, respectively. McC. Gamble (1898) performed one of the first studies that applied the W-F Law.

In general, humans can discriminate well between odors but are less precise in their ability to estimate odor intensity. Often, the perceived odor intensity of a compound will vary greatly within a group of individuals. In extreme cases, an individual may be scent-blind to a compound and not perceive its odor at all. This phenomenon is called "anosmia." Further discussion of anosmia is widely available (Harper et al. 1968; Engen 1982; Wright 1982; Amoore 1986; Smith and Duncan 1992).

The variability among individuals in ability to perceive odors may prove frustrating in evaluating the data from scientific studies, because "it has been fashionable in scientific circles to pursue quantitative precision regardless of whether the thing being measured is in fact precisely measurable"(Wright 1982). In spite of the subjective nature of the intensity measurement, the averages of the responses, when plotted, depict fairly well the straight-line relation defined by the W-F Law (Wright 1982).

The plateau that is sometimes observed at both ends of the W-F plot may be explained by relating the perceived odor intensity to the fraction of nasal receptors sending a signal to the central nervous system (Wright 1982). In this case, the intensity "S" is substituted in the W-F Law by "Log^{-1} r", where "r" is the fraction of receptors sending impulses to the brain. The resultant plot indicates that even sub-threshold odors may contribute to the overall odor perception, and, conversely, a maximum may be reached where further increases in concentration no longer result in a perceived increase in odor intensity.

Water Industry Methods of Sensory Analysis

United States' water utilities routinely employ the threshold odor number (TON) method to evaluate water samples (APHA, AWWA, and WPCF 1992). This method determines the degree of dilution necessary to produce a water sample of barely

perceptible odor. Drawbacks of the method include: (1) using an open-ended scale where the TON assigned may be greatly influenced by an individual's personal sensitivity to the odor (Mallevialle and Suffet 1987), (2) the odor is not broken down into individual components (Mallevialle and Suffet 1987), and (3) the dilution process may change the character of the odor (Wright 1982; Engen 1982; Mallevialle and Suffet 1987).

A more recent addition to the water industry's roster of odor-evaluation techniques is the flavor profile analysis (FPA) method. Although it was initially developed for use by the food industry (Cairncross and Sjöström 1950), the method was later adapted for use by water utilities (Krasner et al. 1985). The method has been standardized, and instructions for both training and usage are available (APHA, AWWA, and WPCF 1992; AWWA 1993). Meng and Suffet (1992) analyzed water sample FPA data and suggested some quality assurance-quality control procedures.

The FPA method requires a panel of at least four members, who undergo substantial training to familiarize them with the odor intensity scale and the commonly used odor descriptors. An advantage of the FPA method is that it is sometimes possible for panelists to separate the sample odor into individual odors and their relative intensities. It also is theoretically possible to estimate the concentrations of odorants present in a sample by relating the odor intensities to the W-F plot obtained from FPA evaluation of compound standards; however, interferences (e.g., masking, antagonism, or synergism) among compounds may make a direct comparison of FPA odor intensities to odorant concentration impractical.

ENVIRONMENTAL FACTORS AFFECTING ALGAL PRODUCTIVITY

Nitrogen and phosphorus compounds can stimulate the growth of large populations of planktonic algae. Sources of these compounds include human sewage, industrial wastes, agricultural runoff, and urban runoff. Efforts to control inputs of nitrogen and phosphorus include the operation of advanced wastewater treatment (AWT) facilities and best management practices (BMP) for point-source and nonpoint-source pollution control, respectively. Additionally, the present ban on phosphorus in synthetic laundry detergents in many areas of the United States is a direct result of efforts to reduce the phosphorus content of domestic wastewater. In the late 1960s and early 1970s, research indicated that the use of phosphate detergents caused domestic wastewaters to

contain from two to three times as much phosphorus as they otherwise would (Sawyer 1968; Lee et al. 1978).

The forms of nitrogen (N) commonly found in water supplies are organic-N, nitrite-N (NO_2-N), nitrate-N (NO_3-N), and ammonia-N (NH_3-N). Ammonia-N usually exists in equilibrium with ammonium ion (NH_4-N). There are several sources of these compounds. The forms and concentrations of nitrogen that occur in a wastewater treatment plant effluent depend upon the degree of treatment received at the facility. Surface runoff may also contribute all of the commonly observed forms of nitrogen. Some blue-green algae do not depend upon external sources of nitrogen, because they can use atmospheric nitrogen.

The forms of phosphorus (P) commonly found in water supplies are soluble orthophosphate (o-PO_4-P), particulate and precipitated phosphorus, and organic-P. These phosphorus compounds can occur in both wastewater effluents and surface runoff. Other sources of nitrogen and phosphorus are precipitation and dry fallout, both of which may contribute significantly to the nutrient loadings within a region (Novotny and Chesters 1981; Jones and Lee 1982). Several studies have shown that phosphorus loading has a direct relationship to phytoplankton productivity in freshwater lakes and impoundments (Dillon and Rigler 1974; Schindler 1978; Lee et al. 1978; Williams et al. 1978).

Ammonium ion, nitrate-N, and biologically available phosphorus are the nutrient forms that are most readily assimilated by phytoplankton (Lee et al. 1978; Sawyer 1968). Studies have demonstrated that algae have different nutrient preferences. For example, the green algae (*Chlorophyceae*) prefer NH_3-N to oxidized-N (Reynolds 1984; Harris 1986; Fogg and Thake 1987); whereas the diatoms (*Bacillariophyceae*) prefer NO_3-N to NH_3-N (Patrick 1977; Rashash 1991).

Water utility managers have, at times, relied on chemical methods of algal control. Copper sulfate is often mentioned as the compound used. The literature contains much information on the effectiveness of copper sulfate for taste-and-odor control, limitation of algal blooms, recommended means of application, and potential drawbacks (Monie 1956; Tuwiner 1976; McGuire et al. 1984; Hanson and Stefan 1984; Raman 1985; Raman and Cook 1988).

The amount of copper sulfate required to control algal blooms has been the subject of several research efforts. In general, the effectiveness of copper sulfate decreases with increasing pH, alkalinity, and concentrations of organic and inorganic matter in water

(Raman and Cook 1988; Hering and Morel 1988). The algae themselves may reduce the toxicity of copper sulfate through the release of copper-complexing agents (McKnight and Morel 1979).

As the preceding discussion demonstrates, algae, the compounds they produce, the odors associated with those compounds, methods of detection, and means of treatment and control are all areas of continuing interest to both water industry researchers and personnel. Once the algae have developed, it is necessary to have safe, efficient means of removing both them and their by-products from the water prior to distribution. Although the presence of algae in water is not regulated, both they and their metabolites may have impacts upon drinking water constituents that are regulated (e.g., trihalomethane formation). The potential for asthetic problems associated with tastes-and-odors in drinking water is considered in that a Secondary (i.e., unenforcable) Maximum Contaminant Level exists on threshold odor number. Tastes and odors imparted to the water by algal metabolites can cause consumer dissatisfaction that results in rejection of the public water supply in favor of an alternative, though not necessarily safe, drinking water source. As stated in the Introduction, the current information on algae contains many gaps in regard to the influence of environmental factors on both algal population growth and the production of odorous algal metabolites. This research is intended to fill some of those gaps.

CHAPTER 3
METHODS AND MATERIALS

ALGAL CULTURES

The algae included in this research investigation are depicted in Table 3.1. The formulations of the media in which the algae were grown are presented in Appendix A, Tables A.1-A.3. Each of the nine cultures was unialgal, but only one culture, *Chlamydomonas peterfii*, was axenic. Dr. B. Parker (Biology Dept., Virginia Tech) examined each of the blue-green algae cultures for actinomycetes microscopically and by growth on actinomycete agar plates (Difco Laboratories) incubated with parafilm seals at 30°C for five days. No actinomycetes were observed.

Near completion of the research project, the axenicity of the *C. peterfii* culture was re-checked using three plates each of SMA and R2A agar. The agar media were manufactured by BBL and Difco Laboratories, respectively, and reconstituted according to manufacturer instructions. The agar plates were streaked with aliquots from a mature, odorous culture of *C. peterfii* and incubated at 25°C for three weeks. No growth was observed on any of the plates.

Table 3.1
Algae used for AWWARF odor project

Alga	Source	Medium	Code
Anabaena laxa	G. Izaguirre*	ASM-1	
Microcystis aeruginosa	U. Texas, Austin†	ASM-1	UTEX LB2388
Oscillatoria sp.	G. Izaguirre	ASM-1	
Oscillatoria tenuis	R. Hoehn‡	ASM-1	
Phormidium sp.	G. Izaguirre	ASM-1	
Chlamydomonas peterfii	U. Texas, Austin	OCM + peptone	UTEX 2400
Dinobryon cylindricum	U. Texas, Austin	DY III	UTEX LB2265
Synura petersenii	U. Texas, Austin	DY III	UTEX LB2406

* G. Izaguirre, Metropolitan Water District of Southern California

† University of Texas Culture Collection at Austin

‡ R. Hoehn, The Charles E. Via, Jr. Dept of Civil Engr., VPI&SU

The *Oscillatoria* sp. contributed by Izaquirre did not survive in the 25°C incubator. Temperature was not believed to be the problem as the alga was originally isolated from a Mississippi catfish pond and maintained at 25°C by Izaguirre. The alga also failed to grow when incubated in either larger test tubes or Erlenmeyer flasks. The incubator lights may have been deficient in a light wavelength required by the alga, and attempts were made to correct the situation by supplementing the cool-white fluorescent lamps located near the *Oscillatoria* sp. culture racks in the incubator with 25-watt and 60-watt incandescent bulbs. The alga still did not grow in the incubator, but it could be successfully grown on a north-facing window sill at ambient temperature.

The *O. tenuis* was originally isolated by D. E. Henley (1970) and has been maintained at Virginia Tech since 1971. It was contaminated with a fungus, and therefore, was treated with a 0.05% cycloheximide solution. After three transfers to ASM-1 medium containing 0.05% cycloheximide, the alga was transferred to regular ASM-1 medium. The growth of the algal cultures that were subjected to cycloheximide became sluggish, but returned to normal vigor after transfer back into regular ASM-1 medium. This culture was maintained on a north-facing window sill along with the *Oscillatoria* sp.

The *Anabaena laxa* was originally identified only as *Anabaena* sp. when it was received from Izaguirre, who had maintained it in BG-11 medium. The culture developed akinetes when transferred to ASM-1 medium, and the presence of akinetes enabled Parker (Biology Dept., Virginia Tech) to identify the culture as *Anabaena laxa*. The identity was later confirmed by Dr. G. Dillard, Western Kentucky University.

CULTURE EXPERIMENTS

The culture experiments were carried out in two phases. The first phase involved growth of each culture under fairly optimal conditions. The samples collected during the first phase were evaluated by FPA and extracted for analysis of organic compounds that were either retained within the algal cells or were released into the medium. The second phase involved the growth of only three of the algae, *A. laxa*, *Phormidium* sp., and *S. petersenii*, under six additional growth regimens. The actual growth conditions employed during both phases of the research are presented in Table 3.2. The samples collected during the second phase were extracted for analysis of organic compounds, but no FPA evaluations were performed. Instead, the FPA panel evaluated several concentrations of standards made from purchased compounds that had been identified in the GC-MS analyses of algal extracts during the first phase of the project.

Table 3.2

Algal growth conditions and media nutrient concentrations at t_0

Alga	Scheme	Light ($\mu E/m^2/s$)	Nutrients* NO_3-N (mg/L)	NH_3-N (mg/L)	o-PO_4-P (mg/L)	Temp. (°C)
D. cylindricum	DY III	8	6.82	0.87	0.73†	20
C. peterfii	OCM+‡	36	142.91	-----	26.58	25
M. aeruginosa	ASM-1	33	32.40	-----	5.47	25
O. tenuis	ASM-1	30§	32.09	-----	8.41	25**
O. sp.	ASM-1	30§	32.84	-----	8.14	25**
P. sp.	ASM-1††	8	32.39	-----	5.50	25
"	mid-N	8	5.10	-----	5.35	25
"	low-N	8	2.51	-----	4.18	25
"	low-P	9	26.80	-----	0.42	25
"	high-light	28	34.23	-----	4.98	25
"	low-temp	8	30.92	-----	5.59	15
A. laxa	ASM-1††	32	36.10	-----	6.28	25
"	mid-N	32	5.36	-----	5.53	25
"	low-N	32	2.84	-----	4.20	25
"	low-P	36	26.42	-----	0.36	25
"	low-light	7	31.86	-----	5.24	25
"	low-temp	28	31.08	-----	5.77	15
S. petersenii	DY III††	8	4.19	1.37	1.16†	20
"	mid-N	8	1.53	0.45	0.84†	20
"	low-N	8	0.83	0.36	0.96†	20
"	low-P	8	3.83	0.90	0.55†	20
"	high-light	45	2.93	0.75	0.98†	20
"	low-temp	8	2.47	0.71	0.85†	10

* Nutrient values represent the average of three replicates

† Represents the acid hydrolyzable phosphorus

‡ OCM+ = OCM plus peptone

§ Average ambient light during daylight hours at north-facing window sill

** Average ambient room temperature

†† Variations in culture conditions were relative to these treatment conditions

Experimental Preparation

The inoculum for each experiment was prepared by aseptically transferring 20-40 mL of each experimental alga to a sterile Erlenmeyer flask that contained 40 mL of the appropriate medium. The volume to be transferred from the algal cultures was determined by the apparent culture densities. If growth in the initial culture tube (parent tube) was abundant, a smaller aliquot was transferred. If the alga formed either clumps or long filaments as it grew, it was blended (aseptically in a blender for ~20 s) as part of the dilution process. The blending produced a more homogeneous inoculum for transfer and enumeration. Approximately 0.3 mL of the suspension was then added by pipette to each of 126 30-mL, screw-top test tubes containing 15 mL of recently-made sterile medium. A 5-mL portion of each inoculum was reserved for cell-density determination (to be described). The tubes were placed in three, 40-tube racks that were labeled on one end A, B, and C, respectively. The racks of tubes were then placed in the appropriate temperature incubator, and light-intensity measurements were made with a Model Li-185B Quantum-Radiometer-Photometer (Licor, Inc.) light meter. If necessary, the racks were shaded to prevent excessive illumination. The incubator lights were on continuously.

The experimental algae were inspected, shaken, and the tube locations rotated within the racks each Monday, Wednesday, and Friday. Each tube rack was removed from the incubator, the caps tightened, and the rack covered by an aluminum pan. The entire rack was then inverted several times to mix the algae within the tubes. Light illumination effects resulting from tube location relative to the incubator lights were lessened by changing the positions of all the tubes within the rack (e.g., tubes located at the inside center of the racks were moved to the outside corners). The caps were loosened and each rack rotated 180° prior to being placed back in the incubator. The A, B, C labels on the racks facilitated the proper end-to-end rotation of the racks. As the racks were shifted within the incubator with respect to one another, they were arranged as ABC, BCA, CAB, ABC, etc.

Additional Methods

Dinobryon cylindricum

An additional evaluation of *D. cylindricum* cultures was performed. When the culture was treated in the previously described manner, few organic compounds were detected during GC-MS analyses even though the FPA panelists perceived fishy odors; therefore, a batch culture of the alga was grown, and larger volumes were sacrificed for compound extraction and GC-MS analysis.

A 1-L Erlenmeyer flask containing 500 mL sterile DY III medium was inoculated with a known volume of inoculum. A 100-mL portion of the culture was aseptically removed after 13, 23, and 33 days of incubation at 20°C, and a 5-mL portion of each 100-mL sample was prepared for cell-density determination (to be described). The remainder of each sample was extracted for GC-MS analysis of organic compounds.

Chlamydomonas peterfii

An additional evaluation of *C. peterfii* cultures was also conducted. Several odoriferous extracellular compounds (e.g., isovaleric acid, benzyl acetate, and phenethyl alcohol) were present in *C. peterfii* cultures that were grown in medium that contained peptone. The influence of peptone on the extracellular products that appeared in *C. peterfii* cultures was evaluated by growing the alga in OCM medium without peptone. The alga was cultured in tubes containing 15-mL portions of medium and handled in the manner previously described.

Enumeration

Each 5-mL portion of the inoculum that had been reserved at t_0 for enumeration was examined microscopically and the cell density determined. Motile algae cultures were preserved with a nondisruptive fixative (either FAA (formalin-acetic acid-ethyl alcohol) or acid Lugols). Algal enumeration was performed according to the Palmer-Maloney Counting Chamber Method, as described in Section 10200F.2b, *Standard Methods* (APHA, AWWA, and WPCF 1992). Although tedious and time consuming, the Palmer-Maloney direct cell count method was used instead of either particle counting or optical

density methods because it enabled both the differentiation between living cells and cell debris and the observation of either akinetes or heterocysts in the applicable cultures. The method also enabled the observer to check for extraneous algae or foreign matter. The individual cells of all algal morphologic types (i.e., filamentous, colonial, and unicellular) were counted. Seven people trained in algae-counting procedures performed the algae counts over a 1.3 year period.

Three chambers were filled with approximately 0.1 mL of the reserved inoculum and viewed at 200x magnification through light microscopes fitted with calibrated ocular micrometers that had ocular grids of known area. The algal cells in at least ten grid areas were then counted. More grid areas (up to 50) were counted if cell densities were low such that no algae were observed in several grid areas. The numbers of cells and filaments (or colonies) were recorded, and the cell density of each inoculum suspension was then calculated. The equation used was:

$$D = CA(NaV)^{-1} \tag{3.1}$$

where D = cell density, cells/mL
C = total number of algal cells counted, cells
A = area of Palmer-Maloney chamber, mm^2
N = number of ocular grid areas counted
a = area of ocular grid at 200x, mm^2
V = volume of Palmer-Maloney chamber, mL

The three cell densities were averaged and the algal cell concentration in the freshly inoculated tubes was then calculated from knowledge of the cell density in the inoculum, volume of inoculant, and total volume of medium in the freshly inoculated tubes. The equation used was:

$$D_o = DV_i (V_t + V_i)^{-1}$$

where D_o = cell density in experiment culture tubes at 0 days, cells/mL
D = cell density in inoculant, cells/mL
Vi = volume of inoculant placed in experiment culture tube, mL
Vt = volume of medium in experiment culture tube, mL

SELECTION OF TUBES FOR ANALYSIS

The cultures were sampled several times during their population growth curves; these times were approximately t_1 = day 3, t_2 = day 7, t_3 = day 12, t_4 = day 19, t_5 = day 28, and t_6 = day 40. The tubes were selected by the following procedure:

- The area of each of the three racks was bisected along its major and minor axes to form four quadrants, for a total of 12 quadrants. The quadrants were labeled A1, A2, A3, A4, B5, B6,...C12, respectively.
- Each quadrant contained 10 possible culture-tube positions; identified as A1-1, A1-2, A1-3, A1-4, A1-5,...A1-10, A2-1, A2-2, A2-3,...A2-10,...C12-10.
- On each sampling date, a random-number program was used to generate two lists; one for quadrants and one for tubes, with the lists containing the number ranges 1-12 and 1-10, respectively.
- Tubes were withdrawn from the racks by pairing the random quadrant-tube numbers. If a position were empty, the next quadrant-tube pair was used.
- A total of 18 tubes was selected; nine tubes for applicable nutrient analyses and enumeration of culture density, the other nine tubes for FPA and organics extraction. (The nutrient analyses were conducted by M. Beaty, Biology Dept., Virginia Tech.)

The nine culture tubes selected for nutrient analyses and enumeration were grouped by threes. The culture tubes in each grouping were combined into a larger, sterile tube to produce three composite samples and then blended for about 20 s to improve homogeneity. A 5-mL aliquot from each combined, blended sample was placed into another sterile tube for enumeration. The samples were preserved either to prevent algal motility or if the samples could not be counted promptly. The remainder of each sample was used for applicable nutrient analyses.

Each of the three algal composites was counted three times by the Palmer-Maloney Chamber method. The three cell densities calculated for each sample were averaged (within tube average). The average and standard deviation of the cell densities among the three samples were then calculated (overall average and the associated standard error, respectively) and plotted to depict the algal population growth curves.

NUTRIENT ANALYSES

All of the methods described below were from *Standard Methods* (APHA, AWWA, and WPCF 1992).

Nitrate Nitrogen (NO_3-N)

Nitrate nitrogen concentrations were determined by a modified version of *Standard Methods* Section 4500-NO_3^- E. Cadmium Reduction Method that utilized Hach (Hach Chemical Company, Loveland, CO) powder pillows.

Ammonia Nitrogen (NH_3-N)

Ammonia nitrogen concentrations were determined by the method described in *Standard Methods* Section 4500-NH_3 D. Phenate Method.

Orthophosphate Phosphorus (o-PO_4-P)

Orthophosphate-phosphorus concentrations were determined by the method described in *Standard Methods* Section 4500-P E. Ascorbic Acid Method.

Acid Hydrolyzable Phosphorus (PO_4-P)

Acid hydrolyzable phosphorus concentrations were determined by the method described in *Standard Methods* Section 4500-P B. Sample Preparation.

Algal Culturing and Nutrient Analyses Equipment

The algae were grown in four incubators: (1) Percival model 135-LVL (at 20°C), (2) Percival model 1-60LLVL (at 25°C), (3) Sherer model 4-4 (at 20°C), and (4) Sherer model R1-25 (15°C). A Perkin Elmer model 552 dual beam spectrophotometer with a 5 cm pathlength was utilized for all nutrient analyses that required spectrophotometric measurements.

SAMPLE PREPARATION FOR ORGANICS EXTRACTION AND FPA EVALUATIONS

On sample-collection days, the nine culture tubes selected for organic compound extraction and FPA analyses were grouped by threes and each group transferred into a clean (i.e., distilled water washed, acetone rinsed, and oven dried) glass, 50-mL centrifuge tube (Pyrex #8424) to produce a composite sample. The composite sample tubes were stoppered and then shaken to thoroughly mix the algae. A 15-mL aliquot from each of the three large tubes was then placed into a clean 25-mL centrifuge tube (Corex #8446).

If the algal culture cell-density permitted the samples to be readily filtered, the three 15-mL samples were filtered through polycarbonate filters (25 mm x 0.8 μm, Poretics #11050). Each medium filtrate was collected in a clean Erlenmeyer flask and then placed into a clean 30-mL test tube. Each sample filter was placed in a tube containing 10 mL of sterile media of the same type used to culture the alga, and the tubes were vigorously shaken to dislodge the algal cells from the filters.

If the algal cultures were so dense that they could not be filtered, which was generally the situation, the three 15-mL sample portions were centrifuged at 8,000 rpm for one hour. A rubber sleeve insert (Corning) was placed in the centrifuge vanes to prevent tube breakage. After centrifugation, the supernatant (growth medium) was removed by pipette and placed into clean 30-mL test tubes. The contents of a sterile tube, containing 10 mL of media of the same type used to culture the alga, was used to resuspend the algal pellet remaining in each centrifuge tube. The resuspended cells and fresh media were then placed back into the sterile media tubes.

Nine samples were prepared on each algae-sampling date (Table 3.3). The samples were placed in a 4°C refrigerator to await the isolation of organic compounds by continuous liquid-liquid extraction and Kuderna-Danish evaporation (CLLE-KD). The algal cell duplicate, medium blank, and incubator blank served as quality assurance-quality control (QA-QC) samples.

Table 3.3

Description of organic extraction samples collected on each sampling-date

No. of samples	Sample description
3	Algal cell samples - filtered or centrifuged to separate the cells from a 15-mL portion of the growth medium and then resuspended in 10 mL of fresh sterile medium
3	15-mL portion of growth medium filtrate
1	Algal cell duplicate sample - a duplicate of one of the algal cell samples
1	Media blanks - media stored in 4°C refrigerator since sterilization
1	Incubator blanks - blank sterile media tubes placed in incubator with the experimental cultures to check for possible contamination of samples by volatile compounds

The samples generally were extracted within four days after collection, and no preservative was added to the samples. Mercuric chloride was mentioned in the literature as a sample preservative (Krasner et al. 1983); however, an organomercury complex formed that obscured a large region of the GC-MS scan. The complex eluted later than either MIB or geosmin, so it may not be a problem for researchers primarily interested in those two compounds. Several of the compounds of interest in this research did elute within the region obscurred by the organomercury complex; therefore, mercuric chloride was not added to the samples. If samples could not be extracted within four days, they were frozen. Freezing was not considered to be a good option, however, because of the potential for breakage of the glass tubes, so every effort was made to extract all samples within the four-day limit.

ORGANIC COMPOUND EXTRACTION

Prior to extraction, a 30-μL portion of an internal standard solution (Table 3.4) was added to each sample. The source and purity of the compounds shown in Table 3.4, as well as the others used during the course of this research, are presented in Appendix B, Table B.1. A 30-μL portion of a spike solution (Table 3.5) also was added to the duplicate algal-cell sample.

Table 3.4

Internal standard solution

Compound	Concentration ng/μL
n-chlorooctane	29.84
n-chlorodecane	28.55
n-chlorododecane	29.88
n-chlorohexadecane	31.66
n-chlorooctadecane	30.83

Table 3.5

Spike solution

Compound	Concentration ng/μL
MIB-d3	4.8
geosmin-d_3	5.1
palmitic acid	83.8

After the internal standards and spike solutions were added to their respective samples, the algal organic compounds were isolated by 16-hr continuous liquid-liquid extraction (CLLE) of the prepared 10-15 mL samples in 45 mL methylene chloride (Optima grade); followed by Kuderna-Danish evaporation of the methylene chloride fraction to a volume of approximately 1 mL. During the 16-hr extraction, the condenser portion of the CLLE apparatus was cooled by a recirculating chiller (Lauda k-2/RD, manufactured by Brinkmann Instruments) set at 14°C. The concentrated samples were placed in 1.8-mL glass vials (Supelco) that had Teflon-lined caps. Once in place, the caps were wrapped with Teflon tape and the vials placed in a 4°C refrigerator. Immediately prior to GC-MS analysis, each sample was further concentrated to a final volume of approximately 0.07 mL by evaporating the methylene chloride with a light stream of nitrogen gas (0.2 total hydrocarbon content grade).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

A Hewlett-Packard 5890 series II GC coupled to a Hewlett-Packard 5970 mass selective detector was used for analysis of the methylene chloride extracts of the experimental culture samples. Mass spectra were compared to the Wiley and NBS75K mass spectral libraries. The following protocol was followed each day that samples were analyzed by GC-MS:

- Execute the "clean-up" program to remove anything that may have accumulated in the capillary column or other parts of the system since the instrument was last used. The program entailed a temperature ramp from 40 to 280°C at 12°C/min. The injection port temperature, detector temperature, and column linear velocity were the same as for algal sample runs (Table 3.6).
- Calibrate the GC-MS system by running the autotune program supplied by Hewlett-Packard. The reference standard used to calibrate ion abundance was perfluorotributylamine (PFTBA).
- Analyze a check standard solution. There were four different check standard solutions analyzed during the investigation. The composition of each of these standards is presented in Appendix C, Table C.1. The results from the analyses of the standards were used to evaluate the status of both the instrument and the capillary column, and also to obtain the response factors of the various compounds so that the concentration of compounds within the algal samples could be determined. The GC-MS operating conditions were the same as those used for the analyses of algal samples (Table 3.6).
- Analyze experimental samples. The instrument operating conditions are presented in Table 3.6.

Table 3.6

Gas chromatography-mass spectrometry instrument settings

Instrument parameter	Setting
Helium linear velocity	31±0.5 cm/s
Injection port temperature	220°C
Transfer line	280°C
Column temperature program	2 min at 35°C; 35-260°C at 5°C/min; 13 min at 260°C
Purge off time	3 min
Ion scan range	45 to 400 amu
Scan threshold (i.e., lower abundance limit)	1,000
Source pressure	Approx. $4x10^{-5}$ torr
Sample size	Approx. 3.0 µL; splitless injection
Column	30-m x 0.25-mm i.d. x 0.25 µm film DB-5 and DB-5ms fused silica capillary column*

*J & W Scientific, Folsom, CA

During the first few months of the investigation, the method described by Eichelberger et al. (1975), which used decafluorotriphenylphosphine (DFTPP) as the standard, also was used to calibrate ion abundance in the GC-MS system. When the GC-MS system was tuned by PFTBA, the ion-abundance range requirements for DFTPP were satisfied; therefore, the use of two ion-abundance calibration standards was considered to be redundant, and the use of DFTPP was discontinued..

Response Factors

Response factors (RWR) were determined for the compounds presented in Appendix C, Table C.1. The concentrations of and peak areas associated with the two internal standards that eluted nearest the compound in question were used in calculating the RWR. The equation used was:

$$RWR = (A_c/C_c)\,(C_{STD}/A_{STD}) \qquad (3.2)$$

where RWR = response factor or "relative weight response"

A_c = integrated area of compound peak

C_c = known concentration of compound in the standard solution, ng/μL

C_{STD} = $C_{std1} + C_{std2}$ = sum of the known concentrations of the two internal standards eluting nearest to the compound, ng/μL

A_{STD} = $A_{std1} + A_{std2}$ = sum of the integrated areas of the two internal standards eluting nearest to the compound

Five fused-silica capillary columns were used for this investigation; three were DB-5 and two were DB-5ms, each as described in Table 3.6. Some response differences were noted among the five columns, so individual response factors were calculated. The appropriate column's response factors were used to calculate the concentrations of compounds in the experimental samples. Not all of the compounds that were identified by the GC-MS spectral libraries could be acquired; therefore the RWR of compounds that were not obtained were assumed to be the same as the RWR of structurally similar compounds that had been obtained and confirmed (i.e., the RWR for β-cyclocitral was used as the RWR for hydroxy-β-cyclocitral; the RWR for 2t,6c-nonadienal was used as the RWR for 2t,4c,7c-decatrienal).

Quantification of Algal Sample Compounds

The quantities of the various compounds located both within the cells and in the growth medium were calculated by the following equation:

$$C_u = [(A_u)(V_{is})(C_{is})] \div [(A_{STD})(RWR)(M)(KD_f)] \quad (3.3)$$

where C_u = concentration of compound present in either algal cells or cell-free media, ng/mL

A_u = integrated area of compound peak

V_{is} = volume of internal standard solution added to the sample prior to extraction, μL

C_{is} = $C_{std1} + C_{std2}$ = sum of the known concentrations of the two internal standards eluting nearest the compound, ng/μL

A_{STD} = $A_{std1} + A_{std2}$ = sum of the integrated areas of the two internal standards eluting nearest the compound

RWR = relative weight response for the compound

M = volume of sample that was extracted by CLLE-KD, mL

KD_f = Kuderna-Danish correction factor to account for different loss rates from the evaporation apparatus.

The procedure that was used to determine the KD_f is described in the next section.

DETERMINATION OF CLLE-KD EXTRACTION EFFICIENCY

The 24 compounds that were evaluated are presented in Appendix C, Table C.2. A 30-μL aliquot of the internal standard solution was added to each of the sterile, 15-mL samples of ASM-1 media along with the appropriate volume of recovery solution. The samples were extracted and concentrated as previously described for the algal samples.

Effects of Concentration on Compound Recovery (Scheme I)

This experiment is designated "Scheme I" in Appendix C, Table C.2. The objective was to determine the effect, if any, of concentration on compound recovery. Specifically, 2.5, 5, 10, 15, 20, 25, 30, 40, 50, and 60-μL portions of recovery solutions #1 and #2 (Appendix C, Table C.2) were extracted for 16 hours, for a total of 20 samples. The concentrations obtained after CLLE-KD isolation and GC-MS analyses were compared to the concentrations of compounds originally added to the sterile ASM-1 media; the regression lines were then plotted.

The Kuderna-Danish correction factors (KD_f) were obtained from the slopes of the regression lines. For example, if the recovered amount of a compound for each extraction concentration was calculated to be twice the amount originally added, then both the regression-line slope and the KD_f equaled 2. When the slopes of the regression lines were within the range of 0.73 to 1.17 (e.g., from 73 to 117 percent recovery), no correction was made. The detection limits for each of the 24 compounds were obtained by application of the method described by Sharaf et al. (1986), which incorporated the lower limit of the confidence interval about the regression lines from the recovery data.

Effects of Extraction Time on Compound Recovery (Scheme II)

This experiment is designated "Scheme II" in Appendix C, Table C.2. The objective was to determine the effect of extraction time on compound recovery. The samples were extracted for 4, 8, 12, 16, 20, and 24 hours, instead of the usual 16 hours. Each 15-mL sample of sterile ASM-1 medium was spiked with 30 μL of either recovery solution #1 or #2. The treatment scheme was performed twice, with two samples at each of the six extraction times for both recovery solutions. The influence of extraction time on compound recovery was observed by plotting the concentrations obtained after CLLE-KD isolation and GC-MS analyses against extraction time.

FLAVOR PROFILE ANALYSIS (FPA)

The preliminary screening of a group of graduate student volunteers from the Charles E. Via, Jr. Department of Civil Engineering at VPI&SU took place during December, 1991. The students' abilities to discriminate odors of reference compounds were evaluated. Additionally, the "triangle-test" was conducted to determine if the students could determine which sample in a set of three smelled differently than the other two. This testing was conducted by Webster (1992) in preparation for her research with odor oxidants, and followed the methods detailed in Section 2170B of *Standard Methods* (APHA, AWWA, and WPCF 1992) and AWWA (1993). Students selected for further FPA training were those who were suitable for odor-evaluations and who would be at VPI&SU long enough to serve continuously on the panel.

The FPA panel was trained February 3-5, 1992, by G. Burlingame of Philadelphia Water Department. Afterwards, from five to seven members of the seven member FPA panel convened weekly to evaluate samples prepared from either algal cultures or odor standards. Odor standards were presented only at concentrations well below the Material Safety Data Sheet exposure warning levels to avoid potential health hazards.

Algal Sample FPA

The 500-mL stoppered flasks used for FPA were cleaned by rinsing with distilled water and oven drying. The clean FPA flasks were rinsed three times with Milli-Q reagent water, filled with 200 mL of Milli-Q water, and placed in a 45°C water bath with their

stoppers ajar. The flasks were allowed to "steam" for approximately one hour. A volume of the water was then removed and replaced with an equal volume of algal sample. The volumes of algal samples used ranged from 1 to 100 mL.

FPA of Compound Standards

The glassware was prepared as previously described. After the 200 mL of Milli-Q water had steamed for one hour, 1 to 15 μL of the compound stock solutions (Appendix C, Table C.2), which were dissolved in acetone, were added to the FPA flasks. No more than 15 μL of the compound solutions was added, as it was determined that larger volumes of acetone interfered with the FPA evaluations.

FPA Sessions

A typical FPA session involved the evaluation of from five to seven samples, which were maintained at 45°C in a water bath. At least one of the samples was a blank consisting only of Milli-Q water. A flask containing steamed Milli-Q water was present also and labeled as "odor-free" (OF). Prior to a session, panelists were asked if any were on medication, had a cold, were currently troubled by allergies, or had any other problem that may influence their ability to discern odors.

The FPA sessions were held in a room that was as odor-free as possible. Prior to the session, a list of odor descriptors was written on the blackboard; however, panelists were told that they could use additional descriptors, as necessary. The panelists evaluated the samples and recorded the descriptor and intensity of each. If desired, they sniffed the OF bottle between samples. Once the evaluations were complete, the descriptors and intensity results determined by each panelist were written on the blackboard.

Discussion ensued about the descriptors used, particularly for the compound standards. It was sometimes necessary to develop a group of descriptors (e.g., earthy, corn, and musty for geosmin) for a particular odor. An intensity rating of zero was assigned to a panelist's response if he did not include a member of the descriptor group used by the rest of the panelists for that compound. The averages of the odor intensity for each sample were then calculated for each descriptor group. If the panelists could not reach agreement on the descriptors, even so far as to group, the average intensity rating was recorded with the descriptor "no consensus." Typically, this occurred only when unfamiliar standards were presented to the panelists at near-threshold concentrations.

QUALITY ASSURANCE-QUALITY CONTROL (QA-QC) PROTOCOLS

Algal Cultures

The cultures were evaluated for the presence of actinomycetes, and, in the case of the axenic *Chlamydomonas peterfii*, for continued axenicity. The algal cultures were maintained by transferring from 0.2 to 0.5 mL aliquots of each culture to three fresh, sterile media tubes once every 30 days. The old cultures were kept until the next transfer date. In this way, backup cultures were available, if needed. The cultures were checked every Monday, Wednesday, and Friday and the incubator temperatures recorded.

Algal Culture Extractions

Nine experimental culture tubes were selected for analysis on each sample date. The tubes were grouped into sets of three, to create three composite samples (see section "Sample preparation for organics extraction and FPA analyses"). One of the algal cell samples was evaluated twice, with one sample spiked with low concentrations of MIB-d_3, geosmin-d_3, and palmitic acid. Media blanks and incubator blanks were collected also on each sample date (Table 3.3).

GC-MS

The complete description of the protocol that was followed each day that samples were analyzed was presented in the section entitled "Gas chromatography-mass spectrometry;" a condensed version is as follows:

- Execute clean-up program
- Calibrate GC-MS with PFTBA
- Analyze a check standard solution
- Analyze experimental extracts

Flavor Profile Analysis

A blank consisting only of Milli-Q water was presented to the FPA panelists, along with the other samples, for evaluation.

CHAPTER 4
RESULTS AND DISCUSSION

CHAPTER OVERVIEW

A brief summary of the QA-QC results is presented for both the algal extractions and the FPA evaluations. The ability of the CLLE-KD method to extract and concentrate twenty-four selected compounds (of the 63 compounds that were detected during the investigation) was evaluated, because the results of the investigation were based upon the reliability of the method for collecting the organic compounds from both the algal cells and the cell-free media. The selected compounds represented a variety of molecular weights and compound types (e.g., alkanes, alkenes, aldehydes, alcohols, carbon rings, and acids). Several of the compounds were: (1) known to cause odor problems in surface waters (e.g., geosmin, MIB, and 2t,6c-nonadienal), (2) described in fragrance literature as having odors (e.g., phenethyl alcohol, benzyl acetate, and isovaleric acid), or (3) ascertained to have an odor when the compound standard was obtained (linolenic acid). These compounds also were selected for evaluation by FPA.

The GC-MS and FPA results associated with each alga are discussed, including the comparisons between the FPA evaluations of the algal cultures and those of the compound standards. The average amounts (ng/10,000 cells) of the compounds that were detected frequently in either the algal cells or the cell-free medium are presented, while compounds that were detected only sporadically are just listed. The possible significance of both the frequently and sporadically detected compounds is discussed in a later section. The results from the statistical analyses of the data obtained from the environmental-variation experiments conducted with three of the algae are discussed in detail and include the discussion of possible methods for lessening the potential for odor problems associated with the algae.

QUALITY ASSURANCE-QUALITY CONTROL

As stated in the previous chapter, there were several QA-QC protocols observed during this investigation. A brief summary of the QA-QC results from the algal culture extractions and the FPA evaluations is presented here.

Algal Culture Extractions

The samples that were collected to be extracted were described in Table 3.3. Odor compounds were not observed in any of the media blanks that were kept in either the refrigerator or the incubators; therefore, it is highly unlikely that cross-contamination of odor compounds occurred in the algal samples while they were in the incubators. Low concentrations (e.g., less than 0.5 ng/mL) of the 17- through 21-carbon n-alkanes were detected in a few of the incubator blanks and in even fewer of the refrigerator blanks. Several organic compounds were observed in virtually all of the extracts and were regarded either as present in the media or as method artifacts; examples included toluene, cyclohexanone, phenol, and acetophenone.

The MIB-d_3 and geosmin-d_3 were detected in all of the spiked algal-cell samples. The palmitic acid spikes were detected in nearly all of the samples; the exceptions being some of the samples analyzed with one of the DB-5 columns. That particular column likely was slightly basic (personal communication, J & W Scientific representative), and the peaks associated with the fatty acids were wider than they were when analyzed with the other columns; therefore accurate quantification of the fatty acids was more difficult.

Each of the five capillary columns columns that was used for this investigation analyzed approximately 400 samples. The source, electron multiplier, and injection port of the GC-MS were properly maintained during the course of the investigation.

FPA Evaluations

The same group of people were FPA panelists during this investigation, and the sessions were performed consistently. The blank samples were usually described by the FPA panelists as "odor-free", with "warm glass" and "chalky" at FPA odor intensities of two or less being reported occasionally.

DETERMINATION OF CLLE-KD EXTRACTION EFFICIENCY

The CLLE-KD method was suitable for the extraction of a wide variety of organic compounds from algal cultures. Recoveries of the compounds were linear over the ranges of concentrations evaluated and, generally, were independent of extraction time. The fatty acids and chlorophene (o-benzyl-p-chlorophenol) were exceptions, because the extraction

results were highly variable when the samples were extracted for only four hours. The recoveries of the phenolic compounds and fatty acids were greater than those of the other compounds, and the recovery of isovaleric acid was low (i.e., approximately 10 percent).

Effects of Concentration on Compound Recovery (Scheme I)

The recoveries of the selected compounds were determined relative to n-chloroalkanes that were added as internal standards to the ASM-1 media. The experimental conditions are presented as "Scheme I" in Appendix C, Table C.2. The recovered concentrations of the compound standards were plotted against the added concentrations. Regression lines were drawn and the results are presented in Appendix D, Table D.1. The recoveries of most of the compounds were between 73 to 117 percent. Isovaleric acid is a short chain acid and was apparently lost during the KD concentration at a faster rate than the n-chloroalkanes that were used as internal standards, which resulted in only approximately 14 percent of the isovaleric acid being recovered. Conversely, the fatty acids (e.g., linolenic, palmitic, and myristic) were selectively concentrated compared to the internal standards, resulting in recoveries in excess of 100 percent and an apparent increase in the compound concentrations compared to those originally added. Recoveries of the alcohols and phenolic compounds were between 100 to 180 percent, with the recovery variation likely a result of differences in molecular weight and functional groups.

The overall linearity of the data for the ten concentrations of each compound, as described by the R-squared values presented in Appendix D, Table D.1, was satisfactory for all compounds over the range of concentrations tested. The final calculated concentrations of the compounds detected in the algal extractions were corrected by application of the KD_f (see Eq. 3.3) for compounds that had regression slopes that were outside of the 0.73 to 1.17 range.

The detection limits ranged from less than 7 ng/mL for the n-alkanes, 2t,6c-nonadienal, β-cyclocitral, and geosmin, to greater than 150 ng/mL for the fatty acids. Detection limits were calculated by application of the method described by Sharaf (1986) to the 90 percent confidence interval obtained by plotting the five lowest concentrations that were extracted. Repeated measurements at low concentrations were not performed;

therefore, the detection limits presented in Appendix D, Table D.1 are likely conservatively high. Appendix D, Figure D.1 presents an example of how the detection limits were calculated.

Effects of Extraction Time on Compound Recovery (Scheme II)

The algal extractions were all conducted using a 16-hr extraction time; however, an investigation of the effect of extraction time on compound recovery was undertaken near the end of the project. The experimental conditions are presented as "Scheme II" in Appendix C, Table C.2. The recoveries of the compound standards, expressed as the percentages of the added concentrations, were plotted against the extraction times (Appendix D, Figures D.2 to D.8) which ranged from 4 to 24 hours. The recoveries of the straight-chain hydrocarbons (Appendix D, Figure D.2) were between 75 to 100 percent, regardless of extraction time, for the range of extraction times evaluated. The recoveries of geosmin, MIB, and 2t,6c-nonadienal (Appendix D, Figure D.3) were between 100 to 125 percent, and were both very consistent and reproducible over the range of extraction times evaluated. The recoveries of indanone, phenethyl alcohol, squalene, and phytol (Appendix D, Figure D.4) also appeared to be time-independent over the range of extraction times evaluated.

The recoveries of benzyl alcohol, benzyl acetate, and β-cyclocitral (Appendix D, Figure D.5) were between 90 to 110 percent, and were not dependent upon extraction time. The recoveries of 3-furfural and 1-heptadecene (Appendix D, Figure D.6A and B) were 80 percent and 65 percent, respectively. The recovery of isovaleric acid (Appendix D, Figure D.6C) was only 10 percent. The recoveries for these three compounds did not appear to be time dependent.

The recoveries of diphenylamine, chlorophene, 3-methlythio-1-propanol, and 2-phenylphenol (Figure 4.7) were between 150 to 300 percent; apparently they were retained more readily during the CLLE-KD process than the n-chloroalkanes that were used as internal standards. Recovery of the phenolic compounds was more variable than for 3-methlythio-1-propanol; otherwise the recoveries of the four compounds appeared to be independent of extraction time. Myristic, palmitic, and linolenic acid (Figure 4.8) were also selectively retained, as the recoveries of these compounds ranged from 200 to 300

percent during the 8- to 24-hour extraction time range. The recoveries of the fatty acids were more variable than those of the other compounds, especially at the 4-hour extraction time.

FPA OF COMPOUND STANDARDS

Several of the odorous compounds that were extracted from either the algal cells or the cell-free media were: (1) known to cause odor problems in surface waters (e.g., geosmin, MIB, and 2t,6c-nonadienal), (2) described in fragrance literature as having odors (e.g., β-cyclocitral, benzyl acetate, isovaleric acid, and phenethyl alcohol), or (3) ascertained to have an odor when the compound standard was obtained (e.g., linolenic acid). These compounds were selected to be presented to the FPA panel for odor evaluation. The Weber-Fechner (W-F) plots representing the evaluation of the compound standards are presented in Figures 4.1 and 4.2. The data appear to fit the expected W-F plot well, as indicated by their respective R-squared values.

Geosmin (Figure 4.1A) was described by the FPA panelists as having an "earthy-corn-musty" odor; whereas MIB (Figure 4.1B) had an "earthy-musty" odor. The additional "corn", "corn silk", and "corn shuck" overtones enabled several of the panelists to immediately determine that they were evaluating geosmin, rather than MIB. Literature references were not found for the various corn descriptors applied to the geosmin odors. The W-F plots for geosmin and MIB indicate that the threshold odor concentrations of both compounds was approximately 10 ng/L; these agreed with literature values (Krasner et al. 1983; AWWA 1993). The threshold odor concentration was estimated to be the concentration of a compound that was needed in a FPA sample to evoke an average intensity rating of 2 from the panelists when the sample temperature was 45°C.

The average odor intensity rating of 2t,6c-nonadienal (Figure 4.1C) at 50 ng/L was 3.5. The panelists had no difficulty detecting the cucumber odor and were consistent in their responses over the concentrations evaluated. The threshold odor concentration for 2t,6c-nonadienal was approximately 4 ng/L, which agreed with values obtained by other researchers [e.g., 10 ng/L by Whitfield and Last (1991), and from 2 to 5 ng/L by Burlingame, Philadelphia Water Department (personal communication)]. β-cyclocitral (Figure 4.1D) was described as "sweet-fruity-chocolate-pipe tobacco", with the pleasant

"pipe tobacco" term being applied most consistently by all of the panelists. The threshold odor concentration for this compound was approximately 3 μg/L.

Benzyl acetate (Figure 4.2A) was described as "artificial banana" by the panelists and its threshold odor concentration was approximately 30 μg/L. Isovaleric acid (Figure 4.2B) was described by the panelists as "rancid", "cheesy", "dirty socks", and "sour." These descriptors correspond well with those applied by other researchers to related compounds such as isobutanoic acid, butanoic acid, and pentanoic acid (Hsieh et al. 1989). The threshold odor concentration for isovaleric acid was approximately 20 μg/L.

Phenethyl alcohol (Figure 4.2C) was a pleasant smelling compound, described by the panelists as "floral" and "rose." The threshold odor concentration was approximately 45 μg/L. The FPA panelists described the odor of linolenic acid (Figure 4.2D) as "sweet", "melon", and "watermelon", with the descriptive combination "sweet-watermelon" predominating. The odor intensity of this compound increased more gradually with increased concentrations than the odor intensities of the other compounds that were evaluated; however, the threshold odor concentration of 5 μg/L was lower than those of the other compounds presented in Figure 4.2.

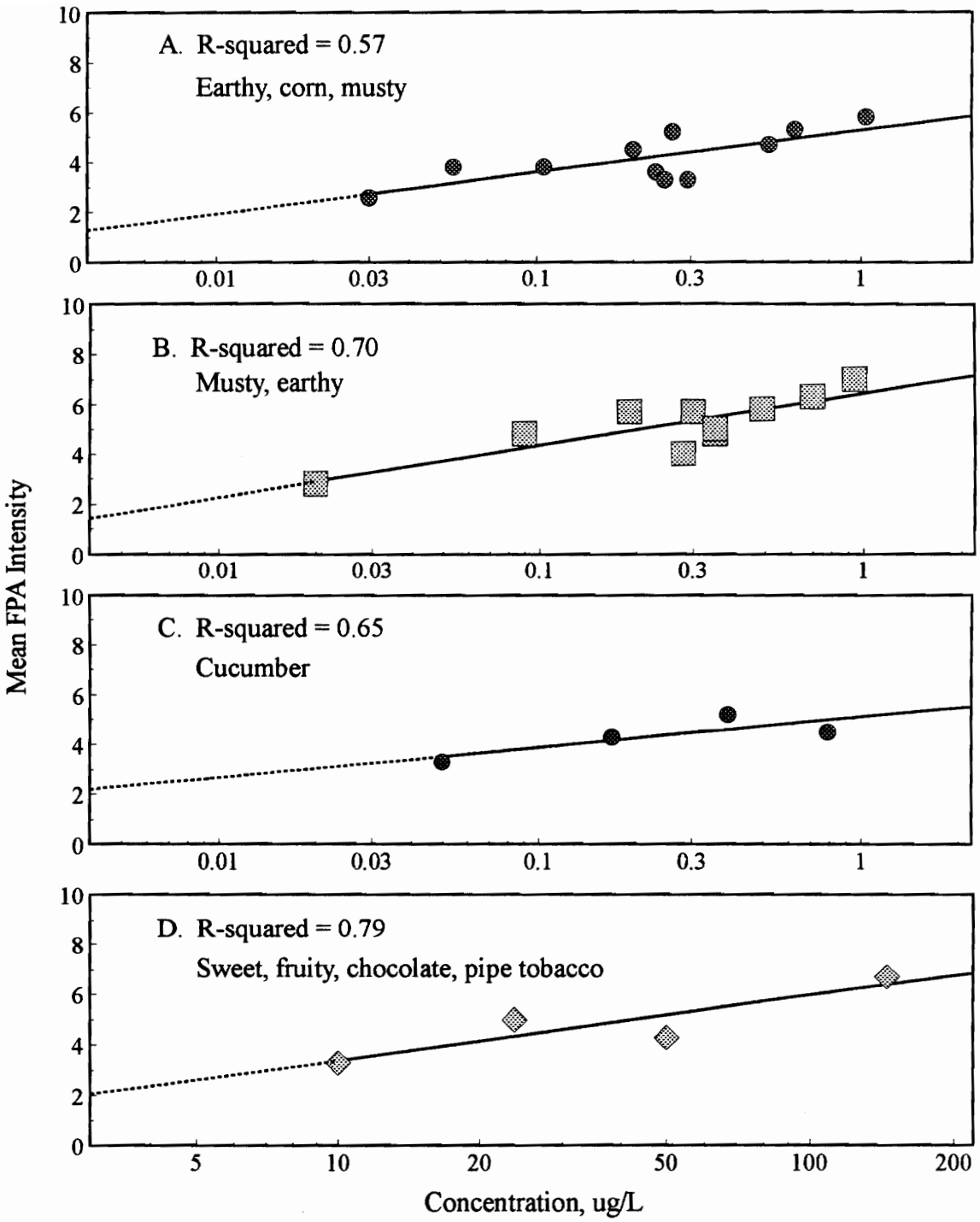


Figure 4.1 Weber-Fechner plots from FPA evaluations of (A) geosmin, (B) MIB, (C) 2t,6c-nonadienal, and (D) *B*-cyclocitral. The dashed line represents the theoretical extension of W-F line beyond the concentrations evaluated by the FPA panelists.

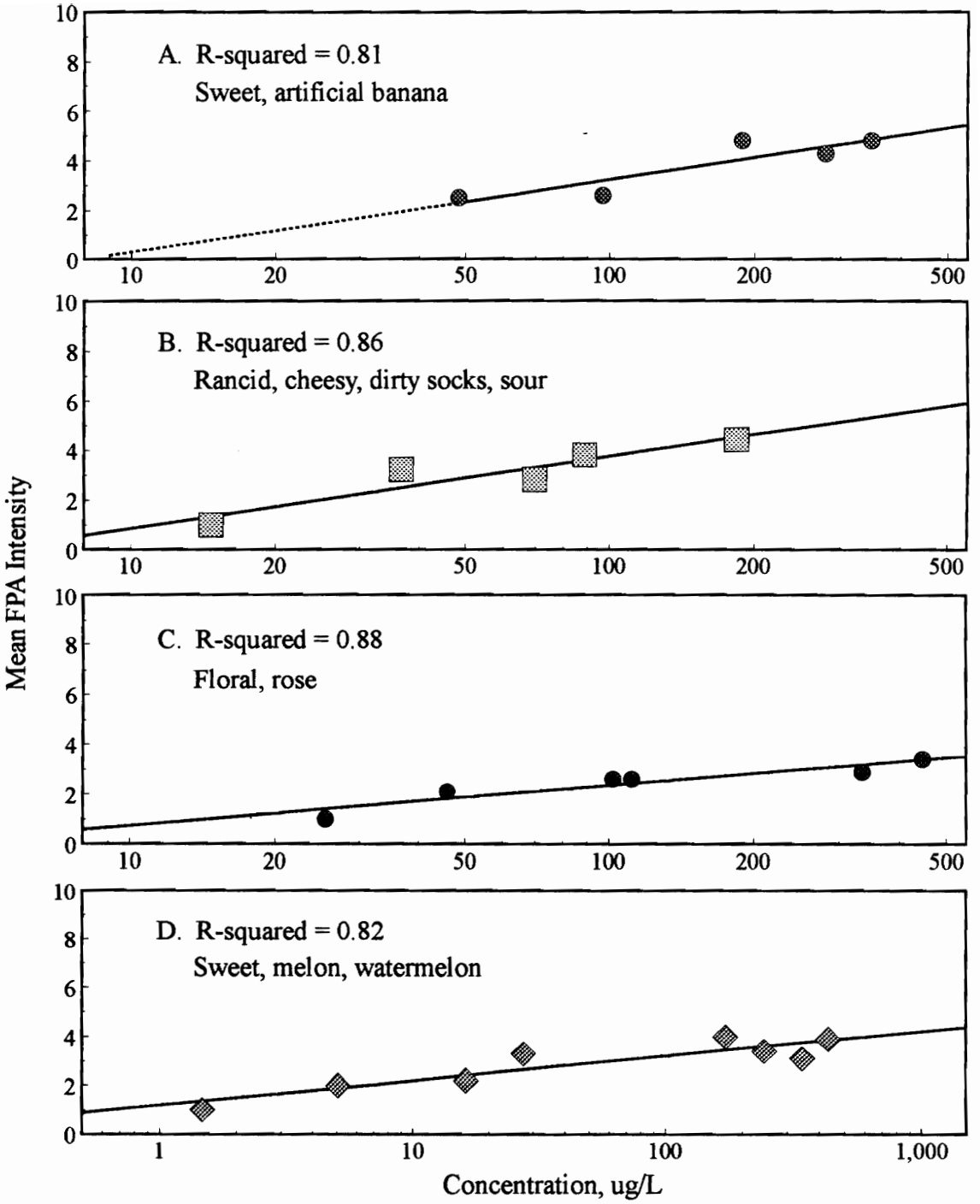


Figure 4.2 Weber-Fechner plots from FPA evaluations of (A) benzyl acetate, (B) iso-valeric acid, (C) phenethyl alcohol, and (D) linolenic acid. The dashed line represents the theoretical extension of W-F line beyond the concentrations evaluated by the FPA panelists.

INSTRUMENTAL AND SENSORY EVALUATIONS OF ALGAL CULTURES

Sixty-three compounds were detected in the algal-culture extracts either frequently or sporadically. Some of the compounds were distinctly odorous (e.g., geosmin, 2t,4c,7c-decatrienal, and linolenic acid), others were potential precursors of odorous compounds (e.g., myristic acid, linoleic acid, and linolenic acid), some were possibly bacteriacidal (e.g., chlorophene and 2-phenylphenol), and some could not be identified. The locations of the compounds (e.g., intra- or extracellular) and the concentrations of those that were detected frequently are presented in this section, while the possible significance of both the frequently and sporadically detected compounds is discussed in a following section. Algal samples were collected on seven sampling dates, starting with t_0, and the detected concentrations were reported. The FPA panelists frequently detected odors in young cultures that contained compound concentrations too low to be detected by GC-MS. The critical population densities, which are the theoretical population densities of algal cells at which odors become perceptible and are based upon both the amounts of compounds produced and the threshold odor concentrations, are presented in the next section. The mass spectra of the compounds are presented alphabetically in Appendix F.

Anabaena laxa

Anabaena laxa is a geosmin producer. The FPA odor descriptors for this culture were "earthy", "sweet-earthy-corn-grassy", and, at 40-days culture age only, "earthy-corn-rotten grass". No compounds that could have directly caused the "sweet-corn-grassy" odors other than geosmin were identified by GC-MS, although the possibility of synergistic and additive effects with other compounds should not be eliminated.

The intensities of the odors and the corresponding geosmin concentrations in the algal samples are compared in Figure 4.3 to the W-F plot for those of the geosmin standard. The panelists' intensity ratings were generally lower than those that would be predicted from the geosmin concentrations detected in the FPA samples. The lower responses may be the result of interaction with other compounds.

The *A. laxa* population growth curve is presented in Figure 4.4A. The plots of the concentrations of geosmin, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.4B and 4.4C, respectively. Other compounds that were frequently

identified in sample extracts are presented in Table 4.1, while compounds that were detected only sporadically are listed in Table 4.2.

Figure 4.4B indicates that in the early stages of population growth, most of the geosmin was retained within the algal cells; however, by 20-days culture age, the geosmin concentrations in the filtrate almost equalled those within the cells. The average production of geosmin, calculated from adding the amounts of geosmin in the cells and cell-free media, was approximately 0.026 ng per 10,000 algal cells (Figure 4.4C).

Table 4.1

Compounds frequently detected in cultures of *Anabaena laxa*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
chlorophene	0.304	0-1.308
geosmin	0.026	0.007-0.063
n-heptadecane	0.884	0.243-2.142
palmitic acid	0.11	0.01-0.28
2-phenylphenol	2.530	0-12.987
phytol	0.193	0.013-0.528
squalene	0.026	0-0.091

*Range of error measurements were determined as described by Taylor (1982)

Table 4.2

Compounds detected sporadically in cultures of *Anabaena laxa*

Compound	Location (cells or filtrate)	Identification*
4H-1,3-benzodioxin	cells and filtrate	s
n-docosane	cells	s
n-eicosane	cells and filtrate	c
2-furfural	filtrate	s
n-heneicosane	cells	c
n-hexadecane	cells and filtrate	s
isopropyl myristate	cells and filtrate	s
isopropyl palmitate	cells and filtrate	s
n-nonadecane	cells	c
n-octadecane	cells and filtrate	c
n-octadecene	cells and filtrate	c
n-pentadecane	cells and filtrate	s
5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	cells and filtrate	s
unknown @ 30.5 min	cells and filtrate	

*c = confirmed with standard; s = spectral library identification

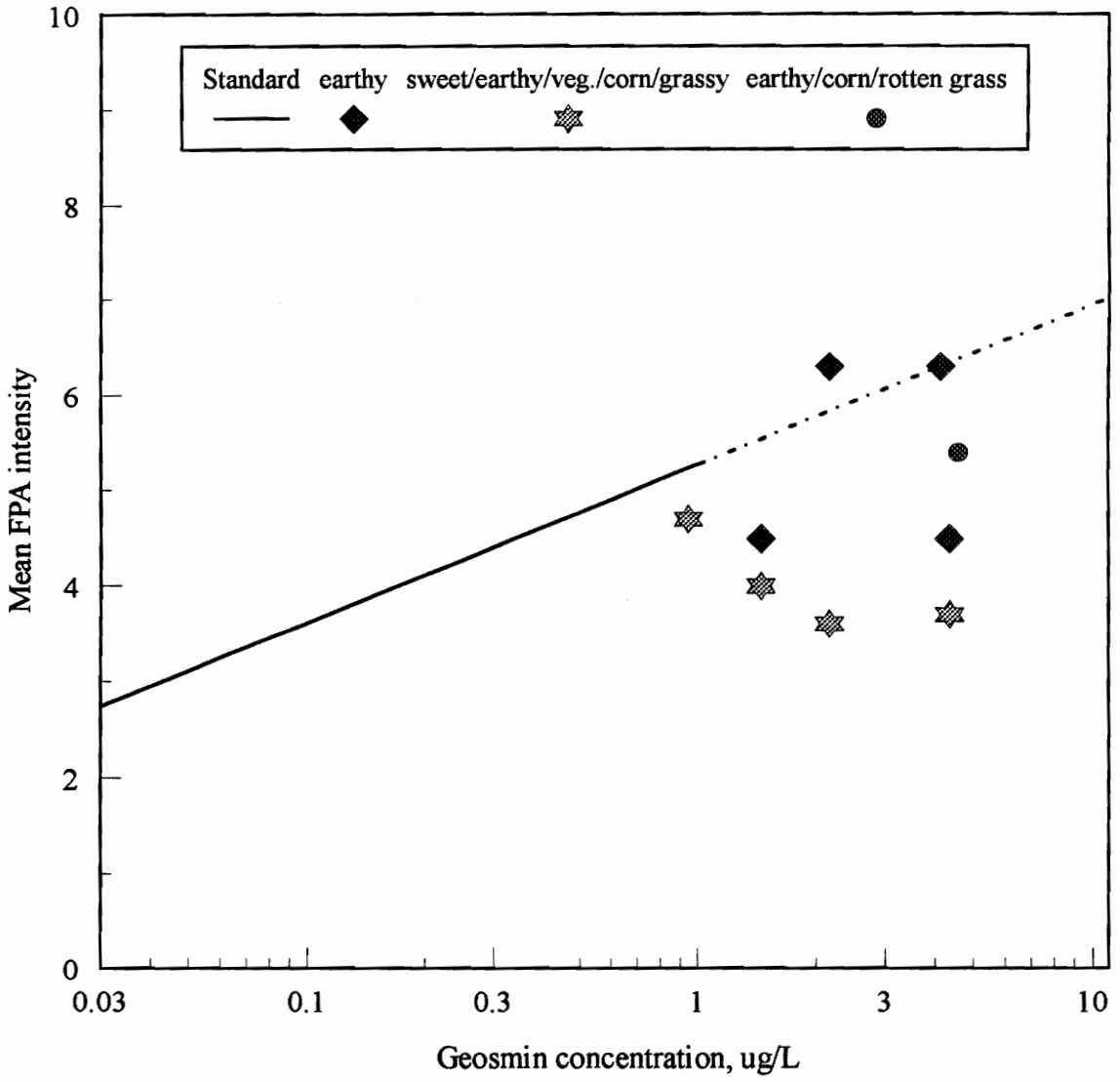


Figure 4.3 Comparison between FPA evaluations of geosmin detected in *Anabaena laxa* and those of geosmin standards (from Figure 4.1A). The dashed line represents the theoretical extension of W-F line beyond the concentrations evaluated by the FPA panelists.

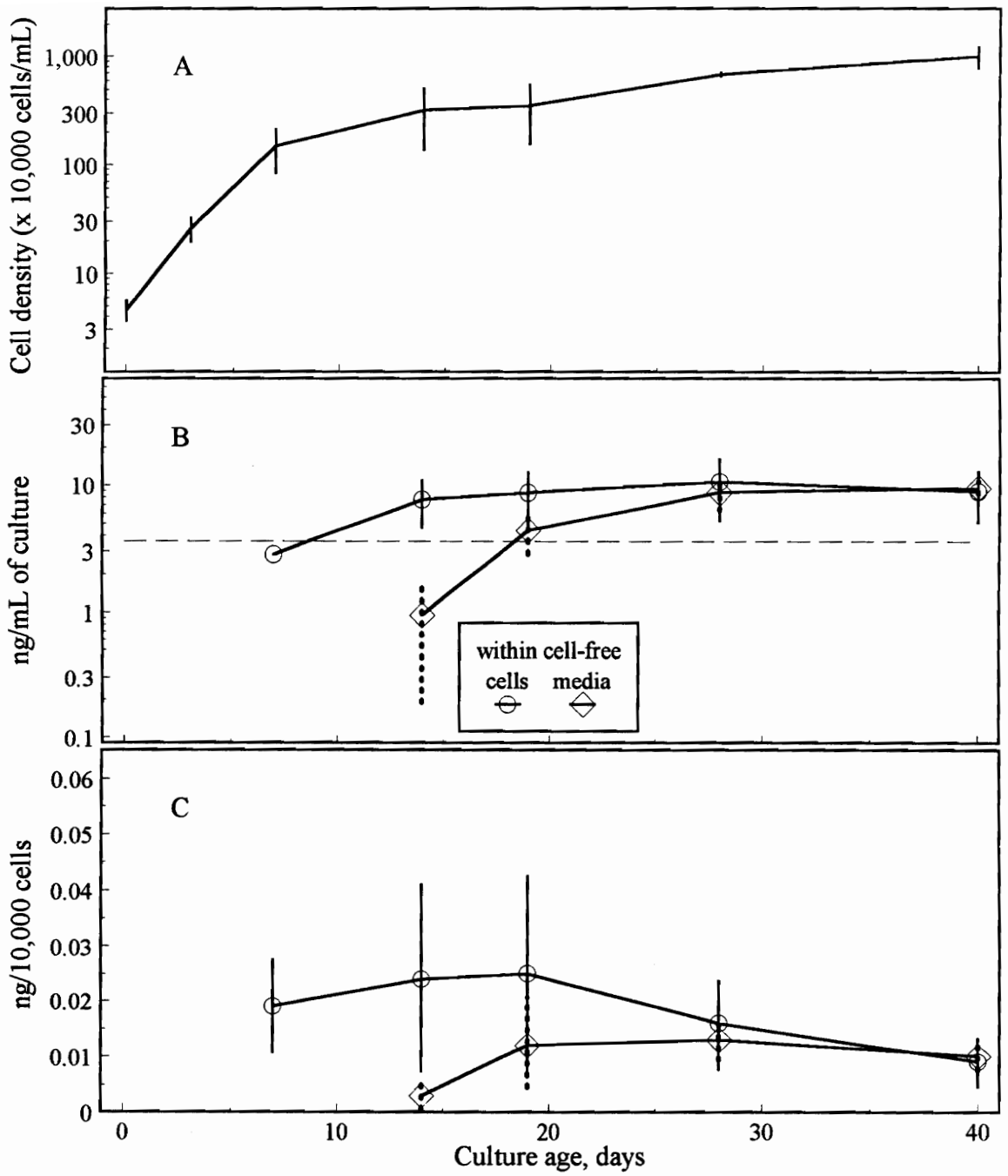


Figure 4.4 Concentrations of geosmin associated with *Anabaena laxa* as a function of culture growth: (A) culture growth, (B) concentrations of geosmin in cells and cell-free media, and (C) concentrations of geosmin produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Chlamydomonas peterfii

The FPA odor descriptors for this culture were meadow-like (e.g., "sweet hay", "vegetation", and "clover") for the cells and media at young culture ages; "fruity-sweet-floral" for the cells at advanced age; and "rancid-rotting hay or clover" for the cell-free media from 18 to 39 days culture age.

The pleasant odors of the cell fractions likely were caused by linolenic acid, which has a watermelon odor. The filtrate contained several odorous compounds: benzyl acetate (artificial banana odor), phenethyl alcohol (rose odor), and isovaleric acid (rotten, rancid, dirty socks). The intensity of the "rotten-rancid" and "sweet" odors and the corresponding isovaleric acid and linolenic acid concentrations present in the algal samples are compared to the W-F plot for the isovaleric and linolenic standards in Figure 4.5. The W-F plots of the standards indicate that at lower concentrations the linolenic acid odors will dominate and that the isovaleric acid odors will dominate at higher concentrations.

Linolenic acid was detected in the cell extracts, and isovaleric acid was detected in the cell-free media. When the FPA panelists evaluated samples of cell-free media (i.e., "isovaleric only" in Figure 4.5) they responded with "rotten" odor intensities that corresponded fairly well to the W-F plot obtained from FPA of the isovaleric acid standards. When the panelists evaluated samples of resuspended algal cells (i.e., "linolenic only" in Figure 4.5), they responded with "sweet" odor intensities that were well above the intensities predicted by the W-F plot obtained from FPA of the linolenic acid standards. The reason for the higher odor intensities is not known.

The FPA samples made from whole algal culture (e.g., containing cells and their culture media) contained both linolenic acid and isovaleric acid, as would be expected. The intensities of the odors associated with the isovaleric acid and linolenic acid (i.e., "rotten(both)" and "sweet(both)", respectively) were both lower than when the cell and cell-free fractions were evaluated (Figure 4.5). Other odoriferous compounds in the samples included benzyl acetate and phenethyl alcohol. The reduction in odor intensities that occurred when several odoriferous compounds were present may have been due to interactions between the compounds, their respective odors, or a combination of both, but further analysis was beyond the scope of this work.

Isovaleric acid was not detected in cell-free media extracts during the supplemental experiment that studied *C. peterfii* grown in OCM medium without added peptone. It is likely that the axenic *C. peterfii* was somehow metabolizing the peptone. Benzyl acetate and phenethyl alcohol were also absent in cell-free media extracts during the supplemental

experiment, but benzyl alcohol was present in cell-free media extracts of cultures grown in OCM medium both with and without added peptone. It is possible that isovaleric acid, benzyl acetate, and phenethyl alcohol are formed in surface waters from the metabolism of naturally occurring organic compounds, but no literature references were found that attributed odor episodes in surface waters to any of these compounds.

The *C. peterfii* population growth curve is presented in Figure 4.6A. The plots of the concentrations of isovaleric acid, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.6B and 4.6C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.3, while compounds that were detected only sporadically are listed in Table 4.4. The average production of isovaleric acid, calculated from adding the amounts of isovaleric acid in the cells and cell-free media, was approximately 4 ng per 10,000 algal cells (Figure 4.6C).

Table 4.3

Compounds frequently detected in cultures of *Chlamydomonas peterfii*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
benzyl acetate	0.655	0-5.374
benzyl alcohol	4.318	0.930-21.076
n-heptadecene	0.351	0.117-0.703
isovaleric acid	4.44	0-12.84
linolenic acid	1.70	0.27-3.04
palmitic acid	0.93	0.61-1.48
phenethyl alcohol	0.523	0.117-2.224
phytol	2.324	0.278-5.685

*Range of error measurements were determined as described by Taylor (1982)

Table 4.4

Compounds detected sporadically in cultures of *Chlamydomonas peterfii*

Compound	Location (cells or filtrate)	Identification*
unknown @ 28.8 min	cells	s
unknown @ 33.3 min	cells	
unknown @ 35.1 min	cells	

*s = spectral library identification

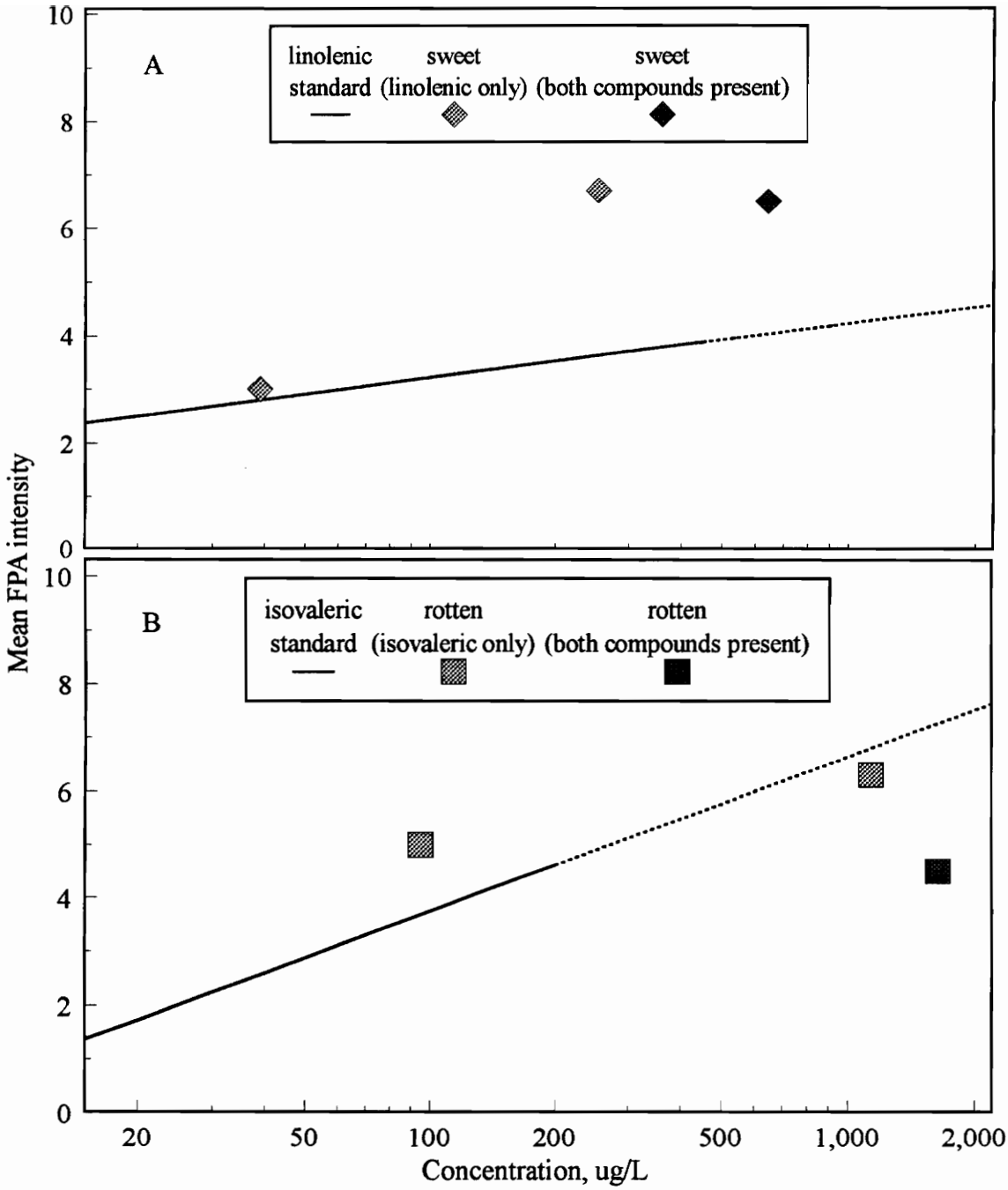


Figure 4.5 Comparison between FPA evaluations of (A) linolenic acid and (B) isovaleric acid detected in *Chlamydomonas peterfii* and those of linolenic acid and isovaleric acid standards (from Figures 4.2D and 4.2B, respectively). The dashed lines represent the theoretical extension of W-F lines beyond the concentrations evaluated by the FPA panelists.

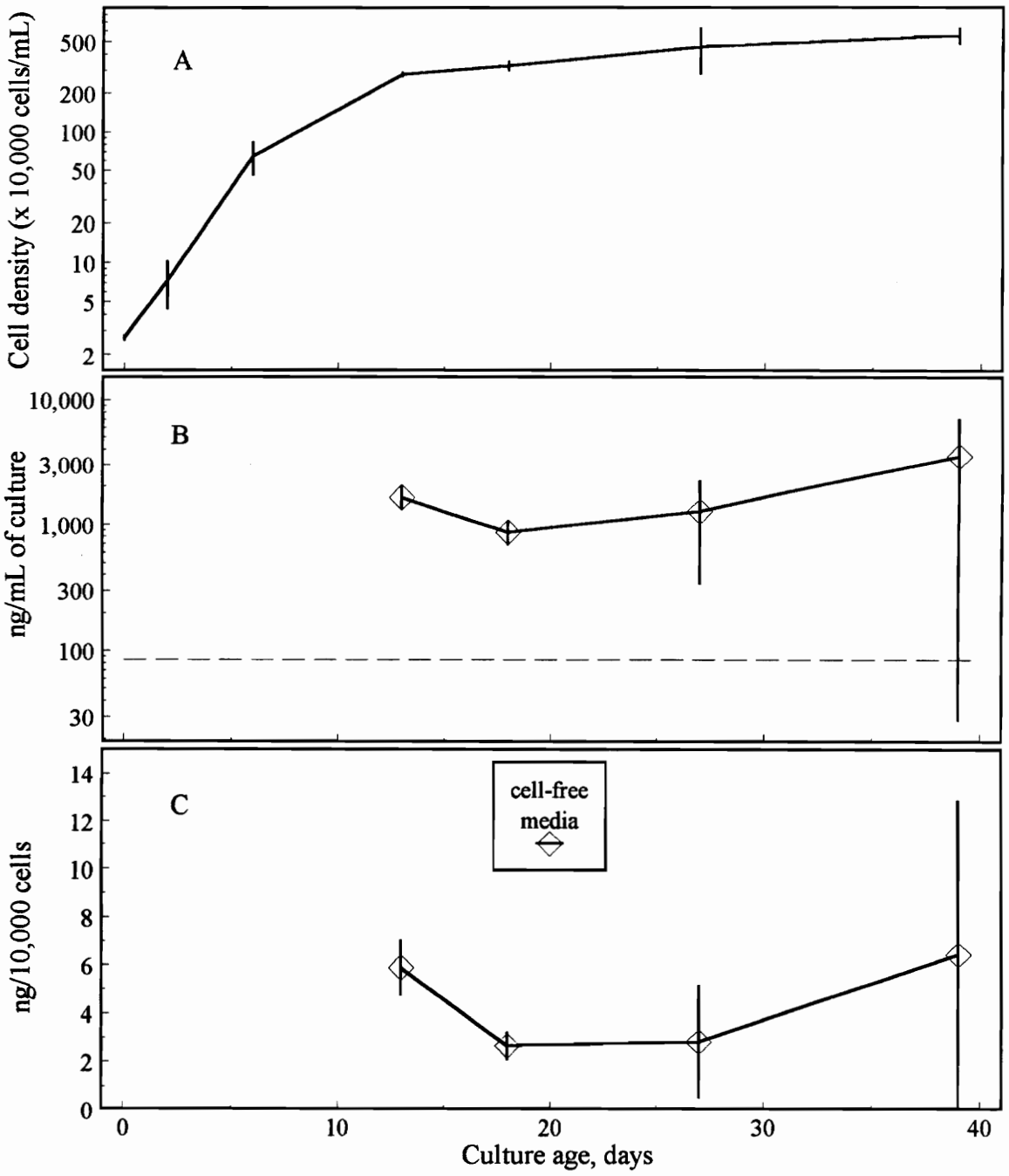


Figure 4.6 Concentrations of isovaleric acid associated with *Chlamydomonas peterfii* as a function of culture growth: (A) culture growth, (B) concentrations of isovaleric acid in cell-free media, and (C) concentrations of isovaleric acid produced per cell. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B and the error associated with C as described by Taylor (1982).

Dinobryon cylindricum

In the first experiment, the *D. cylindricum* cultures were grown in 15-mL of medium. The FPA panelists detected fishy odors (e.g., "fishy", "fish tank", and "swampy") when they evaluated culture samples; however, the GC-MS analyses did not detect any compounds that could be associated with those odors. The odor intensities ranged from 4 to 6.7, and the cell densities in the FPA sample flasks ranged from 400 to 61,600 cells/mL, respectively.

In the second experiment, the alga was grown in a 1-L Erlenmeyer flask that contained 500-mL sterile DY III medium. Larger sample sizes (e.g., the cells centrifuged from 40-mL portions rather than 15-mL portions) were sacrificed for the extraction of organic compounds. The compound 2t,4c,7c-decatrienal, which has been positively identified as having a fishy odor (Karahadian and Lindsay 1989), was confidently identified on the basis of retention order and mass spectra match (C. Karahadian, Dept. of Foods and Nutrition, Purdue University, personal communication). The cell extracts had a very intense fish, shark-oil, cod liver oil odor.

The *D. cylindricum* population growth curve during the second experiment is presented in Figure 4.7A. The plots of the concentrations of 2t,4c,7c-decatrienal, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.7B and 4.7C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.5, while compounds that were detected only sporadically are listed in Table 4.6. The possible significance of these compounds is discussed in a later section.

The majority of the 2t,4c,7c-decatrienal the alga produced was retained within the cells (Figures 4.7B and C). The average production of 2t,4c,7c-decatrienal, calculated from adding the amounts of 2t,4c,7c-decatrienal that were detected in the cells and cell-free media, was approximately 4 ng per 10,000 algal cells. No standard was available for FPA evaluation; therefore, the threshold odor concentration was not determined. It is likely, however, that the algae produce detectable odors while at very low cell densities, because, as described above, an FPA sample that contained 400 cells/mL evoked an odor intensity of 4.

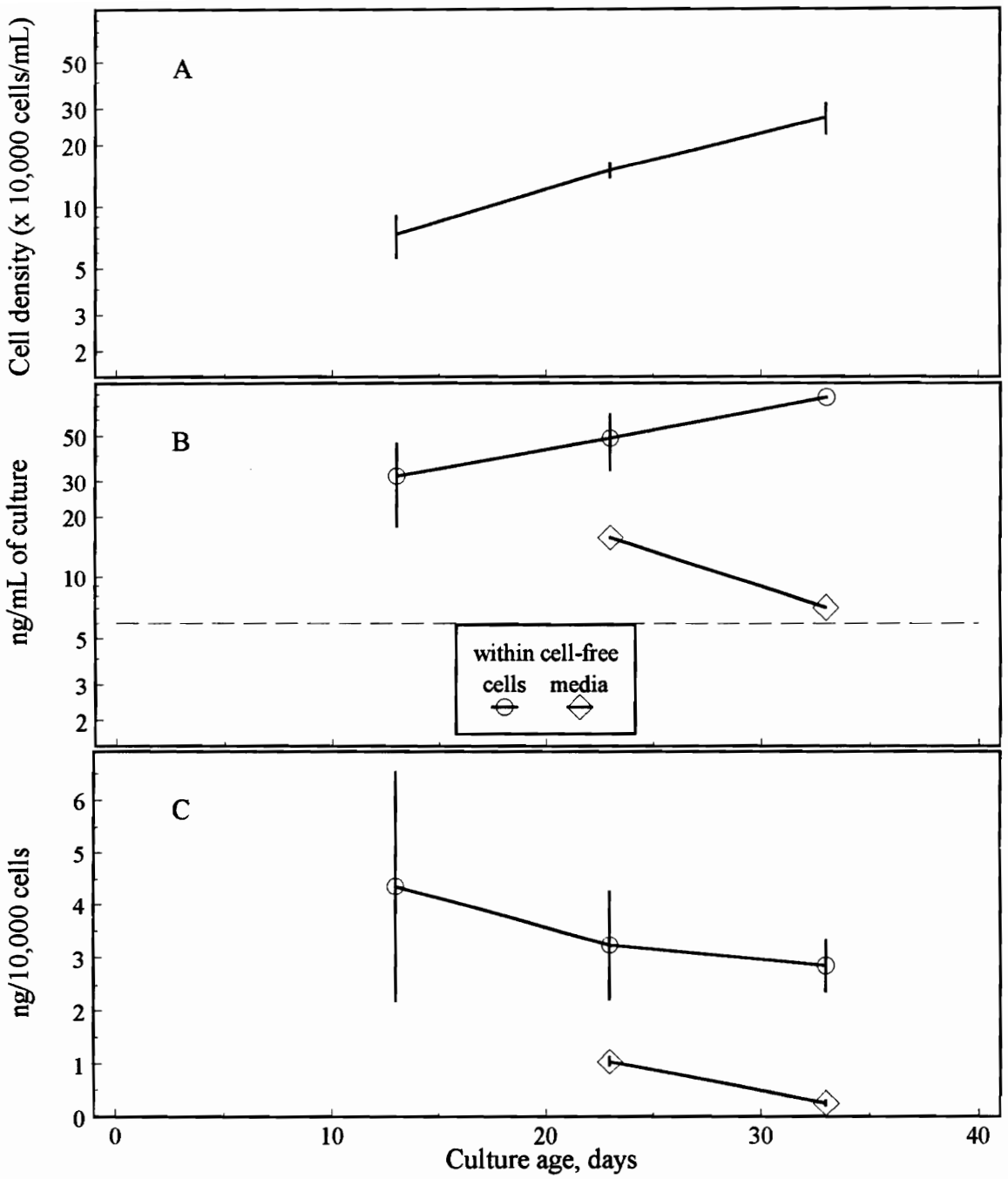


Figure 4.7 Concentrations of 2t,4c,7c-decatrienal associated with *Dinobryon cylindricum* as a function of culture growth: (A) culture growth, (B) concentrations of 2t,4c,7c-decatrienal in cells and cell-free media, and (C) concentrations of 2t,4c,7c-decatrienal produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Table 4.5

Compounds frequently detected in cultures of *Dinobryon cylindricum*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
18C fatty acids†	9.67	0.16-17.23
2t,4c,7c-decatrienal	3.918	2.20-6.522
myristic acid	4.84	3.70-5.18
palmitic acid	5.98	2.46-8.67

* Range of error measurements were determined as described by Taylor (1982)

† Mainly linolenic and linoleic, but also some oleic and stearic acids

Table 4.6

Compounds detected sporadically in cultures of *Dinobryon cylindricum*

Compound	Location (cells or filtrate)	Identification*
n-docosane	cells and filtrate	s
n-eicosane	cells and filtrate	c
n-heneicosane	cells and filtrate	c
n-heptacosane	cells	s
n-heptadecane	cells and filtrate	c
n-hexacosane	cells and filtrate	s
hexadecenoate	cells	s
isopropyl myristate	cells	s
n-nonadecane	cells and filtrate	c
n-octadecane	cells and filtrate	c
n-pentacosane	cells and filtrate	s
n-pentadecane	filtrate	s
squalene	cells and filtrate	c
stearic acid	cells and filtrate	s
n-tetracosane	cells and filtrate	s
n-tricosane	cells and filtrate	s
5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	cells	s
unknown @ 30.5 min	filtrate	
unknown @ 35.1 min	cells and filtrate	
unknown @ 39.3 min†	cells	
unknown @ 39.5 min	cells	

* c = confirmed with standard; s = spectral library identification

† likely an isomer of the unknown @ 39.5 min

Microcystis aeruginosa

Microcystis aeruginosa produced both β-cyclocitral and hydroxy-β-cyclocitral. These compounds have been identified by Jüttner (1976; 1983; 1984a; 1987) in cultures of *M. aeruginosa*. The FPA panel described the culture samples as "sweet-melon-grassy-corn" for both young culture ages and diluted older cultures, and as either "oily-fishy" or "rancid-fishy" for less-diluted samples that were older than 19 days culture age. These descriptors do not correspond to the "sweet-tobacco" odors associated with β-cyclocitral; however, the "sweet-melon" descriptors do correspond to odors ascribed to linolenic acid.

The intensities of the "sweet-melon-grassy" odors and the corresponding linolenic acid concentrations in the algal samples are compared to the W-F plot for the linolenic standard in Figure 4.8A. The "pipe tobacco" odor of β-cyclocitral was not detected, even though there were sufficient concentrations of the compound to produce perceivable odors (Figure 4.8B). The odor of β-cyclocitral was likely masked by the odors of linolenic acid. The source of the "oily-rancid-fishy" odors was not determined.

Although both β-cyclocitral and linolenic acid have odors, no references were found that attributed odor episodes in surface waters to either of these compounds. The FPA panelists did not describe any sulfurous or petroleum odors, which have been reported by other researchers (Jüttner 1984a and Persson 1988, respectively). The hydroxy-β-cyclocitral likely has an odor, because it is structurally similar to other compounds that have known odors. For example, β-cyclocitral has "tobacco-like" odors (Jüttner 1976) and dehydro-β-cyclocitral (a.k.a. safranal) has "sweet", "green-floral", and "tobacco-herbaceous" odors (Arctander 1969).

The *M. aeruginosa* population growth curve is presented in Figure 4.9A. The plots of the concentrations of β-cyclocitral, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.9B and 4.9C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.7, while compounds that were detected only sporadically are listed in Table 4.8. Three odorous compounds that were detected sporadically and may have contributed to the culture odor were dehydro-β-cyclocitral, 3-methylthio-1-propanol, and diphenylamine. The possible significance of these compounds is discussed in a later section; however, none of the individual compounds was known to have either an "oily-fishy" or an "oily-rancid-fishy" odor ascribed to it.

Greater than 95 percent of the β-cyclocitral produced by the alga was retained within the cells (Figure 4.17b and c). The average production of β-cyclocitral and linolenic acid, calculated from adding the amounts of the compounds in the cells and cell-free media, was approximately 0.105 ng per 10,000 algal cells and 1.758 ng per 10,000 algal cells, respectively (Table 4.8).

Table 4.7

Compounds frequently detected in cultures of *Microcystis aeruginosa*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
β-cyclocitral	0.105	0.045-0.192
hydroxy-β-cyclocitral	0.243	0.128-0.473
n-heptadecane	0.397	0.167-0.961
linoleic	1.16	0.35-3.79
linolenic	1.76	0.33-6.22
myristic acid	0.08	0-0.60
n-octadecane	0.083	0.009-0.486
palmitic acid	0.59	0.15-1.73
phenethyl alcohol	0.039	0.002-0.102
phytol	0.069	0-0.198
squalene	0.043	0.001-0.229

* Range of error measurements were determined as described by Taylor (1982)

Table 4.8

Compounds detected sporadically in cultures of *Microcystis aeruginosa*

Compound	Location (cells or filtrate)	Identification*
3-methylthio-1-propanol	filtrate	c
benzyl alcohol	filtrate	c
chlorophene	cells	c
diphenylamine	filtrate	c
n-docosane	cells and filtrate	s
dodecanoate	cells	s
n-eicosane	cells and filtrate	c
n-heneicosane	cells and filtrate	c
hexadecenoate	cells	s
isopropyl palmitate	cells and filtrate	s
n-nonadecane	cells and filtrate	c
nonanoate	cells	c
n-pentacosane	cells	s
2-phenylphenol	cells	c
safranal	cells	s
squalene	cells	c
5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	filtrate	s
unknown @ 10.8 min (β-cyclocitral-like)	cells and filtrate	
unknown @ 13 min (β-cyclocitral-like)	cells and filtrate	
unknown @ 15.7 min (β-cyclocitral-like)	cells	

*c = confirmed with standard; s = spectral library identification

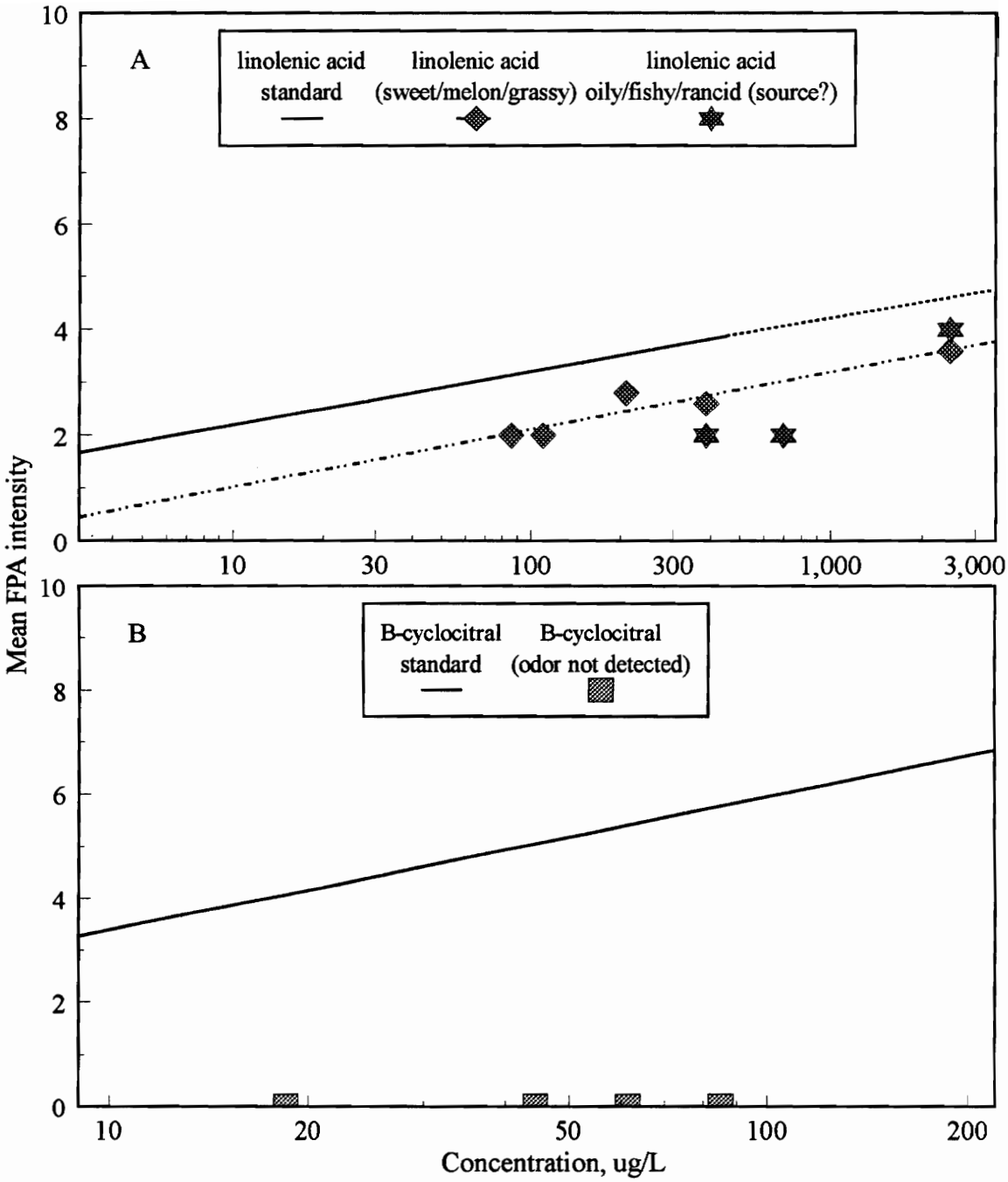


Figure 4.8 Comparison between FPA evaluations of (A) linolenic acid and (B) *B*-cyclocitral detected in *Microcystis aeruginosa* and those of linolenic acid and *B*-cyclocitral standards (from Figures 4.2D and 4.1D, respectively). The dashed line for the linolenic acid standard represents the theoretical extension of the W-F line beyond the concentrations evaluated by the FPA panelists.

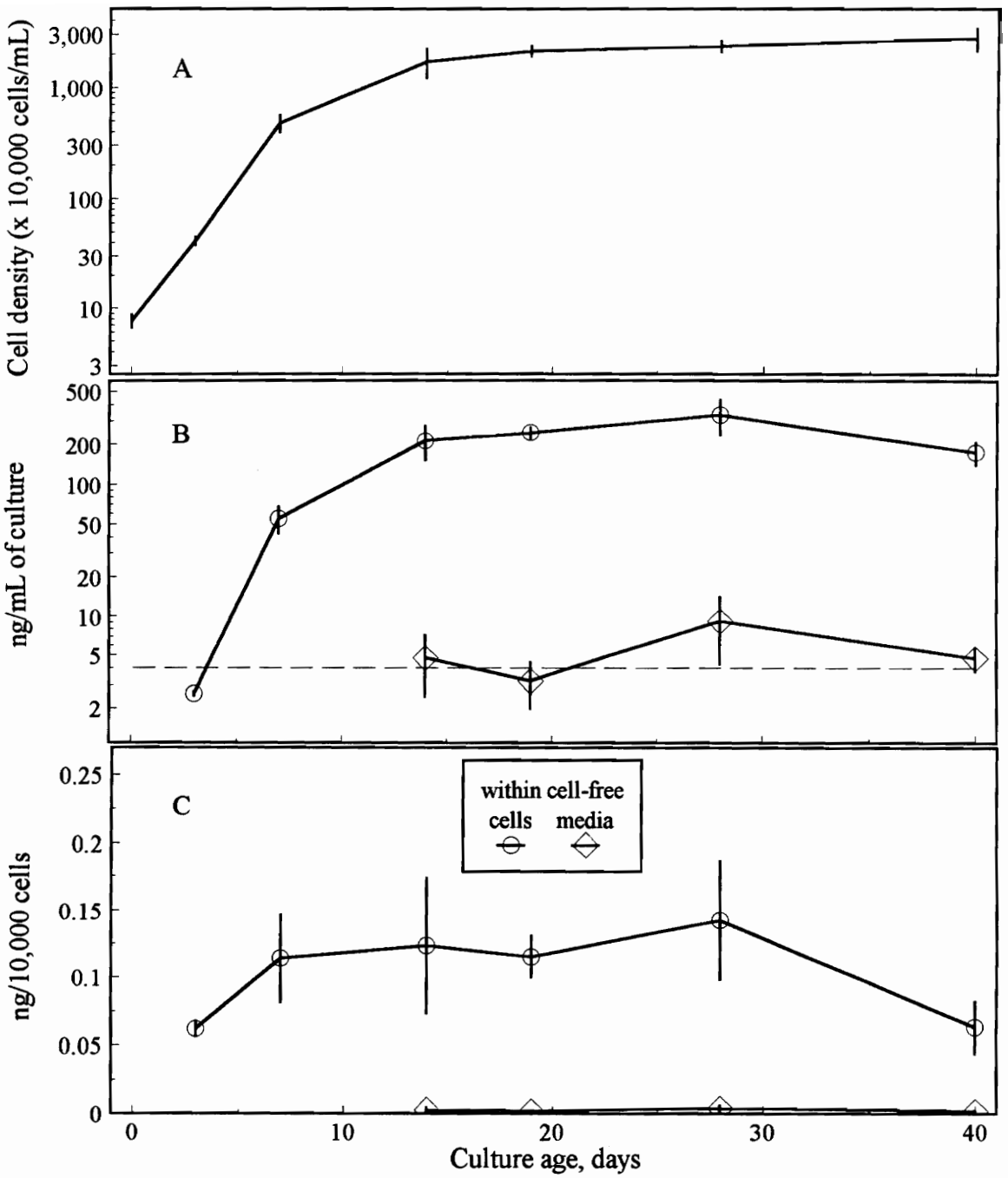


Figure 4.9 Concentrations of *B*-cyclocitral associated with *Microcystis aeruginosa* as a function of culture growth:- (A) culture growth, (B) concentrations of *B*-cyclocitral in cells and cell-free media, and (C) concentrations of *B*-cyclocitral produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Oscillatoria sp.

This strain of *Oscillatoria* produced MIB but no detectable geosmin. The FPA odor descriptors for this culture were "earthy-musty" and also a slight (e.g., intensities between 1 to 3) "sweet-melon-corn-grassy". Linolenic acid was detected in the culture extracts and may have contributed to the "sweet-melon" odors perceived by the FPA panelists. The intensities of the "earthy-musty" odors and the corresponding MIB concentrations in the algal samples are compared to the W-F plot for the MIB standards (Figure 4.10). The odor intensities were generally less than those predicted on the basis of the MIB concentrations. The compounds that contributed to the "sweet-melon" odors may have interferred with the ability of the panelists to perceive the "earthy-musty" odors, with the result being the lower-than-expected odor intensities for the MIB concentrations in the algal cultures.

The *Oscillatoria* sp. population growth curve is presented in Figure 4.11A. The alga did not grow very readily and was maintained on a north-facing window sill because it died when placed in the 25°C incubator. Growth was still erratic, as can be seen in Figure 4.11A. The plots of the concentrations of MIB, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.11B and 4.11C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.9, while compounds that were detected only sporadically are listed in Table 4.10. The possible significance of these compounds is discussed in a later section.

The algal cells retained approximately one-half to two-thirds of the MIB (Figures 4.11B and C). The average production of MIB, calculated from adding the amounts of MIB in the cells and cell-free media, was approximately 2 ng per 10,000 algal cells. No MIB was detected in the GC-MS extracts of either algal cells or cell-free media obtained from cultures less than 13-days old, which was likely because the cell densities were relatively low; however, the FPA panelists did detect "musty-earthy" odors in every culture sample that was evaluated during the 40-day incubation. The odor intensities of FPA samples evaluated after the cultures had incubated for only 0- and 3-days were 2.7 and 3.7, and the cell densities in the FPA samples were 5,400 and 21,600 cells/mL, respectively.

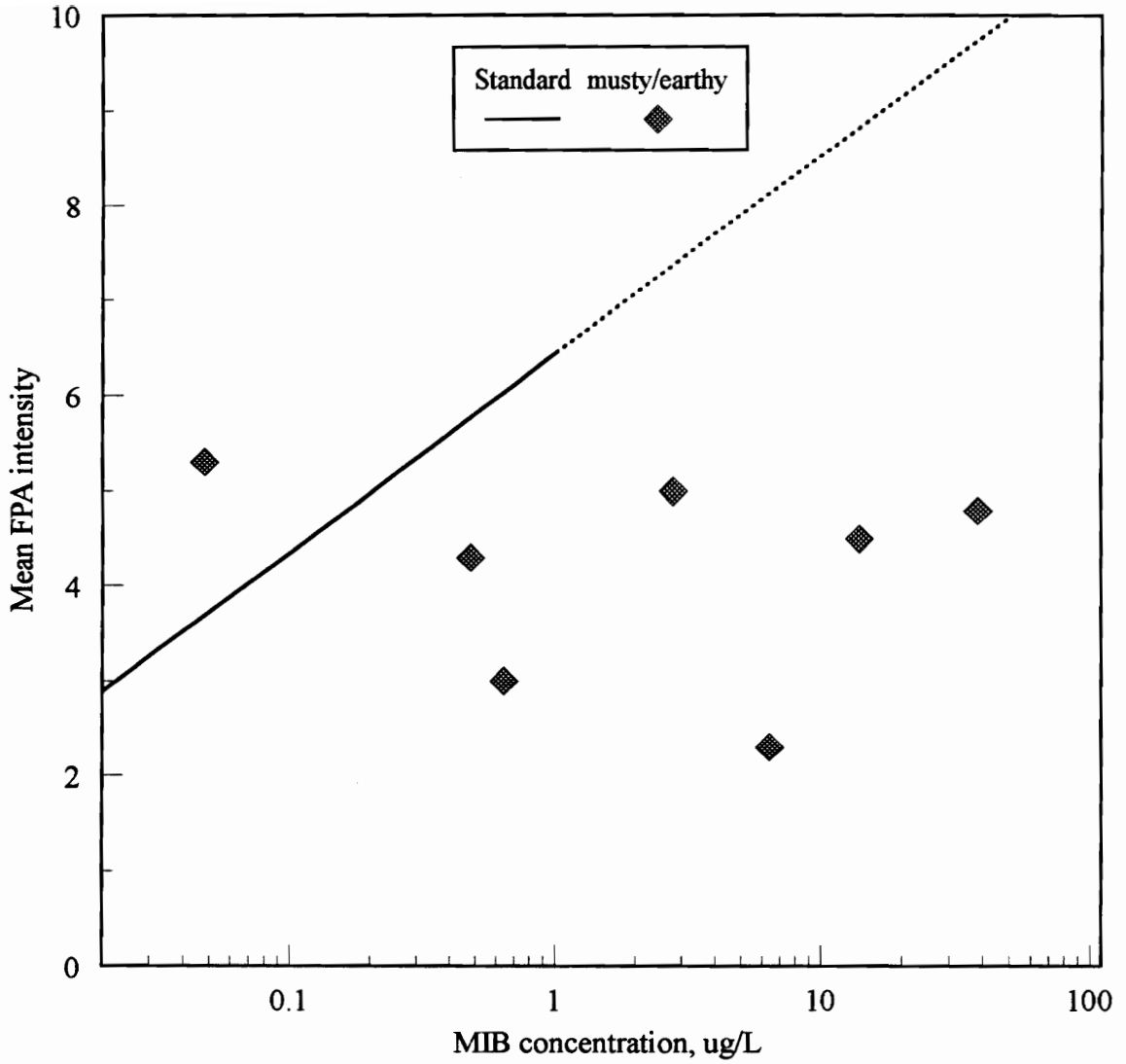


Figure 4.10 Comparison between FPA evaluations of MIB detected in *Oscillatoria* sp. and those of MIB standards (from Figure 4.1B). The dashed line represents the theoretical extension of W-F line beyond the concentrations evaluated by the FPA panelists.

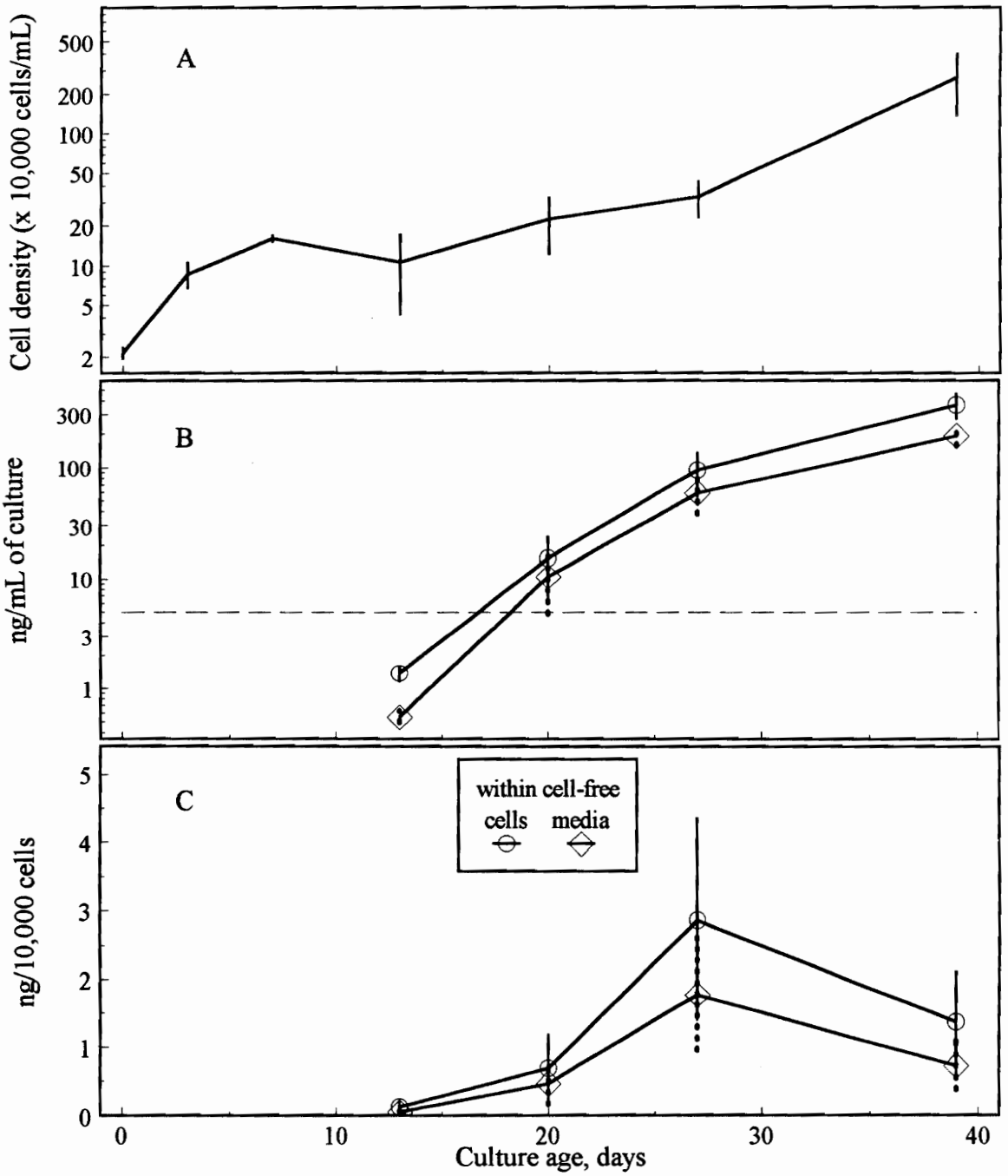


Figure 4.11 Concentrations of MIB associated with *Oscillatoria* sp. as a function of culture growth: (A) culture growth, (B) concentrations of MIB in cells and cell-free media, and (C) concentrations of MIB produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Table 4.9

Compounds frequently detected in culutures of *Oscillatoria* sp.

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
n-heptadecane	2.186	0.029-9.714
linoleic acid	18.49	0-44.53
linolenic acid	10.94	1.84-21.44
MIB	2.017	0.069-6.978
myristic acid	1.22	0-2.86
palmitic acid	16.33	0-39.11
phytol	1.756	0.150-4.491

*Range of error measurements were determined as described by Taylor (1982)

Table 4.10

Compounds detected sporadically in cultures of *Oscillatoria* sp.

Compound	Location (cells or filtrate)	Identification*
chlorophene	cells and filtrate	c
dodecanoate	cells and filtrate	s
n-eicosane	cells and filtrate	c
nonanoate	cells	c
n-octadecane	cells and filtrate	c
octadecanoate	cells	s
2-phenylphenol	cells and filtrate	c
squalene	filtrate	c

* c = confirmed with standard; s = spectral library identification

Oscillatoria tenuis

Oscillatoria tenuis produced geosmin, but no detectable MIB. The FPA odor descriptors for this culture were "sweet-melon-corn" and "earthy-corn-musty". No references were found in the literature where the odor descriptor "corn" was applied to geosmin. The intensities of the earthy-type odors and the corresponding geosmin concentrations in the algal samples are compared to the W-F plot for the geosmin standards (Figure 4.12). The odor intensities of the culture samples almost paralleled the W-F plot, but were approximately 1.5 to 2 FPA intensity scale units lower than the intensities predicted by the predicted intensities. The "sweet-melon-corn" odors were slightly stronger than the "earthy-corn-musty" odors, and more-nearly paralleled the W-F line for the geosmin standards. Linolenic acid, which may have contributed to the "sweet-melon" odors, was detected in cell extracts after 20-days incubation.

The *O. tenuis* population growth curve is presented in Figure 4.13A. The plots of the concentrations of geosmin, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.13B and 4.13C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.11, while compounds that were detected only sporadically are listed in Table 4.12. The possible significance of these compounds is discussed in a later section.

Oscillatoria tenuis retained approximately 50 percent of the geosmin (Figures 4.13B and C) throughout its population growth. The average geosmin production, calculated from adding the amounts of geosmin in the cells and cell-free media, was slightly greater than 0.03 ng per 10,000 algal cells.

Table 4.11

Compounds frequently detected in cultures of *Oscillatoria tenuis*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
geosmin	0.031	0.004-0.086
n-heptadecane	0.065	0.035-0.117
heptadecene	0.093	0.036-0.168

* Range of error measurements were determined as described by Taylor (1982)

Table 4.12

Compounds detected sporadically in cultures of *Oscillatoria tenuis*

Compound	Location (cells or filtrate)	Identification*
chlorophene	cells	c
dodecanoate	cells and filtrate	s
n-eicosane	cells and filtrate	c
n-heneicosane	filtrate	c
hexadecenoate	filtrate	s
linoleic acid	cells	c
linolenic acid	cells	c
myrcenol	cells and filtrate	s
myristic acid	cells and filtrate	c
N-cyclohexyl-acetamide	filtrate	s
nonanoate	cells and filtrate	c
palmitic acid	cells and filtrate	c
2-phenylphenol	cells	c
squalene	cells	c

* c = confirmed with standard; s = spectral library identification

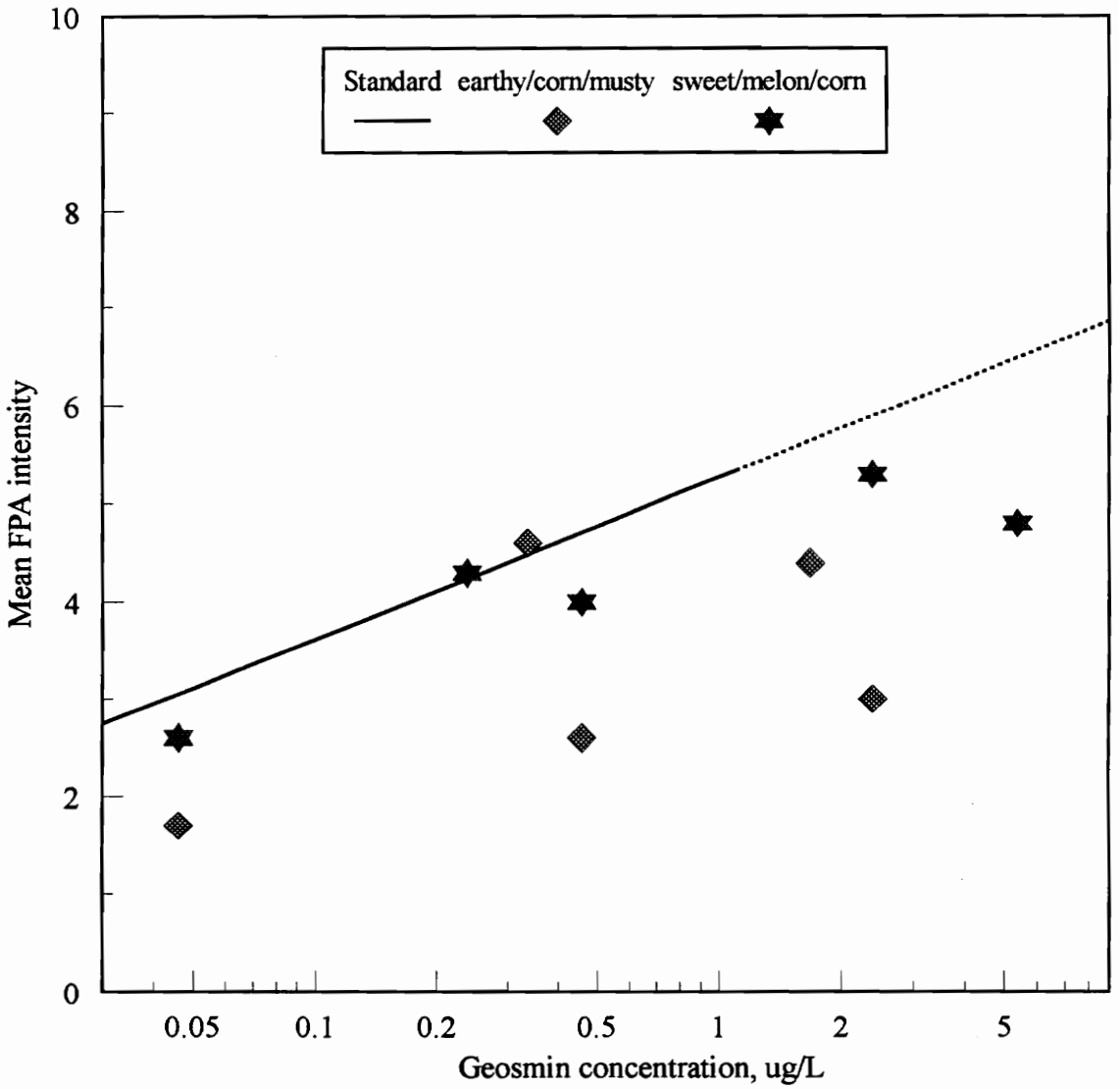


Figure 4.12 Comparison between FPA evaluations of geosmin detected in *Oscillatoria tenuis* and those of geosmin standards (from Figure 4.1A). The dashed line represents the theoretical extension of W-F plot beyond the concentrations evaluated by the FPA panelists.

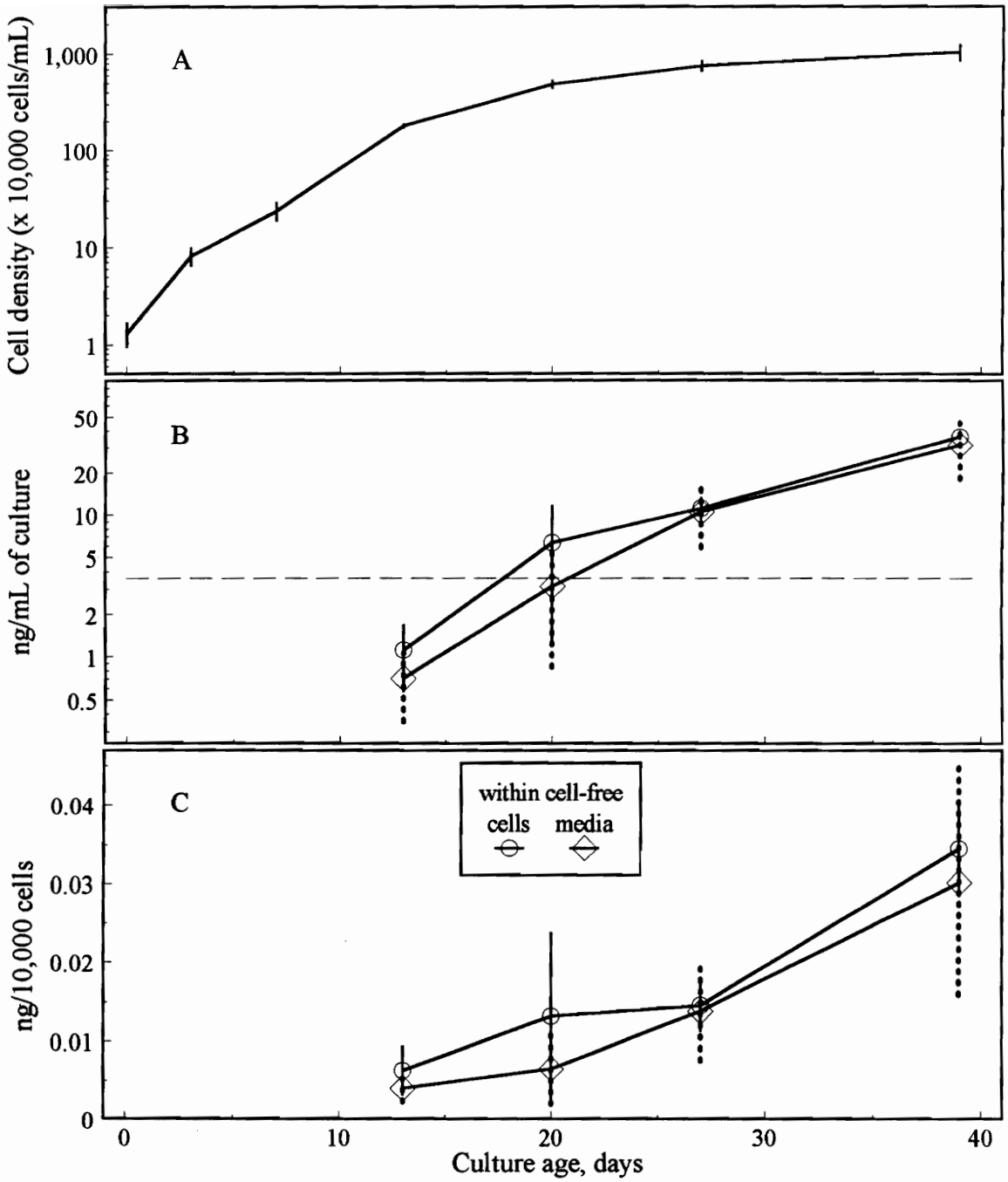


Figure 4.13 Concentrations of geosmin associated with *Oscillatoria tenuis* as a function of culture growth: (A) culture growth, (B) concentrations of geosmin in cells and cell-free media, and (C) concentrations of geosmin produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Phormidium sp.

Both MIB and geosmin were detected in extracts from this alga; however, the MIB was present in much larger concentrations. Although the geosmin concentrations were less than those of the MIB, the alga produced more geosmin (Table 4.13) than did either *A. laxa* (Table Table 4.1) or *O. tenuis* (Table 4.11). The FPA odor descriptors for this culture were "earthy-musty" and "earthy-corn-musty". The intensities of the earthy-type odors and the corresponding MIB concentrations in the algal samples are compared to the W-F plot for the MIB standards (Figure 4.14). The "earthy-musty" and "earthy-corn-musty" odor intensities almost paralleled the intensities predicted by the W-F plot for MIB.

The "earthy-corn-vegetable-cucumber-grassy-musty" odors, which were perceived in 40-day-old cultures, were likely a result of the geosmin produced by the alga. The FPA panelists ascribed "earthy-corn-musty", "sweet-melon-corn", and "sweet-earthy-corn-grassy" odors both to the other algal cultures that contained geosmin and to the geosmin standards. No references were found that ascribed either "vegetable" or "cucumber" odors to geosmin; however, neither 2t,6c-nonadienal ("cucumber" odor) nor linolenic acid ("melon-watermelon" odor) were detected in any of the extracts.

The *Phormidium* sp. population growth curve is presented in Figure 4.15A. The plots of the concentrations of MIB, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.15B and 4.15C, respectively. Other compounds that were frequently identified in sample extracts are presented in Table 4.13, while compounds that were detected only sporadically are listed in Table 4.14. The possible significance of these compounds is discussed in a later section.

Throughout its population growth, the alga released more than 80 percent of the MIB that was produced (Figures 4.15B and 4.15C). The average MIB production, calculated by adding the amounts of MIB in the cells and cell-free media, was approximately 0.6 ng per 10,000 algal cells.

Table 4.13

Compounds frequently detected in cultures of *Phormidium* sp.

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
geosmin	0.129	0.036-0.308
n-heptadecane	0.810	0.153-2.141
MIB	0.612	0.298-1.193

* Range of error measurements were determined as described by Taylor (1982)

Table 4.14

Compounds detected sporadically in cultures of *Phormidium* sp.

Compound	Location (cells or filtrate)	Identification*
4H-1,3-benzodioxin	cells and filtrate	s
chlorophene	cells	c
n-eicosane	cells and filtrate	c
2-furfural	filtrate	s
n-heneicosane	cells and filtrate	c
hexadecenoate	cells and filtrate	s
isopropyl myristate	cells and filtrate	s
myrcenol	filtrate	s
myristic acid	cells and filtrate	c
n-nonadecane	cells and filtrate	c
n-octadecane	cells and filtrate	c
octadecene	cells	s
palmitic acid	cells and filtrate	c
2-phenylphenol	cells	c
squalene	cells and filtrate	c

* c = confirmed with standard; s = spectral library identification

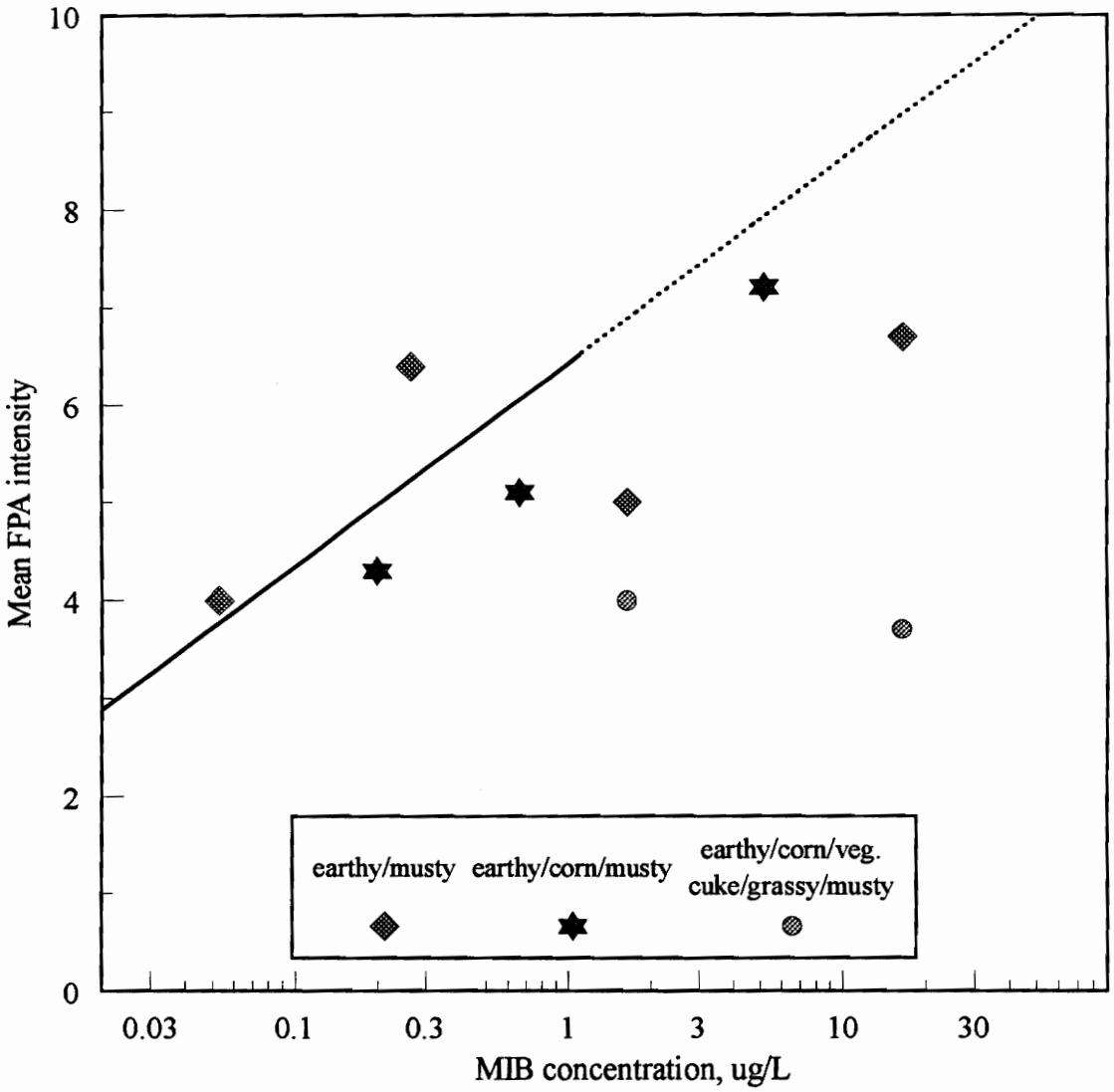


Figure 4.14 Comparison between FPA evaluations of MIB detected in *Phormidium* sp. and those of MIB standards (from Figure 4.1B). The dashed line represents the theoretical extension of W-F plot beyond the concentraions evaluated by the FPA panelists.

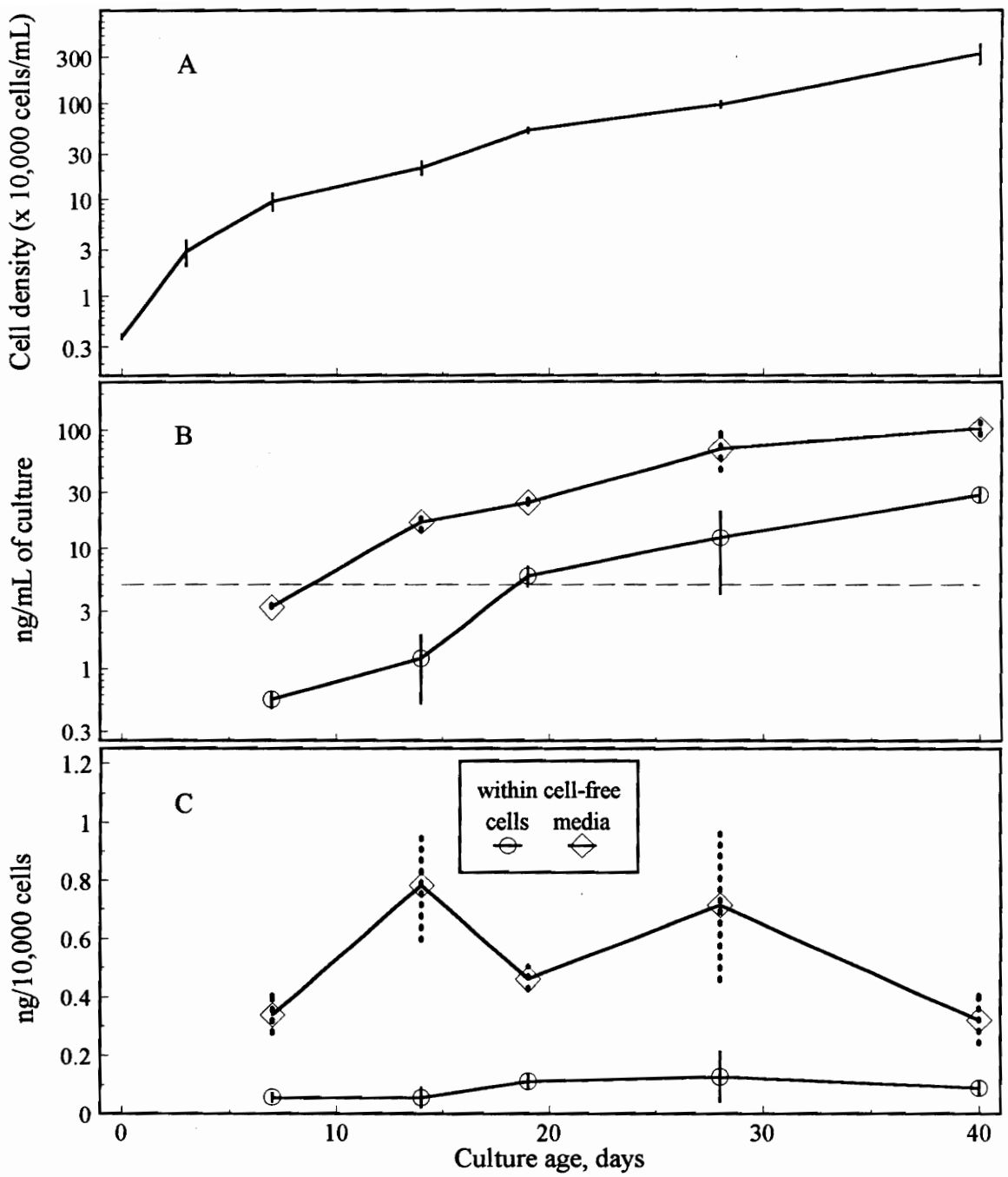


Figure 4.15 Concentrations of MIB associated with *Phormidium* sp. as a function of culture growth: (A) culture growth, (B) concentrations of MIB in cells and cell-free media, and (C) concentrations of MIB produced per cell and either retained or released. Horizontal dashed line in B represents a conservative detection limit. Vertical bars represent ±1 STD for A and B, and the error associated with C as described by Taylor (1982).

Synura petersenii

Synura petersenii produced both 2t,6c-nonadienal (cucumber odor) and 2t,4c,7c-decatrienal (fishy odor). The FPA odor descriptors for this culture were "sweet-melon-cucumber" at very young culture ages (e.g., 3 days) and "fishy-fishtank" at culture ages from 3 to 40 days. The intensities of both the cucumber and fishy odors and the corresponding concentrations of the 2t,6c-nonadienal and 2t,4c,7c-decatrienal in the algal samples were compared to the W-F plot for the 2t,6c-nonadienal standard (Figure 4.16). A standard of the 2t,4c,7c-decatrienal could not be obtained for FPA evaluation. The cucumber odor of 2t,6c-nonadienal was not detected beyond 3 days culture age, even though sufficient 2t,6c-nonadienal was present. Apparently, the cucumber odor was masked by the fishy odor of the 2t,4c,7c-decatrienal. At the times when the FPA panelists actually detected the cucumber odor (i.e., from 0 to 3 days incubation), the culture contained insufficient 2t,6c-nonadienal to be detected by GC-MS analysis.

The *Synura petersenii* growth phase curve is presented in Figure 4.17A. The plots of the concentrations of 2t,4c,7c-decatrienal, both per milliliter of algal culture and per 10,000 cells, are presented in Figures 4.17B and 4.17C, respectively. Other compounds that were frequently identified in algal sample extracts are presented in Table 4.15, while compounds that were detected only sporadically are listed in Table 4.16. The possible significance of these compounds is discussed in a later section.

Synura petersenii retained nearly 90 percent of the 2t,4c,7c-decatrienal (Figures 4.17B and 4.17C) throughout its population growth. From Figure 4.17C, it may be seen that the production of 2t,4c,7c-decatrienal appeared to be the greatest between 7 to 19 days culture age (approximately 10 ng per 10,000 algal cells) and then decreased after the algal culture reached stationary phase (approximately 4 ng per 10,000 algal cells). Although the graphs are not presented, the patterns of 18-carbon fatty acids and 2t,6c-nonadienal production were the same as that of 2t,4c,7c-decatrienal, i.e., the amounts produced (ng/10,000 cells) were greatest at approximately 14 days culture age and then decreased with increased culture age.

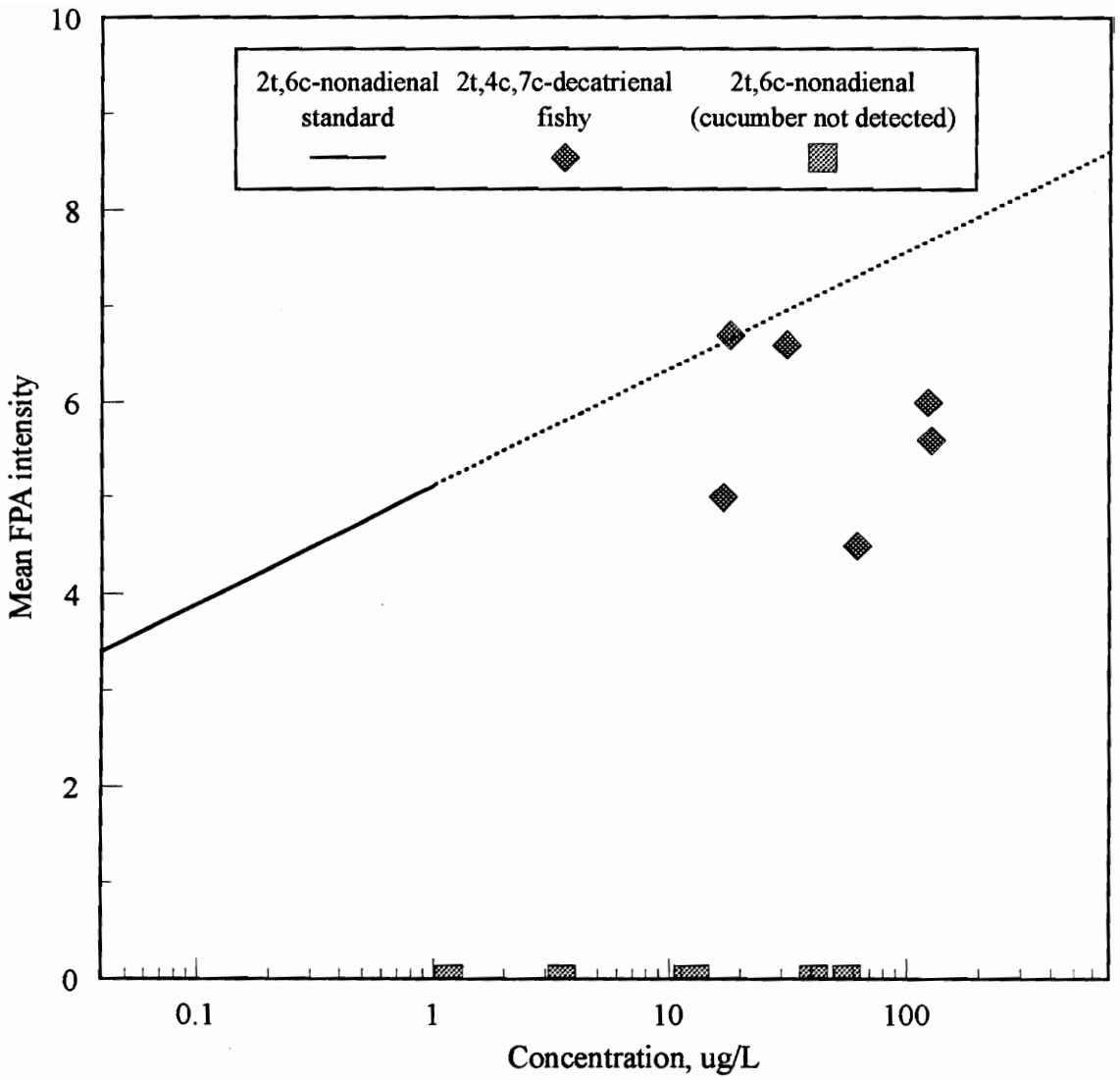


Figure 4.16 Comparison between FPA evaluations of 2t,6c-nonadienal and 2t,4c,7c-decatrienal detected in *Symura petersenii* and those of 2t,6c-nonadienal standards (from Figure 4.1C). The dashed line indicates the theoretical extension of W-F plot for 2t,6c-nonadienal beyond the concentrations evaluated by the FPA panelists.

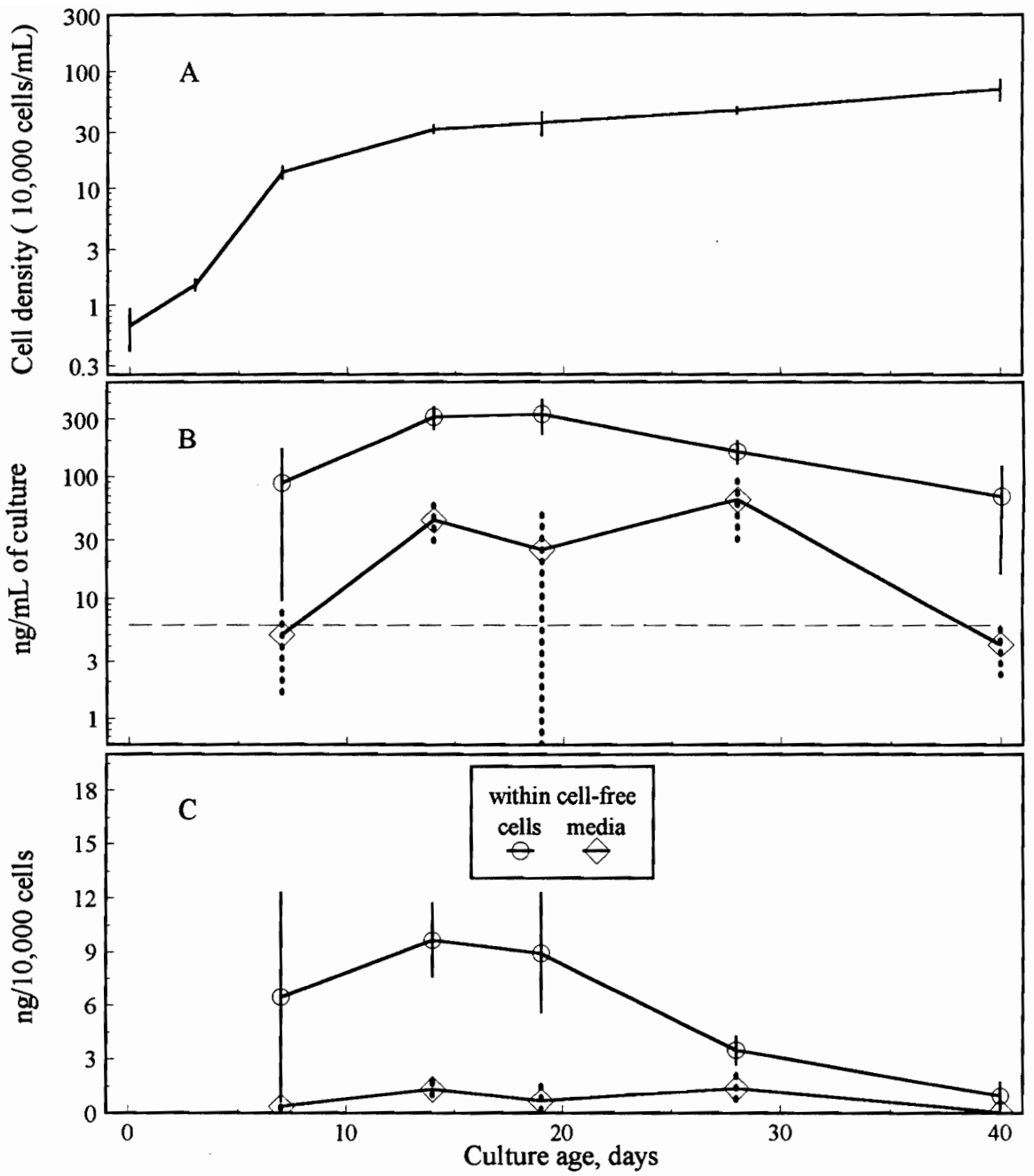


Figure 4.17 Concentrations of 2t,4c,7c-decatrienal associated with *Symura petersenii* as a function of culture growth: (A) culture growth, (B) concentrations of 2t,4c,7c-decatrienal in cells and cell-free media, and (C) concentrations of 2t,4c,7c-decatrienal produced per cell and either retained or released. Horizontal dashed line in B represents conservative detection limit for 2t,4c,7c-decatrienal based upon detection limit for 2t,6c-nonadienal. Vertical bars represent ± 1 STD for A and B, and the error associated with C as described by Taylor (1982).

Table 4.15

Compounds frequently detected in cultures of *Synura petersenii*

Compound	Average production (ng/10,000 cells)	Production range* (ng/10,000 cells)
18C fatty acids†	11.10	0-49.60
2t,4c,7c-decatrienal	6.660	0.231-13.826
2t,6c-nonadienal	2.368	0.023-5.868
myristic acid	8.00	0.91-22.39
palmitic acid	3.35	0.50-9.56
unknown @ 39.5 min‡	5.47	1.33-11.44

* Range of error measurements were determined as described by Taylor (1982)

† Mainly linolenic and linoleic, but also some oleic and stearic acids

‡ Unknown fatty acid with a 39.5 min retention time that is believed to be an 18C fatty acid breakdown product

Table 4.16

Compounds detected sporadically in cultures of *Synura petersenii*

Compound	Location (cells or filtrate)	Identification*
benzoate	cells	s
chlorophene	cells	c
n-heptadecane	cells and filtrate	c
hexadecenoate	cells and filtrate	s
octadecanoate	cells	s
2-phenylphenol	cells	c
squalene	cells	c
unknown @ 15.4 min	cells and filtrate	
unknown @ 22.7 min	cells and filtrate	
unknown @ 35.1 min	cells and filtrate	

* c = confirmed with standard; s = spectral library identification

Critical Population Densities

The term "critical population density," as it was used in this research, is defined as the theoretical population density of algal cells (cells/mL) at which an odor may become perceptible; it was calculated by dividing a compound's threshold odor concentration (μg/L) by the amount of that compound produced by the algae (ng/10,000 cells). The threshold odor concentration was estimated to be the concentration of compound standard in a FPA sample that would evoke an average intensity rating of 2 from the panelists when the sample temperature was 45°C. The actual population density at which an odor is first perceived may be either greater or lower than the critical density because of interactions and masking effects among the other odorous compounds in the sample. The calculated critical population densities of algae studied in this investigation are presented in Table 4.17. These values were calculated for specific compounds that were produced by selected algal cultures. Mallevialle and Suffet (1987) published the critical concentrations of several odorous algae, but the method for estimating the values was different than the method used during this research.

Phenethyl alcohol, benzyl acetate, and β-cyclocitral required relatively high cell densities before their odors were perceived. Isovaleric acid and linolenic acid required moderate cell densities, and geosmin, MIB, and 2t,6c-nonadienal required low cell densities. The high production of MIB by *O.* sp. and of 2t,6c-nonadienal by *S. petersenii*, combined with the very low threshold odor concentrations for these two compounds, resulted in critical cell densities of only 50 and 17 cells/mL, respectively. The FPA of the *O.* sp. algal cultures (Figure 4.10) indicated that a higher concentration of algal cells than indicated by the critical cell density would actually be required to produce perceptible "musty-earthy" odors; however, the required density would still be low (i.e., less than 1,000 cells/mL).

The critical population density associated with 2t,4c,7c-decatrienal was not calculated, because a standard was not available for FPA evaluation. The plot of the 2t,4c,7c-decatrienal data obtained from evaluation of *S. petersenii* samples (Figure 4.16) did not lend itself to a reliable approximation of a threshold odor concentration; however, it can be stated that 10 μg/L of 2t,4c,7c-decatrienal was readily perceived by the FPA panelists as "fishy", with an intensity rating of approximately 6. Both *D. cylindricum* and *S. petersenii* produced large amounts of this compound, 3.92 and 6.66 ng/10,000 cells, respectively. It is very likely that the critical population density associated with this compound is quite low.

Table 4.17

Critical population densities of selected algae that produced odoriferous metabolites

Alga	Compound	Odor* threshold (μg/L)	Average production (ng/10,000cells)	Critical population density (cells/mL)
A. laxa	geosmin	0.010	0.026	3,800
C. peterfii	benzyl acetate	30	0.655	460,000
C. peterfii	isovaleric acid	20	4.44	45,000
C. peterfii	linolenic acid	5	1.70	29,000
C. peterfii	phenethyl alcohol	45	0.93	860,000
D. cylindricum	2t,4c,7c-decatrienal	NA†	3.92	NA†
M. aeruginosa	β-cyclocitral	3	0.105	290,000
M. aeruginosa	linolenic acid	5	1.76	28,000
M. aeruginosa	phenethyl alcohol	45	0.039	11,000,000
O. sp.	linolenic acid	5	10.94	4,600
O. sp.	MIB	0.010	2.017	50
O. tenuis	geosmin	0.010	0.031	3,200
P. sp.	geosmin	0.010	0.129	800
P. sp.	MIB	0.010	0.612	200
S. petersenii	2t,4c,7c-decatrienal	NA‡	6.660	NA†
S. petersenii	2t,6c-nonadienal	0.004	2.368	17

* Odor thresholds were determined by FPA panel evaluations of compound standards

† This compound's threshold odor concentration was not determined

‡ At a concentration of 10 μg/L, the odor was readily perceived by the FPA panel as "fishy", and the odor intensity was rated at approximately 6.

POSSIBLE SIGNIFICANCE OF THE VARIOUS ORGANIC COMPOUNDS

More than sixty compounds were detected during the GC-MS analyses of the algal culture extracts; however, not all of them were confirmed or even identified. Several compounds (e.g., toluene, cyclohexanone, acetophenone, decanal, and phthalates) were detected in virtually every sample. These compounds were most likely artifacts; examples of their possible sources are as follows: (1) the distilled water used to make the media (e.g., decanal), (2) the acetone contained two peaks that eluted shortly after completion of the delay time that was selected to by-pass the acetone and methylene chloride solvent peaks (these peaks were detected in the analyses of both compound standards and extracted samples), (3) the methylene chloride (e.g., cyclohexanone), (4) the extraction method (e.g., an unidentified compound that occurred in the analyses of all extracted samples, but not in the analyses of compound standard solutions that were not extracted), and (5) either the phenolic resin culture-tube caps or the media (e.g., phthalates). This section discusses those compounds that were determined to be algal metabolites, and whose identities were either confirmed with standards or assumed on the basis of their mass spectra and retention times relative to the confirmed compounds.

Alkanes and Alkenes (Hydrocarbons)

Several n-alkanes (17-, 18-, 19-, 20-, 21-, and 22-carbon chains) and alkenes (1-hexadecene) have been extracted from both surface water samples (Slater and Blok 1983b) and surface water samples taken during bloom conditions (Hayes and Burch 1989). A variety of green and blue-green algae produce these compounds (Materassi et al. 1980; Borowitzka 1988b). Materassi et al. (1980) stated that n-heptadecane was a major component of the algae they examined. Alkanes and alkenes were also detected in cultures of *Synura uvella* (Cranwell et al. 1988), with n-heptadecane and heptadecene being identified as major components. Hayes and Burch (1989) identified n-heptadecane as a major component of both *Oscillatoria* sp. and *Anabaena flos-aquae* in samples collected during surface water algal blooms. Odor thresholds, which were evaluated in oils, were greater than 13,000 mg/L for aliphatic hydrocarbons that contained 12 or more carbon atoms (Forss 1973); therefore, it is unlikely that these compounds contribute to odors in surface waters.

The algae studied in this investigation retained within their cells most of the hydrocarbons that were produced. The significance of these compounds is not known. The identities of the 17- to 21-carbon n-alkanes, heptadecene, and squalene were confirmed with standards, while the identities of the other alkanes and alkenes that were detected were assumed on the basis of both their mass spectra and retention times relative to the confirmed compounds. No literature references were found that mentioned the presence of squalene in algal cell extracts; however, The Merck Index (1989) stated that squalene, which is constructed of six isoprene units, has been detected in shark oil in large quantity and also in yeast, olive oil, rice bran oil, and wheat germ oil.

Aldehydes (2-furfural, 2t,6c-nonadienal, and 2t,4c,7c-decatrienal)

2-furfural

The 2-furfural may be produced by condensation of aliphatic aldehydes (Arctander 1969), and was reported to have "sweet, bread-like, caramellic, cinnamon-almond-like" odors. Arctander (1969) also stated that the compound was "widely used in flavor compositions for imitation butter, molasses, fruit, nut, cinnamon" and other flavors. The concentration of 2-furfural in these products ranged from 1 to 30 mg/L.

This compound was detected only in the cell-free media extracts of *Anabaena laxa* and *Phormidium* sp. Possible functions or environmental impacts of the compound are unknown; however, if 0.06 grams of 2-furfural are either ingested or inhaled by a human, severe headaches may result (Arctander 1969). No literature references were found that mentioned the presence of 2-furfural in either algal or surface-water samples.

2t,6c-nonadienal

Hayes and Burch (1989) detected 2t,6c-nonadienal in extracts of *Synura uvella* samples that were collected from a reservoir during bloom conditions. They suggested that the compound was formed by oxidative cleavage of unsaturated fatty acids that had been produced by the alga. Grosch and Schwarz (1971) demonstrated that 2t,6c-nonadienal in cucumbers was derived from linolenic acid. Whitfield and Last (1991) cited several publications that described potential oxidation mechanisms of linolenic acid, with

enzymatic oxidation as the most likely method. It is possible that lipoxygenase produced by *Synura* oxidizes the linolenic acid and forms 2t,6c-nonadienal.

The threshold odor concentration of 2t,6c-nonadienal determined during this study was approximately 4 ng/L, which agreed with values obtained by others [e.g., 10 ng/L by Whitfield and Last (1991), and from 2 to 5 ng/L by G. Burlingame, Philadelphia Water Department (personal communication)]. *Synura petersenii* produced approximately 2.4 ng per 10,000 algal cells, and the greatest production occurred during log-phase growth. Most of the 2t,6c-nonadienal was retained within the algal cells; therefore, removal of the algal cells from intake water in a water treatment plant prior to oxidation would reduce the potential of odor problems. The critical cell density for this alga was only 17 cells/mL (Table 4.17), which makes its early detection in surface waters imperitive if odor episodes are to be minimized. A single Synura colony can consist of more than 17 cells; therefore, the cause of a fishy-odor episode could be easily missed during the examination of surface-water samples.

2t,4c,7c-decatrienal

This aldehyde, like 2t,6c-nonadienal, is formed by the oxidation of unsaturated fatty acids; specifically, linolenic acid (Frankel 1982). Ke et al. (1974) detected 2,4,7-decatrienals in oxidized Mackerel oils. Karahadian and Lindsay (1989) detected both 2t,4t,7c- and 2t,4c,7c-decatrienal in headspace analyses of a commercial cod liver oil.

The 2t,4c,7c-decatrienal was detected in the extracts of both *Synura petersenii* and *Dinobryon cylindricum* during this investigation, which was the first time that the compound has been isolated from algae. Both algae produced large amounts of linolenic acid, which may be the precursor to the 2t,4c,7c-decatrienal. The identity of 2t,4c,7c-decatrienal was not confirmed with a standard because a commercial source was unavailable; however, the retention time was appropriate relative to known compounds in the samples and the mass spectra (Appendix E, Figure E.8) matched that of a compound identified by C. Karahadian (Purdue University, Dept. of Foods and Nutrition, personal communication) as 2t,4c,7c-decatrienal that was confirmed by headspace analysis to have a "fishy" odor .

The FPA panel did not determine an odor threshold concentration for 2t,4c,7c-decatrienal because the compound was unavailable; however, Hsieh and Kinsella (1989)

cited a study where the flavor threshold concentration was 150 µg/L. The odor threshold concentration likely is considerably less than 150 µg/L, because the "fishy" odor intensities that were assigned to the culture samples evaluated by the FPA panelists were quite high even at 10 µg/L (Figure 4.16).

Synura petersenii produced more 2t,4c,7c-decatrienal than 2t,6c-nonadienal (6.7 ng and 2.4 ng per 10,000 algal cells, respectively). *Dinobryon cylindricum* produced 2t,4c,7c-decatrienal (approximately 3.9 ng per 10,000 algal cells) but no 2t,6c-nonadienal. As with 2t,6c-nonadienal, most of the 2t,4c,7c-decatrienal that was produced by both *Synura petersenii* and *Dinobryon cylindricum* was retained within the algal cells throughout their population growth; therefore, removal of the algal cells from intake water in a water treatment plant prior to oxidation would help reduce potential odor problems.

Acids

Acids (benzyl acetate, nonanoate, dodecanoate, and isovalerate)

Slater and Blok (1983b) detected nonanoate in surface water samples that were extracted by CLLE. Rezanka et al. (1983) detected nonanoate and dodecanoate in extracts of several green and blue-green algal cultures. Cranwell et al. (1988) detected dodecanoate in cultures of *Dinobryon divergens*, but not in *Synura uvella*. No references were found that mentioned the presence of either benzyl acetate or isovalerate (a.k.a. 3-methyl butyrate) in algal extracts.

During this investigation, nonanoate (identity confirmed) and dodecanoate (identity assumed) were detected sporadically in the cell and cell-free media extracts of the blue-green algae *M. aeruginosa*, *Oscillatoria* sp., and *O. tenuis*. Benzyl acetate and isovalerate were detected in the cell-free media extracts of axenic *C. peterfii* cultures that were grown in OCM medium that contained peptone. The identification of the compounds was confirmed with purchased standards, and their odors were described as "artificial banana" and "rancid-dirty socks", respectively. It is likely that both compounds were formed when the algae metabolized the peptone, and it also is possible that these compounds can be formed in surface waters when the algae metabolize naturally occurring organics. One possible explanation of why these compounds have not been detected in surface waters is that low-molecular-weight compounds are readily metabolized by bacteria (see Literature Review).

Fatty Acids

Slater and Blok (1983b) detected myristic and palmitic acid in CLLE extracts of surface-water samples. Numerous fatty acids were detected in the extracts of four freshwater chrysophyte cultures (Cranwell et al. 1988) and in selected green and blue-green algal cultures (Rezanka et al. 1983). Kenyon et al. (1972) investigated the fatty acid composition of selected filamentous blue-green algae. Further discussion of the fatty acid content of algae was presented in the Literature Review.

A diversity of fatty acids was detected in the extracts of the algae selected for this investigation. The identities of myristic, palmitic, linoleic, and linolenic acid were confirmed with standards, while the identities of hexadecenoate, isopropyl myristate, isopropyl palmitate, stearic acid, and oleic acid were based on their mass spectra and retention times relative to the confirmed compounds.

Synura petersenii and *D. cylindricum* produced large amounts of the 18-carbon fatty acids (predominantly linoleic and linolenic acid but also some stearic and oleic acid) and lesser amounts of myristic and palmitic acid. They also produced two compounds that were identified only as "unknown @ 39.3 min" and "unknown @ 39.5 min" but that likely were decomposition products of the 18-carbon fatty acids. *Anabaena laxa* produced palmitic acid and small amounts of both isopropyl myristate and isopropyl palmitate. *Chlamydomonas peterfii* produced both palmitic and linolenic acid. *M. aeruginosa* produced linolenic, linoleic, and palmitic acid. *Oscillatoria* sp. produced relatively large amounts of linoleic, palmitic, and linolenic acid, and a small amount of myristic acid. *Oscillatoria tenuis* and *Phormidium* sp. produced only minor amounts of fatty acids. The FPA panelists described the odor of linolenic acid as "sweet-melon-watermelon".

As was previously discussed in the Literature Review, the oxidation of fatty acids (especially linolenic and linoleic acid) produces a variety of compounds. Two compounds produced when linolenic acid is oxidized are 2t,6c-nonadienal and 2t,4c,7c-decatrienal, both of which were discussed earlier in this section. *Oscillatoria* sp. produced large amounts of linolenic acid, but neither 2t,6c-nonadienal nor 2t,4c,7c-decatrienal were ever detected in the extracts of this alga. It is likely that the alga lacks the enzyme(s) required for their formation.

Alcohols (3-methylthio-1-propanol, phytol, and myrcenol)

The 3-methylthio-1-propanol was detected in only the cell-free extracts of 40-day old *M. aeruginosa* cultures, and its identity was confirmed with a purchased standard. Unofficial odor descriptors (i.e., FPA panelists did not evaluate the compound in a formal FPA session) applied to the compound were "German potato salad", "vinegary", "potatoes", and "unpleasant". The 3-methylthio-1-propanol may have contributed to the less-pleasant odor descriptors applied to older *M. aeruginosa* samples.

The Merck Index (1989) described phytol as a chlorophyll decomposition product. This compound was detected frequently in the extracts of *A. laxa*, *C. peterfii*, *Oscillatoria* sp., and *M. aeruginosa*. It was mainly located in the cells, but lesser amounts were also detected in the cell-free extracts. A purchased standard confirmed the identification of the compound. Phytol was not evaluated by FPA, but unofficial evaluation did not detect any significant odor.

A compound that was assumed to be myrcenol (based on the spectral library identification) was detected somewhat sporadically in both cell and cell-free media extracts of *O. tenuis* cultures and very sporadically in cell-free extracts of *Phormidium* sp. cultures. Arctander (1969) described myrcenol as a terpene with an interesting odor, but also with a limited shelf-life because it polymerized readily. It is possible that the compound is an intermediate in geosmin production; however, confirmation of the compound's identity should precede further speculation on its function.

Cyclic Compounds

2-Methylisoborneol (MIB)

The musty-smelling compound, MIB, was produced by *Oscillatoria* sp. and *Phormidium* sp. The average amounts produced by the two algae were 2.01 ng per 10,000 cells (Table 4.9) and 0.61 ng per 10,000 cells (Table 4.13), respectively. The *Oscillatoria* retained approximately 50-70 percent of the MIB it produced, while the *Phormidium* released more than 80 percent of the MIB into the medium. The differences between the two algae in both their compound production and release resulted in actual

concentrations of MIB in the respective cell-free media extracts (ng per mL) that were rather similar (Figures 4.11B and 4.15B). Because the *Oscillatoria* sp. retained more MIB than the *Phormidium* sp. retained, removal of *Oscillatoria* sp. algal cells prior to oxidation in a water treatment plant would likely be a more effective method by which to lessen the potential for odor problems than if the offending alga were *Phormidium* sp.

The odor intensities obtained from the evaluation of MIB standards were more-closely duplicated by the intensities obtained from the evaluations of the *Phormidium* sp. culture samples than those from the *Oscillatoria* sp. culture samples. As stated previously, the compounds (i.e., linolenic acid) that contributed to the "sweet-melon" odors associated with the *Oscillatoria* sp. samples may have interferred with the panelists' abilities to perceive the "earthy-musty" odors, the result being the lower-than-expected odor intensities for the MIB concentrations actually present. Linolenic acid likely did not interfere with the evaluations of *Phormidium* sp., because none was detected in the culture extracts.

Geosmin

Geosmin was produced by *A. laxa*, *O. tenuis*, and *Phormidium* sp. The average amounts of geosmin produced by these algae were approximately 0.03 ng per 10,000 cells by both *A. laxa* and *O. tenuis* (Tables 4.1 and 4.11, respectively) and 0.13 ng per 10,000 cells by *P.* sp. (Table 4.13). The FPA evaluations of the *A. laxa* and *O. tenuis* cultures were presented (Figures 4.3 and 4.12, respectively) and the results were discussed previously.

The relatively large amounts of MIB produced by *Phormidium* sp. prevented an evaluation of the odors contributed by geosmin; however, it is likely that the "earthy-corn-vegetable-cucumber-grassy-musty" odors perceived in 40-day old cultures (Figure 4.14) were caused by geosmin. Other compounds that may have have contributed either "vegetable" or "cucumber" odors (e.g., 2t,6c-nonadienal or linolenic acid) were not detected in extracts of *Phormidium* sp.

The amount of geosmin retained within the algal cells differed among the three algae. *Phormidium* sp. released approximately 80 percent of the geosmin it produced, *O. tenuis* retained approximately 50 percent, and the retention of geosmin by *A. laxa* changed

with culture age (i.e., more was retained by young cultures, and more was released by older cultures). Similar changes in geosmin retention as a function of culture age were observed by Rosen et al. (1992) who studied a culture of *Anabaena circinalis*.

Aldehydes (β-cyclocitral, dehydro-β-cyclocitral, and hydroxy-β-cyclocitral)

The cyclic aldehydes β-cyclocitral, dehydro-β-cyclocitral, and hydroxy-β-cyclocitral were produced by *M. aeruginosa*, and greater than 95 percent of each compound was retained by the algal cells. The identity of β-cyclocitral was confirmed with a purchased standard. The FPA panelists described the odor of β-cyclocitral as "sweet-fruity-chocolate-pipe tobacco", with the descriptors "sweet" and "pipe tobacco" being applied most regularly. The hydroxy-β-cyclocitral was neither confirmed with a standard nor was there an associated mass spectra library match; however, the compound's mass spectra (Appendix E, Figure E.7) and retention time (i.e., a few minutes longer than that of β-cyclocitral) indicated that the likely identity of the compound was hydroxy-β-cyclocitral.

The possible concentrations of hydroxy-β-cyclocitral in the extracts were calculated by applying the response factors obtained for β-cyclocitral, because the response factors associated with similar compounds would be expected to be similar. The hydroxy-β-cyclocitral concentrations in the algal cells were approximately twice those of β -cyclocitral. No literature references that described the odor of hydroxy-β-cyclocitral were found.

The GC-MS peaks associated with the dehydro-β-cyclocitral (a.k.a. safranal) detected in the cell extracts were much smaller than those of the other two compounds, and it likely was present in lower concentrations. Arctander (1969) described the odor of dehydro-β-cyclocitral as "sweet", "green-floral", and "tobacco-herbaceous". The compound was not detected in the cell-free media extracts. Three other compounds that were detected sporadically were assumed to be related to β-cyclocitral, because their mass spectra were similar. These compounds were identified only as "unknown @ 10.8 min", "unknown @ 13 min", and "unknown @ 15.7 min". Possible odors associated with these compounds are not known.

Acids (benzoate and benzyl acetate)

The Merck Index (1989) stated that benzoate is used as an antifungal agent, and Arctander (1969) stated that the pure compound is odorless. Benzoate was detected sporadically in *Synura petersenii* cell extracts during this investigation. Its function, if any, is unknown.

Benzyl acetate is used extensively in perfumes and its odor was described by Arctander (1969) as "sweet floral, fresh, light, and fruity." The FPA panelists described the odors of the purchased standard as "sweet-artificial banana." The compound was detected during this investigation in the cell-free media extracts of axenic *Chlamydomonas peterfii*. Benzyl acetate may have contributed to the pleasant, meadow-like odors of the *C. peterfii* culture.

Alcohols (benzyl alcohol and phenethyl alcohol)

During this investigation, benzyl alcohol was detected frequently in the cell and cell-free media extracts of axenic *C. peterfii*, with the greater concentrations being detected in the cell-free media (4.3 ng/10,000 cells). It was detected sporadically in the cell extracts of *Microcystis aeruginosa*. Although it has a nondescript odor, benzyl alcohol is "used in perfumery as a blender" (Arctander 1969). The identity of the compound was confirmed with a purchased standard.

Aldrich (1994) described the odor of phenethyl alcohol as rose, floral, fragrant, and honey, and Arctander (1969) stated that is was "one of the most widely used of all perfume chemicals." Hayes and Burch (1989) detected minor amounts of phenethyl alcohol in extracts of *M. aeruginosa* samples that were obtained from a reservoir.

The FPA panelists described the odors of phenethyl alcohol standards as "floral-rose." It was detected in both the cell and cell-free media extracts of *M. aeruginosa*, with the greater amounts of the compound being located in the cell-free media (0.04 ng/10,000 cells). Larger amounts of the compound (0.52 ng/10,000 cells) were detected in the cell-free media extracts of axenic *C. peterfii* grown in OCM media that contained peptone. The compound likely was produced during algal assimilation of the peptone, because it was not detected in cultures grown in OCM media that did not contain peptone. The odors ascribed to phenethyl alcohol make it very likely that the compound contributed to the pleasant, meadow-like odors of the algal culture.

Phenolic Compounds (diphenylamine, chlorophene, and 2-phenylphenol)

The identities of these compounds were confirmed with purchased standards. During this investigation, diphenylamine was detected sporadically in the cell-free media extracts of *M. aeruginosa*. Unofficial odors ascribed to it were "floral-artificial rose."

The Merck Index (1989) stated that chlorophene is used as a disinfectant and that 2-phenylphenol is a germicide-fungicide. Unofficial odors (i.e., FPA panelists did not evaluate the compound in a formal FPA session) ascribed to these compounds included "chlorinous, musty, solvent (formaldehyde), and straw" for chlorophene; and "pool odors (e.g., musty, humid, chlorinous), formaldehyde, and vegetable-overtones" for 2-phenylphenol.

Chlorophene and 2-phenylphenol are structurally similar compounds. They were both detected (usually sporadically) in the early- to mid-log-phase cell extracts of *A. laxa*, *M. aeruginosa*, *Oscillatoria* sp., *O. tenuis*, *Phormidium* sp., and *S. petersenii* cultures. The possible anti-microbial function of these compounds was not investigated.

INFLUENCE OF ENVIRONMENTAL FACTORS ON ALGAL POPULATION GROWTH AND ODOROUS COMPOUND PRODUCTION

The multiple regression analyses and Friedman tests (parametric and two-way layout nonparametric analyses, respectively) were performed with SYSTAT 5.02 (Systat Inc., Evanston, IL). The multiple comparisons among treatments were performed according to Hollander and Wolfe (1973). The experiments were designed such that the treatments would be exactly the same except for the one variable being altered for that particular treatment; however, as can be seen in Table 3.2, small variations in the light intensity and the nitrogen and phosphorus concentrations did occur among the treatments. The determination that differences in culture growth and compound production occurred among the treatments was valid; however, the assumption that the differences were due solely to that variable being evaluated by a particular treatment was questionable and complicated the interpretation of the nonparametric analyses.

The multiple regression model applied to the algal data was:

$$Y = \beta_0 x_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \beta_8 x_8 + \beta_9 x_9 + \varepsilon$$

where Y = Population density (cells/mL) or compound production (ng/10,000 cells)

$\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6, \beta_7, \beta_8, \beta_9$ = unknown coefficients (the units are defined in Table 4.19) that are associated with their respective x-values

x_0 = multiple regression model constant (unitless)

x_1 = culture age (days)

x_2 = light intensity ($\mu E/m^2/s$)

x_3 = temperature (°C)

x_4 = age * age, a quadratic term that was included in the model to account for a possible parabolic curve in the Y-data (i.e., Y-data values increased and then decreased with respect to increased culture ages)

x_5 = Initial NO_3-N, the media concentration of NO_3-N in mg/L at t_0

x_6 = Initial PO_4-P, the media concentration of PO_4-P in mg/L at t_0

x_7 = N-to-P ratio, the unitless ratio of initial N and P concentrations at t_0

x_8 = Initial NO_3-N * Initial AHP, an interaction term between the initial concentrations of NO_3-N and AHP in DY III media at t_0

x_9 = Initial NH_3-N * Initial AHP, an interaction term between initial concentrations of NH_3-N and AHP in DY III media at t_0

ε = The random error or residual that represents all other factors that were not included in the model but which may have affected the response variable, Y

The significance of each of the environmental variables (x-values) included in the multiple regression model was evaluated following the guidelines presented in Table 4.18. The units for the various multiple regression model coefficients (β-values), which were obtained by applying the above model, are presented in Table 4.19. The regressions applied the nutrient concentrations and N-to-P ratios that were obtained at t_0. In all cases, the significance of each of the model variables was determined for the model only as written above and over the concentrations evaluated. Other models and concentrations would likely produce different results.

The results obtained from the light and temperature experiments may be applied to the environment; however, the direct application of the results obtained from the statistical evaluations of the nutrient treatments to environmental conditions may be inappropriate because the nitrogen and phosphorus concentrations in the media were higher than those likely to be observed in natural situations. The statistical evaluations of the nutrients, however, can indicate the relative importance of the various environmental conditions on both population growth and compound production.

Table 4.18
Evaluation of multiple regression model p-values

p-value	Evaluation
$p > 0.1$	likely not significant
$0.05 < p < 0.1$	possibly significant
$p < 0.05$	significant

Table 4.19

Units of regression model coefficients

Model variables (x-values)	Units of β-values	
	Population density (Y = Cells/mL)	Compound production (Y = ng/10,000 cells)
constant	log cells/mL	ng/10,000 cells
age	log cells/mL/day	ng/10,000 cells/day
light intensity	(log cells/mL)/(μE/m^2/s)	(ng/10,000 cells)/(μE/m^2/s)
temperature	log cells/mL/°C	ng/10,000 cells/°C
age * age	log cells/mL/day^2	ng/10,000 cells/day^2
initial NO_3-N	(log cells/mL)/(mg/L)	(ng/10,000 cells)/(mg/L)
initial PO_4-P	(log cells/mL)/(mg/L)	(ng/10,000 cells)/(mg/L)
N to P ratio	log cells/mL	ng/10,000 cells
(initial NO_3-N * initial AHP)	(log cells/mL)/$(mg/L)^2$	(ng/10,000 cells)/$(mg/L)^2$
(initial NH_3-N * initial AHP)	(log cells/mL)/$(mg/L)^2$	(ng/10,000 cells)/$(mg/L)^2$

ANABAENA LAXA

Figure 4.18 presents the algal population growth curves for *Anabaena laxa* obtained from the six different treatment schemes previously described (Table 3.2). The growth curves are very similar for regular ASM-1, low-temperature, and mid-nitrogen treatments. The growth observed during the low-phosphorus treatment was similar, also, for the first 14 days of incubation; then, the population cell density decreased. The algal population attained the greatest cell densities during the low-nitrogen treatment, possibly a result of the nitrogen-fixation capability of the alga. The ratio of the number of heterocysts to the number of algal cells, however, did not indicate that there were more heterocysts available in the low-nitrogen treatment algal cultures than in those of the mid-nitrogen treatment. Although the ratio increased over the duration of both the low- and mid-nitrogen experiments, it remained comparable between the two treatments. It is possible that there is a critical nitrogen concentration below which the heterocysts either begin to fix molecular nitrogen or become more active. Further investigation of this possibility was beyond the scope of this investigation. The culture grew slowly, but steadily, under low-light conditions. The final densities after 40-days incubation were similar in all cultures except the one with low phosphorus concentrations.

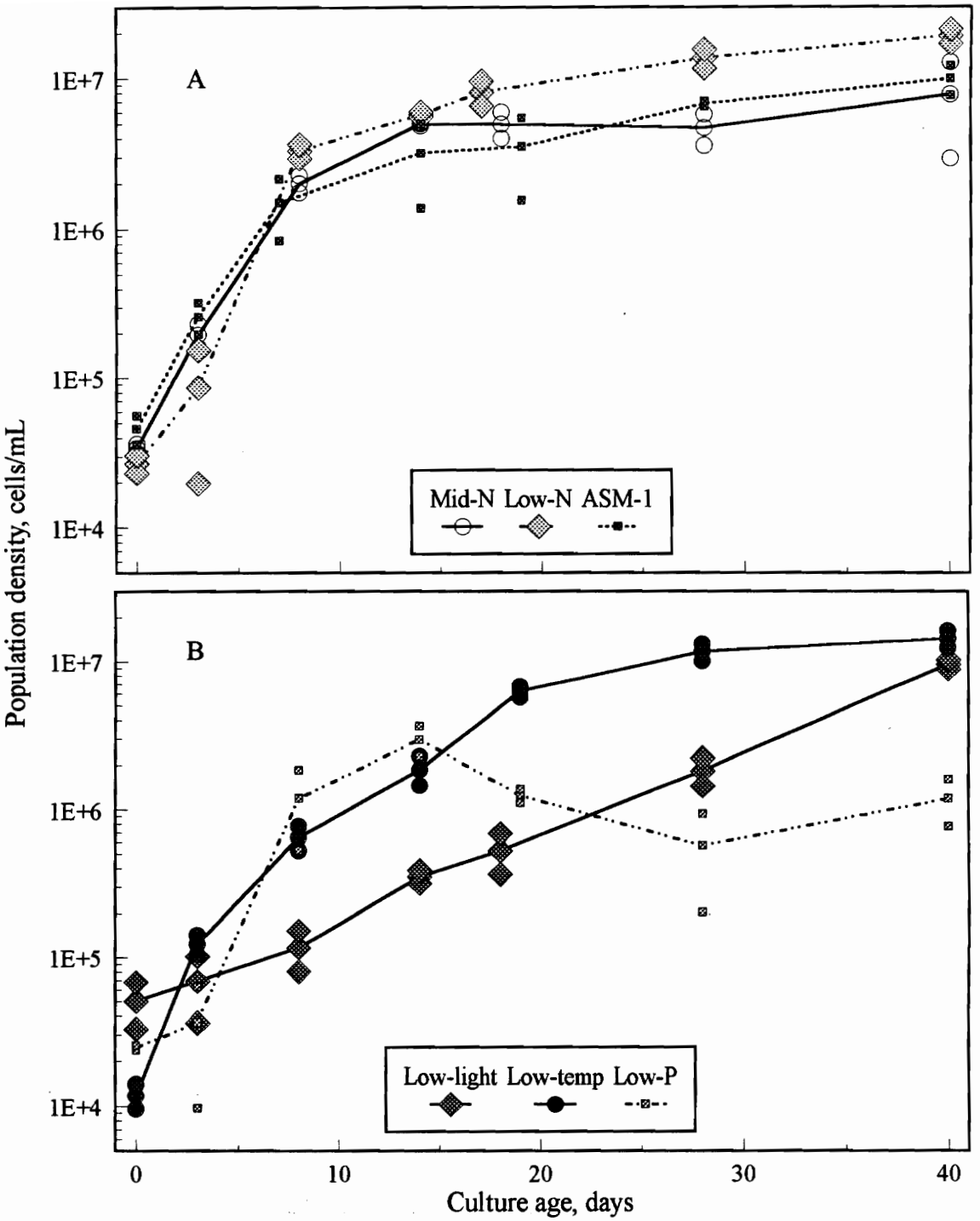


Figure 4.18 *Anabaena laxa* population growth curves grown under six different treatment schemes: (A) mid-N, low-N, and regular ASM-1, and (B) low-light, low-temperature, and low-P ASM-1 (see Table 3.2).

Nonparametric Evaluations of Treatment Effects on *Anabaena laxa*

The Friedman two-way layout results and multiple comparison analyses for *A. laxa* are presented in Table 4.20. The nonparametric results indicated that both the population cell densities ($p = 0.008$) and the total geosmin production ($p = 0.068$) were significantly different among the treatments. The rank sum that was calculated for each treatment (Table 4.20) indicates how the treatments compared to each other. A relatively large rank sum means that the values (i.e., either population density or total geosmin production) attained during that treatment were greater than the values attained during a treatment that had a smaller rank sum.

The multiple comparisons test evaluated the absolute differences in the rank sums obtained by the treatments. The solid line beneath the treatment names (Figure 4.20, multiple comparisons section) means that the values attained during those treatments were not significantly different from each other at the indicated α-level. The population cell densities that were attained during the low-phosphorus and low-light treatments were significantly different than those attained during the low-nitrogen treatment at the $\alpha = 0.049$ confidence level. Total geosmin productions in the cultures subjected to low-light were significantly different than those produced during the mid-nitrogen treatments, also at the $\alpha = 0.049$ confidence level.

As previously stated, further interpretation of the nonparametric results was confounded by the fact that more than one variable was altered within a treatment. The nonparametric results, however, can be compared to the results obtained by the multiple regression analyses in the sense that they will either agree or disagree. Nonparametric methods are more robust (i.e., less influenced by non-conformance with statistical assumptions) than parametric methods; therefore, there tends to be greater assurrance in the validity of parametric results (e.g., light intensity was a significant variable) when their results agree with those of the nonparametric analyses.

Multiple Regression Evaluations of Population Growth and Geosmin Production by *Anabaena laxa*

The regression analyses evaluated four dependent variables: (1) cell density, (2) intracellular geosmin, (3) extracellular geosmin, and (4) total geosmin production. The multiple regression results are presented in Table 4.21 through Table 4.24. The data used for the regressions are presented in Appendix F, Table F.1.

Table 4.20

Nonparametric results for *Anabaena laxa*

Treatment	Rank sum for population cell density	Rank sum for total geosmin production
low light (LL)	16.0	15.0
low temperature (LT)	24.0	23.0
low phosphorus (LP)	13.0	27.0
mid nitrogen (MN)	28.0	35.0
low nitrogen (LN)	36.0	28.0
regular DY III (Reg)	30.0	19.0
Friedman's S	15.490	10.265
P	0.008	0.068
	Multiple comparisons*	
Treatments: (for k = 6, n = 7, and α = 0.049)	LP LL LT MN Reg LN	LL Reg LT LP LN MN
Conclusion:	LP, LL ≠ LN	LL ≠ MN

* The lines beneath the treatments indicate that the attained values were not significantly different

Population density (cells/mL)

The multiple regression analysis that evaluated population density (Table 4.21) indicated that nutrient concentrations were not factors in the attained population densities; however age, age^2, and light intensity were significant. Phosphorus concentration was not significant in the regression; however, Figure 4.18B showed that the population density in the media containing low-phosphorus concentrations declined after 14 days of incubation, likely a result of lack of PO_4-P. The nonparametric analyses also indicated that lower population densities were attained under low-phosphorus conditions (Table 4.20). Further experiments at lower phosphorus concentrations may result in phosphorus being significant to the model. Nitrogen concentration was not a factor in determining the population density attained, likely because *A. laxa* can utilize molecular nitrogen.

Temperature also was not significant, and, as was seen in Figure 4.18, the alga grew as well at 15°C as it did at 25°C.

Light was a significant variable in that increased light intensity resulted in increased population density; however, the coefficient (Table 4.21) was much less than that for age, and, as was seen in Figure 4.18, the population growth at low-light was slow, but steady, over the entire 40-day incubation period. The alga did not reach stationary phase at 14-18 days as it did during the other treatments; therefore, in natural low-light situations, the alga could create problems over a longer period of time. *A. laxa*, as well as other blue-green algae, is capable of buoyancy regulation (Rijn and Shilo 1985); therefore, it may adjust it's depth below the surface and thereby improve the conditions for photosynthesis. If the alga is at a depth at which rapid population growth may occur, the population may be expected to grow rapidly for 14-18 days if other environmental requirements are met, then reach stationary phase.

Table 4.21

Anabaena laxa population density regression results

Dependent variable cell density (log cells/mL)	R-squared = 0.857	Adjusted R-squared = 0.828	Standard error of estimate = 0.386
Model variables	Coefficient	T	P (2 tail)
constant	4.410	5.450	< 0.001
age	0.146	9.392	< 0.001
light intensity	0.026	2.796	0.008
temperature	0.007	0.365	0.718
age * age	-0.002	-6.138	< 0.001
initial NO_3-N	0.006	0.516	0.609
initial PO_4-P	-0.109	-0.641	0.526
N to P ratio	-0.019	-1.289	0.206
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	30.4	7	< 0.001
residual	5.06	34	

Intracellular, extracellular, and total geosmin production (ng/10,000 cells)

The intracellular geosmin production (ng/10,000 cells) was fairly consistent among the treatments (Appendix F, Table F.1), and only age, age^2, and initial nitrogen were significant in the regression (Table 4.22). The multiple regression analysis that evaluated extracellular geosmin (Table 4.23) indicated that both the culture age and the N-to-P ratios were possibly significant to the model; and that the model itself was only fair, when judged by both the adjusted R-squared (i.e., a low value even for a biological system) and the sum-of-squares. The algal data (Appendix F, Table F.1) show that during the low-phosphorus treatment the extracellular geosmin concentrations (ng/mL) were greater than the intracellular geosmin concentrations. This was the only treatment during which extracellular geosmin production (ng/10,000 cells) and concentrations (ng/mL) exceeded those of intracellular geosmin. It is possible, therefore, that relatively greater geosmin production occurs in surface waters when the phosphorus concentrations are reduced; however, as was previously discussed, the algal population densities were significantly lower during the low-phosphorus treatment.

Table 4.22
Anabaena laxa intracellular geosmin regression results

Dependent variable intracellular geosmin (ng/10,000 cells)	R-squared = 0.622	Adjusted R-squared = 0.545	Standard error of estimate = 0.024
Model variables	Coefficient	T	P (2 tail)
constant	0.028	0.561	0.579
age	0.005	4.909	< 0.001
light intensity	-1.75×10^{-4}	-0.297	0.768
temperature	-0.002	-1.495	0.144
age * age	-9.06×10^{-5}	-3.859	< 0.001
initial NO_3-N	-0.002	-2.196	0.035
initial PO_4-P	0.008	0.777	0.443
N to P ratio	3.94×10^{-4}	0.441	0.662
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	0.032	7	< 0.001
residual	0.020	34	

The extracellular geosmin regression results indicated that although the N-to-P ratios were possibly significant, neither initial nitrogen nor initial phosphorus concentrations were significant. The significance of the N-to-P ratios may have occurred because there was greater treatment variety attained in the N-to-P ratios, with roughly four ratios being evaluated: (1) normal ASM-1 concentrations (e.g., the regular ASM-1, low-temperature, and low-light treatments), (2) a low level of nitrogen, (3) a middle level of nitrogen, and (4) a reduced level of phosphorus. The initial N-to-P ratios ranged from approximately 0.5 to 100, representing the low-nitrogen and the low-phosphorus treatments, respectively. The regression coefficients for the N-to-P ratios did support the observation, which was previously discussed, that greater amounts of extracellular geosmin were detected in the cultures that contained the lower phosphorus concentrations.

Table 4.23

Anabaena laxa extracellular geosmin regression results

Dependent variable extracellular geosmin (ng/10,000 cells)	R-squared = 0.490	Adjusted R-squared = 0.385	Standard error of estimate = 0.030
Model variables	Coefficient	T	P (2 tail)
constant	-0.086	-1.354	0.185
age	0.002	1.921	0.063
light intensity	-4.56×10^{-4}	-0.616	0.542
temperature	1.95×10^{-4}	0.137	0.892
age * age	-2.69×10^{-5}	-0.909	0.370
initial NO_3-N	-0.002	-1.669	0.104
initial PO_4-P	0.019	1.398	0.171
N to P ratio	0.002	1.997	0.054
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	0.030	7	< 0.001
residual	0.031	34	

The multiple regression results that evaluated total geosmin production (Table 4.24) indicated that production was influenced by population age, age^2, and initial nitrogen concentration, and also, possibly, by the initial phosphorus concentration and N-to-P ratio. In general, more geosmin (ng/10,000 cells) was observed with increased culture age (Appendix F, Table F.1), although geosmin production declined in some treatments after 19-28 days incubation, hence the significance of age^2. The algal data indicated that more geosmin was produced during the low-phosphorus and mid-nitrogen treatments than during the other treatments. From the regression model, increased N-to-P ratios (e.g., either more nitrogen or less phosphorus) resulted in greater geosmin production; however, neither light intensity nor temperature was significant. The nonparametric analyses (Table 4.20), however, did indicate a significant difference in total geosmin production between the low-light and mid-nitrogen treatments (Appendix F, Table F.1). Evaluations of geosmin production at additional light intensities may result in phosphorus being significant to the regression model.

Table 4.24

Anabaena laxa total geosmin production regression results

Dependent variable total geosmin (ng/10,000 cells)	R-squared = 0.657	Adjusted R-squared = 0.587	Standard error of estimate = 0.035
Model variables	Coefficient	T	P (2 tail)
constant	-0.058	-0.784	0.439
age	0.007	5.017	< 0.001
light intensity	-6.31×10^{-4}	-0.735	0.467
temperature	-0.001	-0.905	0.372
age * age	-1.18×10^{-4}	-3.426	0.002
initial NO_3-N	-0.003	-2.942	0.006
initial PO_4-P	0.027	1.738	0.091
N to P ratio	0.003	2.024	0.051
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	0.089	10	< 0.001
residual	0.032	31	

Implications of Experimental Findings

The conditions that resulted in both the greatest and least concentrations (ng/mL) of geosmin were determined (Appendix E, Table E.1), so that both the conditions during which *A. laxa* was likely to cause odor problems and the possible problem solutions could be evaluated. In general, most of the geosmin remained within the algal cells rather than being released into the media throughout the 40-day incubation period. Maximum concentrations occurred during the low-nitrogen treatment, because average levels of geosmin production occurred in conjunction with very high population densities.

The lowest geosmin concentrations occurred during the low-light, low-phosphorus, and regular ASM-1 treatments. During the low-light treatment, both population densities (cells/mL) and geosmin production (ng/10,000 cells) were low; which resulted in low geosmin concentrations (ng/mL) until the population reached densities as high as in the other treatments. Low geosmin concentrations (ng/mL) were detected during the low-phosphorus treatment, because the impact of high geosmin production (ng/10,000 cells) was outweighed by the relatively low population densities (cells/mL). Low geosmin concentrations were also detected when the algae were grown in regular ASM-1 media, because high population densities occurred in conjunction with low levels of geosmin production.

Apparently, the phosphorus supply was exhausted during the low-phosphorus treatment; therefore, low-phosphorus conditions in the environment likely would also result in relatively lower algal population densities. Although the amount of extracellular geosmin per cell (ng/10,000 cells) would likely be high, the impacts would be reduced by the low population densities. The result would be a lessened geosmin problem. Phosphorus control, treatment of an algal bloom during the first 3-10 days of population growth, and removal of algal cells in a water treatment process prior to oxidation (thereby preventing release of intracellular geosmin into the water) appear to be the most likely methods for lessening the potential odor problems associated with geosmin produced by *A. laxa*.

PHORMIDIUM SP.

Figure 4.19 presents the *Phormidium* sp. population growth curves obtained from the six different treatment schemes previously described (Table 3.2). The highest population cell densities were attained with the regular ASM-1 media. The growth curves are very similar for both the high-light and low-temperature treatments, and those treatments resulted in the next-to-highest cell densities. The low-phosphorus treatment resulted in population densities that possibly were slightly less than those attained from the high-light and low-temperature treatments. The growth curves obtained from the mid- and low-nitrogen treatments were similar to one another, and the cultures attained the lowest population cell densities. A short log-phase that ended after about three days of incubation was observed during each treatment. An extended period of declining growth rate occurred during the incubation period associated with most, but not all, of the treatments.

Nonparametric Evaluations of Treatment Effects on *Phormidium* sp.

The Friedman two-way layout results and multiple comparison analyses for *Phormidium* sp. are presented in Appendix F, Table F.3. The nonparametric results indicated that both the population cell densities ($p = 0.002$) and the total MIB production ($p = 0.001$) were significantly different among the treatments. The multiple comparison results indicated that the population cell densities (cells/mL) attained during the mid-nitrogen, the low-nitrogen, and the low-phosphorus treatments were significantly different at the $\alpha = 0.049$ level than those attained during the regular ASM-1 treatment. Total MIB productions (ng/10,000 cells) during the regular ASM-1 treatment were significantly different at the $\alpha = 0.040$ level than those produced during the high-light and low-nitrogen treatments.

Multiple Regression Evaluations of Population Growth and MIB Production by *Phormidium* sp.

This strain of *Phormidium* produced both MIB and geosmin. The amount of geosmin was consistently less than the amount of MIB (ng/10,000 cells), and greater amounts of both compounds were located extracellularly than intracellularly. The regressions on the geosmin data were not performed, because there were many "zero" observations.

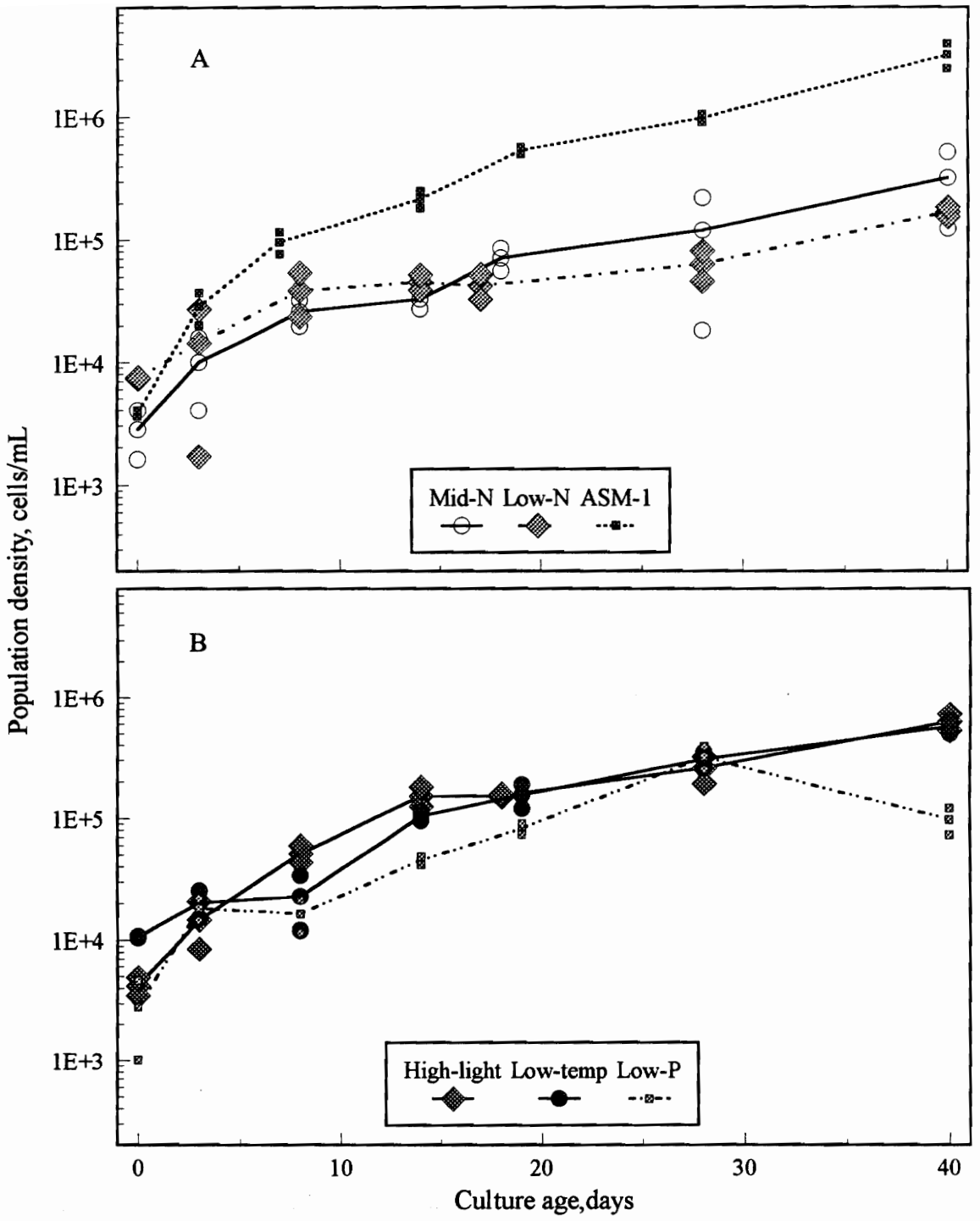


Figure 4.19 *Phormidium* sp. population growth curves grown under six different treatment schemes: (A) mid-N, low-N, and regular ASM-1, and (B) high-light, low-temperature, and low-P ASM-1 (see Table 3.2).

The regression analyses evaluated four dependent variables: (1) cell density, (2) intracellular MIB, (3) extracellular MIB, and (4) total MIB production. The data used for the regressions are presented in Appendix F, Table F.2. Because a few very high MIB values were observed at t_0 days that might distort the results, the regressions did not include t_0. The MIB values at t_0 likely were influenced by both the residual MIB in the inoculum and the low population densities at the beginning of the experiments. The multiple regression results are presented in Appendix F, Tables F.4 through F.7.

Population density (cells/mL)

The multiple regression analysis that evaluated population density (Appendix F, Table F.4) indicate that age, age^2, light intensity, temperature, and initial nitrogen concentration were all significant in the model. Neither phosphorus nor N-to-P ratio were significant in the model. Phosphorus likely would be significant in the model if additional experiments were conducted that evaluated lower phosphorus concentrations.

The light-intensity regression coefficient indicated that decreased light intensity resulted in greater population densities over the intensity range evaluated. Temperature was significant also, and the alga apparently preferred warmer temperatures (e.g., 25°C). The temperature and light intensity results were consistent with information obtained with the culture when it was acquired (Izaguirre, personal communication 1991). Unlike *A. laxa*, *Phormidium* sp. can not utilize molecular nitrogen; therefore, both of the reduced nitrogen treatments resulted in lower population densities (Figure 4.19) and nitrogen was significant in the multiple regression model. These results indicate that the original culturing conditions in ASM-1 media at 25°C and 8 $\mu E/m^2/s$ provided near-optimal growth conditions.

Intracellular, extracellular, and total MIB production (ng/10,000 cells)

The multiple regression analysis indicated that several variables (e.g., N-to-P ratio, age, initial phosphorus concentration, age^2, and temperature) were significant in the model that represented intracellular MIB production (Appendix F, Table F.5). The algal data (Appendix F, Table F.2) show that during the low-phosphorus treatment there was less

intracellular MIB relative to that detected during the other treatments. This result is contrary to that indicated by the phosphorus concentration regression coefficient, which indicated that more intracellular MIB would occur at lower phosphorus concentrations. It is possible that the conflicting phosphorus results occurred because only one low-phosphorus concentration was being compared to the phosphorus concentrations in the media of the other five treatments, all of which were fairly similar. The regression coefficients obtained for the N-to-P ratio likely were more indicative of the actual relationships, and a decrease in N-to-P ratio would result in an increased amount (ng/10,000 cells) of intracellular MIB. The relationship between N-to-P ratio and the amount of intracellular MIB was further indicated by the relatively high MIB detected during the low-nitrogen treatment (Appendix F, Table F.2) relative to the other treatments. The N-to-P ratios ranged from approximately 0.5 to 100, representing the low-nitrogen and the low-phosphorus treatments, respectively.

The extracellular MIB regression results (Appendix F, Table F.6) were very similar to those obtained for intracellular MIB. Two noted differences were: (1) the regression coefficient associated with culture age was much larger in the extracellular MIB regression than in the intracellular MIB regression, 0.124 and 0.031 ng/10,000 cells/day, respectively; and (2) light was a significant variable, and increased light intensity resulted in more extracellular MIB. The amount of extracellular MIB was always several times greater than the amount of intracellular MIB, regardless of either treatment condition or culture age.

The regression results for total MIB production (Appendix F, Table F.7) were extremely similar to those for extracellular MIB (Appendix F, Table F.6). The regression coefficients from both models indicated that decreased N-to-P ratio and increased light intensity were associated with greater total MIB production (ng/10,000 cells). This association agreed with the nonparametric results (Appendix F, Table F.3), which indicated that greater total MIB production occurred during both the high-light and the low-nitrogen treatments. In general, there was more MIB detected as culture age increased; however, a decline in amount (ng/10,000 cells) was observed in some treatments after 28 days of incubation (Appendix F, Table F.2), hence, the significance of age^2.

Implications of Experimental Findings

The conditions that resulted in both the greatest and least concentrations (ng/mL) of MIB were determined (Appendix F, Table F.2), so that both the conditions during which *Phormidium* sp. was likely to cause odor problems and the possible problem solutions could be evaluated. The majority of the MIB was released into the media rather than being retained within the algal cells. Maximum MIB concentrations (ng/mL) occurred during both the high-light and the regular ASM-1 treatments. The high MIB concentrations detected during the high-light treatment occurred because there was very high MIB production (ng/10,000 cells) in conjunction with moderate population densities (cells/mL); however, it occurred during the regular ASM-1 treatment because there was very low MIB production in conjunction with very high population densities. The lowest MIB concentrations occurred during the low-phosphorus, mid-nitrogen, and low-nitrogen treatments. These low MIB concentrations resulted because high MIB production occurred in conjunction with low population densities. Apparently, cell density has greater impact than MIB production per cell on how much MIB will be present. If the cell densities are kept low, odor problems will be reduced, even during periods of high MIB production.

In summary, nitrogen and phosphorus control would be the most likely methods for lessening the potential odor problems associated with MIB produced by *Phormidium* sp. The MIB production per cell might be high, however, there would be fewer algal cells present. Logistical constraints may complicate the treatment of an algal bloom during early log-phase growth, as that ends very early (e.g., within approximately three days in cultures); however, at environmental nutrient levels the log-phase may both last longer and occur at a lower rate than was observed during this investigation. Because *Phormidium* sp. releases most of the MIB and geosmin it produces, the removal of algal cells from intake water in a water treatment plant prior to oxidation would likely be only moderately effective in controlling these odorous compounds.

SYNURA PETERSENII

Figure 4.20 presents the *Synura petersenii* population growth curves obtained from the six different treatment schemes previously described (Table 3.2). The population densities attained during the regular DY III, high-light, low-phosphorus, and mid-nitrogen treatments were all similar; however, the regular DY III final population densities were slightly greater than those attained by the other treatments. The high-light treatment was terminated after approximately 20 days of incubation, because the temperature control failed and the incubator temperature rose markedly.

A lower log rate of population growth was observed during the low-nitrogen treatment than during any other treatment except the one conducted at low-temperature. In addition, the lowest population densities were attained during the low-nitrogen treatment. The initial growth of cultures grown under low-temperature conditions was relatively slow (i.e., a long lag-phase), but log-phase growth persisted longer (i.e., 28 days) than in cultures grown under any other treatment condition.

Nonparametric Evaluations of Treatment Effects on *Synura petersenii*

The Friedman two-way layout results and multiple comparison analyses for *S. petersenii* are presented in Appendix F, Table F.9. The nonparametric results indicated that the population cell densities were significantly different among the treatments but that total 2t,4c,7c-decatrienal production was not significantly different. The multiple comparison analyses were performed twice, once for all six treatments (utilizing only five of the seven sample dates) and once for five of the treatments (utilizing all of the sample dates, but excluding the high light treatment), because the high-light experiment was terminated prematurely.

The population densities attained during the high-light treatment were not significantly different, at the $\alpha = 0.047$ level, from those attained by the other treatment cultures during the first 19 days of incubation. The population densities attained during the low-temperature treatment were significantly different from those attained during the mid-nitrogen and regular DY III treatments, during both the first 19 days and the entire 40 days of incubation ($\alpha = 0.047$ and 0.052, respectively).

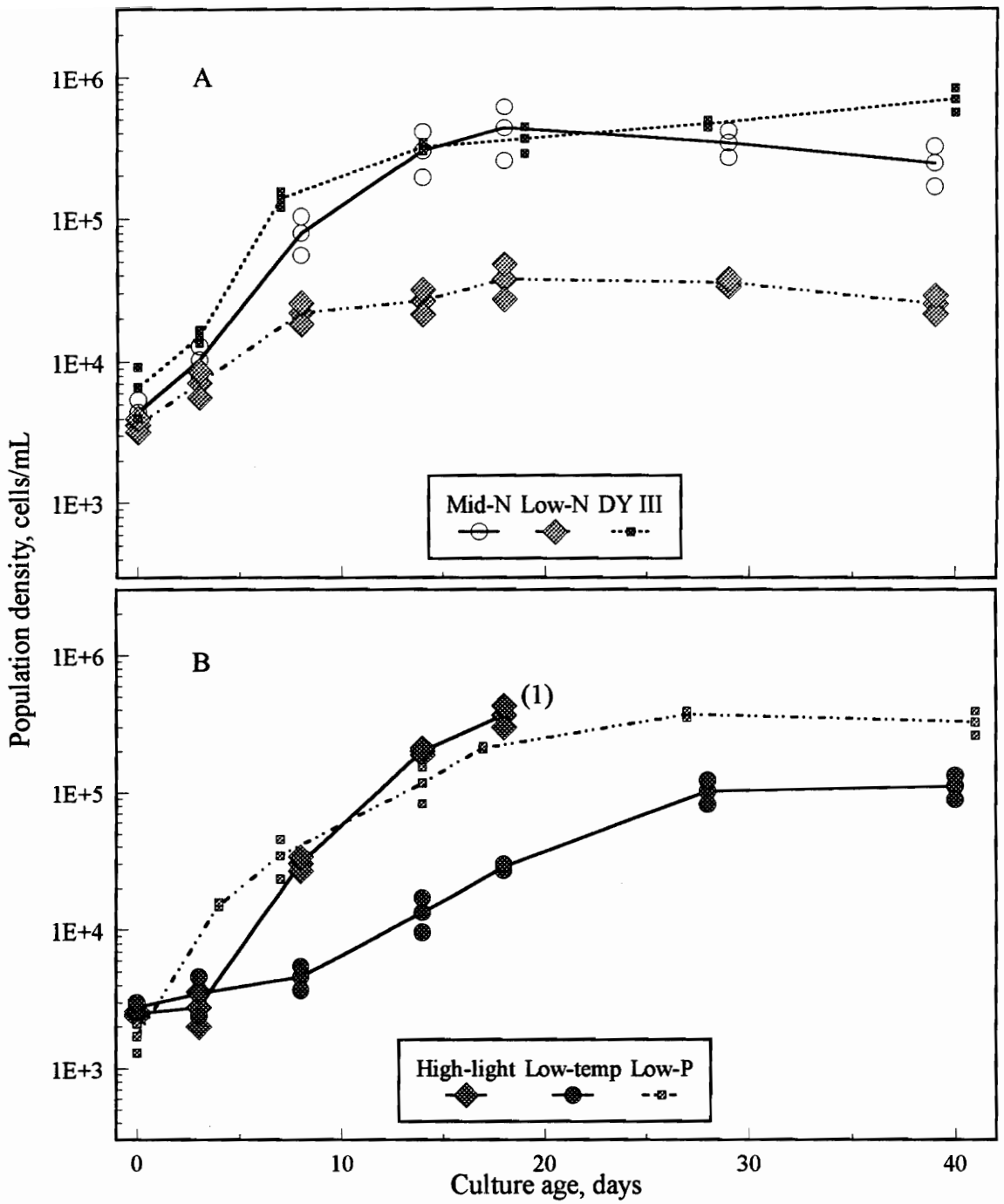


Figure 4.20 *Synura petersenii* population growth curves grown under six different treatment schemes: (A) mid-nitrogen (NO3-N and NH3-N), low-nitrogen (NO3-N and NH3-N), and regular DY III, and (B) high-light, low-temperature, and low-AHP DY III (see Table 3.2). The brackets (1) indicate last sample before incubator temperature rose sharply and severely reduced the number of viable cells present in the culture tubes.

The population densities attained during the low-nitrogen treatment were not significantly different from those attained during the other treatments during the first 19 days of incubation; however, they were significantly different (α = 0.052) from those attained during the regular DY III treatment over the entire 40 day incubation period. The low-nitrogen treatment nonparametric results agreed with the growth curves (Figure 4.20), and indicated that the *Synura* likely ran short of nitrogen after approximately 10 days incubation.

The nonparametric analyses did not detect significant differences in total 2t,4c,7c-decatrienal production, likely because compound production declined once the cultures attained stationary phase. The ages at which total 2t,4c,7c-decatrienal production declined varied among the treatments. The algal data (Appendix F, Table F.8) did indicate that differences in 2t,4c,7c-decatrienal production occurred among the treatments; however, the observation was not supported by the nonparametric results.

Multiple Regression Evaluations of Population Growth and Geosmin Production by *Synura petersenii*

Synura petersenii produced both 2t,4c,7c-decatrienal and 2t,6c-nonadienal. The amount of 2t,6c-nonadienal was consistently less than the amount of 2t,4c,7c-decatrienal (ng/10,000 cells), and greater amounts of both compounds were located intracellularly than extracellularly. The regressions that were performed evaluated six dependent variables: (1) cell density, (2) intracellular 2t,4c,7c-decatrienal, (3) extracellular 2t,4c,7c-decatrienal, (4) total 2t,4c,7c-decatrienal production, (5) intracellular 2t,6c-nonadienal, and (6) total 2t,6c-nonadienal production. The regression was not performed for extracellular 2t,6c-nonadienal, because there were only 5 non-zero values obtained out of a possible 42. The data used for the regressions are presented in Appendix F, Table F.8. The multiple regression results are presented in Appendix F, Table F.10 through Table F.15.

Population density (cells/mL)

The population density multiple regression results (Appendix F, Table F.10) indicated that age, age^2, temperature, and initial NO_3-N * initial AHP (i.e., the interaction between concentrations of NO_3-N and AHP) were significant in the model. Light

intensity was possibly significant. According to the model results, lower population densities were attained during the nitrogen, temperature, and light intensity variations than during the original treatment conditions. These results indicate that the original culturing conditions (i.e., DY III media at 20°C and 8 $\mu E/m^2/s$ light intensity) provided near-optimal growth conditions.

The regression model indicated that decreased light intensity would result in greater population densities; however, the only experiment that was performed at an altered (i.e., increased) light intensity was terminated early. The nonparametric analyses did not indicate that the high-light treatment was significantly different than the other treatments (Appendix F, Table F.9). The regression results indicated that the *Synura petersenii* population densities increased more rapidly at warm temperatures than at cold temperatures, 20°C and 10°C, respectively. Continued increases in temperature would not follow these results, because the alga dies at approximately 22°C.

The nitrogen and phosphorus concentrations in DY III media were much less than those in ASM-1 media, and the range of N-to-P ratios was less than those in either the *A. laxa* or *Phormidium* sp. experiments. The N-to-P ratios ranged from approximately 1.5 to 10, representing the low-nitrogen and low-phosphorus treatments, respectively. The concentration ranges of initial NH_3-N (from 0.36 to 1.37 mg/L) and AHP (from 0.56 to 1.16 mg/L) were very narrow. It is possible that accurate regression coefficients were not obtained because these concentration ranges were too narrow.

Synura petersenii is myxotrophic, and it can assimilate both organic-C and organic-P. The bacteria in the S. petersenii cultures likely provided sufficient organic-P that the algae were not significantly affected by the low-AHP treatment. Additional experiments at lower initial phosphorus concentrations may have provided further information regarding the relationship between nitrogen and phosphorus.

The range of initial NO_3-N concentrations was approximately 0.83 to 4.19 mg/L. The regression results indicate that reduced concentrations of NO_3-N, rather than NH_3-N, would restrict algal population growth. The population growth curve obtained during the low-nitrogen treatment (Figure 4.20A) agreed with the regression coefficient that suggested low-nitrogen concentrations supported a lower population density.

Intracellular, extracellular, and total 2t,4c,7c-decatrienal production (ng/10,000 cells)

Both the adjusted R-squared values, which ranged from 0.35 to 0.38, and the sums-of-squares obtained by the multiple regression results indicated that intracellular,

extracellular, and total 2t,4c,7c-decatrienal production were represented only moderately well by the regression model. The intracellular 2t,4c,7c-decatrienal regression results (Appendix E, Table E.11) indicated that age, age^2, light intensity, and initial NO_3-N * initial AHP (i.e., the interaction between concentrations of NO_3-N and AHP) were significant. Initial NH_3-N * initial AHP (i.e., the interaction between concentrations of NH_3-N and AHP) was possibly significant. The algal data (Appendix F, Table F.8) showed that the amount of intracellular 2t,4c,7c-decatrienal was always several times greater than the amount of extracellular 2t,4c,7c-decatrienal, regardless of either treatment condition or culture age. An abrupt decline in the amount of compound (ng/10,000 cells) was observed in several of the treatments after 14 days incubation (Appendix F, Table F.8), hence the significance of age^2. The point at which a decline in the amount of intracellular 2t,4c,7c-decatrienal was observed corresponded well with the end of log-phase population growth, and the events may be related to each other.

Relatively low amounts of intracellular 2t,4c,7c-decatrienal (Appendix F, Table F.8) were detected during the high-light intensity treatment than during the other treatments. This result was supported by the regression coefficient for low light intensity, which indicated that the algae would produce greater amounts of intracellular 2t,4c,7c-decatrienal when lower light intensities occurred; however, accurate evaluations of the high-light treatment results were hampered by the early termination of the experiment. The effects of nitrogen and phosphorus on the production of 2t,4c,7c-decatrienal were also not readily interpreted. The algal data (Appendix F, Table F.8) showed that large amounts of intracellular 2t,4c,7c-decatrienal were detected during the low-phosphorus treatment. Conversely, lower amounts of intracellular 2t,4c,7c-decatrienal were detected during both of the low-nitrogen treatments relative to all of the other treatments except that of high light intensity. The relationship between nitrogen and phosphorus was not clearly indicated by the regression results, and additional experiments at lower initial phosphorus concentrations may have helped clarify the relationship.

The multiple regression results did not indicate that temperature was significant in the model. From the algal data (Appendix F, Table F.8), it can be observed that the greatest amounts of intracellular 2t,4c,7c-decatrienal were detected during the regular DY III, low-phosphorus, and low-temperature treatments. The production (ng/10,000 cells) of intracellular 2t,4c,7c-decatrienal during the low-temperature treatment differed from that produced during all of the other treatments in that it did not decline with increased culture age. As may be seen in Figure 4.20, there was an extended period of low log-rate

population growth, which lasted from approximately day 7 to day 29 of the incubation period, during the low-temperature treatment. It is possible that the formation of 2t,4c,7c-decatrienal was associated with log-phase population growth.

The extracellular 2t,4c,7c-decatrienal multiple regression results (Appendix F, Table F.12) indicated that age and age^2 were significant in the regression model. This result was supported by the algal data, which showed that extracellular 2t,4c,7c-decatrienal concentrations were typically detected between days 14 and 28 of the 40-day incubation period. The 2t,4c,7c-decatrienal that was released into the media was apparently being removed; likely by either oxidation or bacterial assimilation.

The total 2t,4c,7c-decatrienal production multiple regression results (Appendix F, Table F.13) were extremely similar to the intracellular 2t,4c,7c-decatrienal regression results, and the same evaluations of the regression results are applicable. The similarity between intracellular 2t,4c,7c-decatrienal and total 2t,4c,7c-decatrienal production was likely the result of the large amount of 2t,4c,7c-decatrienal retained within the algae, relative to the amount released; virtually the same values were used in the regressions of both intracellular and total 2t,4c,7c-decatrienal.

Intracellular, extracellular, and total 2t,6c-nonadienal (ng/10,000 cells)

The intracellular 2t,6c-nonadienal and the total 2t,6c-nonadienal production multiple regression results (Appendix F, Tables F.14and F.15) were very similar to each other, as were the regression results obtained from intracellular and total 2t,4c,7c-decatrienal production. Age, age^2, light intensity, and initial NO_3-N * initial AHP were significant in both models, and initial NH_3-N * initial AHP was possibly significant. The alga produced much less 2t,6c-nonadienal than 2t,4c,7c-decatrienal, and, in the case of the low-nitrogen treatment, no 2t,6c-nonadienal was detected during the experiment (Appendix F, Table F.8). The significance of age^2 in the regression model was supported by the fact that the greatest amounts of 2t,6c-nonadienal were generally detected between days 14 and 19 of the 40-day incubation period. The occurrence of many zero data values and the poor overall fit of the regressions, which was based on both the adjusted R-squared values (i.e., less than 0.30) and the sums-of-squares, made a more detailed interpretation of the regression results unrealistic.

Implications of Experimental Findings

The conditions that resulted in both the greatest and least concentrations (ng/mL) of both 2t,4c,7c-decatrienal and 2t,6c-nonadienal were determined (Appendix F, Table F.8), so that both the conditions during which *S. petersenii* was likely to cause odor problems and the potential problem solutions could be evaluated. The majority of both compounds was retained within the algal cells. The greatest concentrations (ng/mL) of 2t,4c,7c-decatrienal and 2t,6c-nonadienal were detected during the regular DY III, low-phosphorus, and mid-nitrogen treatments, between 14 and 29 days of incubation. It is likely that population growth rate influenced compound production, because relatively higher compound production (ng/10,000 cells) occurred during late log-phase growth in the cultures grown under the regular DY III, low-phosphorus, and mid-nitrogen treatments. It is possible that both the population densities and the production of 2t,4c,7c-decatrienal and 2t,6c-nonadienal attained during the high-light treatment would have been similar to those attained during the regular DY III, low-phosphorus, and mid-nitrogen treatments; however, the experiment was terminated after 20-days incubation.

Very low concentrations of 2t,4c,7c-decatrienal and 2t,6c-nonadienal were detected during the low-nitrogen treatment, because low compound production (ng/10,000 cells) occurred in conjunction with low population densities (cells/mL). Low compound concentrations were detected, during 14- to 29-days incubation, in cultures grown under the low-light treatment, because the impact of high 2t,4c,7c-decatrienal production was outweighted by the relatively low population densities. The algal population, however, continued to grow steadily. The combination of continued log-phase growth rate and continued high compound production resulted in high 2t,4c,7c-decatrienal concentrations after 40-days incubation.

In summary, the production of both 2t,4c,7c-decatrienal and 2t,6c-nonadienal was apparently associated with log-phase population growth rather than specific environmental conditions. This association would explain the difficulties in determining differences in compound production based upon treatment (i.e., the nonparametric analyses) and multiple regression analyses of environmental factors. It would account also for the relatively poor fit of the regression models Conditions that prolonged log-phase growth (e.g., low temperature) would, therefore, prolong the potential for odor problems associated with 2t,4c,7c-decatrienal and 2t,6c-nonadienal production.

The results for *Synura petersenii* indicate that much more 2t,4c,7c-decatrienal was produced than 2t,6c-nonadienal and that the majority of both compounds was retained within the algal cells throughout the experiments. *Synura petersenii* could assimilate organic-P (i.e., bacteria and their metabolites), which is likely why the evaluated phosphorus concentrations were not significant to population growth. Nitrogen control and removal of algal cells from intake water in a water treatment plant prior to oxidation appear to be the most likely methods for lessening the potential odor problems associated with 2t,4c,7c-decatrienal and 2t,6c-nonadienal produced by *S. petersenii*.

CHAPTER 5
SUMMARY AND CONCLUSIONS

CLLE-KD METHOD

The CLLE-KD method was both effective and reliable for the extraction and concentration of the organic compounds that were evaluated.

- The recoveries were linear over the concentration ranges evaluated
- The recoveries were generally independent of extraction time during the 4 to 24 hour range evaluated. Recoveries of the fatty acids and chlorophene (o-benzyl-p-chlorophenol) were exceptions in that the recoveries were highly variable if the extraction time was only four-hours. The variability in the recoveries of both the phenolic compounds and the fatty acids was greater relative to the recoveries of the other compounds.

Conclusions from the evaluation of the CLLE-KD method are as follows:

The method is suitable for the extraction and concentration of a wide variety of organic compounds; though recoveries of low-molecular-weight, highly volatile compounds (such as isovaleric acid) will be poor relative to other methods that are designed specifically for their recovery. The method is suitable for the recovery and quantification of geosmin, MIB, 2t,6c-nonadienal, and 2t,4c,7c-decatrienal, among others.

GC-MS AND FPA

The Weber-Fechner Law adequately described the FPA panelists' responses to odor standards over the range of concentrations evaluated. The odor intensities of algal cultures were generally lower than those predicted by the W-F plot associated with the evaluations of compound standards. A few exceptions were:

- The odor of 2t,6c-nonadienal (cucumber) could not be detected in *Synura petersenii* cultures after 3-days incubation, even though the compound was present. The 2t,4c,7c-decatrienal (fishy) apparently masked the cucumber

odors. Masking likely also prevented detection of β-cyclocitral in the presence of linolenic acid in the *M. aeruginosa* cultures.

- Synergistic effects among the pleasant-smelling compounds in cultures of *Chlamydomonas peterfii* were likely responsible for the "sweet-meadow like" odor intensities being greater than predicted on the basis of linolenic acid concentration alone.

Other observations obtained during GC-MS and FPA are as follows:

- Odors produced by young algal cultures (e.g., low population densities) often were detected in FPA samples at compound concentrations too low to be detected by GC-MS.
- The descriptors "corn" and "corn-shucks" were applied to both geosmin standards and algal cultures that contained geosmin, in addition to the usual "earthy-musty" descriptors.
- Several compounds were detected in algal cultures for the first time (2t,4c,7c-decatrienal, safranal, chlorophene, and 2-phenylphenol).
- Several compounds were identified as directly contributing an odor to algal samples (e.g., linolenic acid is generally regarded as a precursor to 2t,6c-nonadienal and 2t,4c,7c-decatrienal; however, it can directly contribute "sweet-watermelon" odors to algal cultures).
- Both the quantity and location (i.e., intra- or extracellular) of many algal metabolites were determined. The concentrations of some compounds were always greater within the algal cells (e.g., fatty acids, alkanes, and 2t,4c,7c-decatrienal). Some compounds were detected only in the media (e.g., isovaleric acid).
- Isovaleric acid, benzyl acetate, and phenethyl alcohol were identified as metabolites that were likely produced by axenic *Chlamydomonas peterfii* during assimilation of peptone.
- The critical population densities of algae that produced odorous compounds were determined. Some of the algae produced more than one odorous compound; however, the effects of interactions among compounds

on either the odors or their intensities was not determined. The compounds associated with the lowest critical population densities would likely be of greatest concern to water utility personnel.

- The critical population densities of *Oscillatoria* sp., *Phormidium* sp., and *Synura petersenii* were extremely low (i.e., as low as 17 cells/mL). Odor problems in water supplies could be reduced markedly if water utility personnel responded promptly (e.g., activated carbon, oxidants, or in-reservoir treatment with $CuSO_4$).

Conclusions regarding GC-MS and FPA are as follows:

In general, the FPA odor intensities assigned by a panel to water samples that contain a mixture of odor compounds would likely be lower than those assigned to compound standards. The GC-MS and FPA evaluations supplement each other: (1) compounds whose odors are not detected during FPA because of masking can be detected during GC-MS analysis, (2) the odors of compounds can be detected in FPA samples at compound concentrations too low to be detected by GC-MS. More useful information, therefore, will be obtained if both methods are utilized than if either one of the procedures is used alone. The GC-MS can also detect if the odorous compounds are within the algal cells or in the water samples. The location of the compounds can influence how a water utility will respond to a potential odor episode.

INFLUENCE OF ENVIRONMENTAL FACTORS ON ALGAL POPULATION GROWTH AND ODOROUS COMPOUND PRODUCTION

The influence of light, temperature, nitrogen, and phosphorus on both algal population growth and odorous compound production were evaluated. The effects of light and temperature on algal population growth and metabolite production may be extrapolated to environmental conditions more readily than those of nitrogen and phosphorus, because the nitrogen and phosphorus concentrations in the media were higher than those likely to be observed in natural situations. The nutrient experiment results, however, can indicate the relative importance of the various environmental conditions on

both population growth and compound production. The conclusions drawn from the environmental experiments are presented for each alga:

Anabaena laxa

Anabaena laxa retains most of the geosmin it produces within its cells, particularly during the early population growth stages. Although large amounts of extracellular geosmin (ng/10,000 cells) were present when the alga was cultured in the low-phosphorus medium, the low population densities (cells/mL) resulted in low geosmin concentrations (ng/mL). Phosphorus control, treatment of an algal bloom during the first 3-10 days of population growth, and removal of algal cells prior to oxidation in a water treatment process (thereby preventing release of intracellular geosmin into the water) appear to be the most likely methods for lessening the potential odor problems associated with geosmin produced by *A. laxa*.

Phormidium sp.

This strain of *Phormidium* produced both MIB and geosmin. The amount of geosmin was consistently less than the amount of MIB (ng/10,000 cells), and greater amounts of both compounds were located extracellularly than intracellularly. Nitrogen and phosphorus control in surface waters are the most likely methods for lessening the potential odor problems associated with MIB produced by *Phormidium* sp. If the cell densities are kept low, odor problems will be reduced even during periods of high MIB production. Logistical constraints may complicate treatment of an algal bloom during early log-phase, as that phase ends very early (e.g., within approximately three days in algal cultures); however, at the nutrient levels expected in lakes and other surface water supplies, the log-phase growth may both last longer and occur at a lower rate than was observed during this investigation. Because *Phormidium* sp. releases most of the MIB and geosmin it produces, the removal of algal cells from intake water in a water treatment plant prior to oxidation would likely be only moderately effective in controlling these odorous compounds.

Synura petersenii

Synura petersenii produced both 2t,6c-nonadienal and 2t,4c,7c-decatrienal and retained most of both compounds within the algal cells throughout the entire population growth period. The alga produced much more 2t,4c,7c-decatrienal than 2t,6c-nonadienal. Production of these compounds was apparently more closely related to log-phase population growth rather than to specific environmental conditions. Conditions that prolonged log-phase growth (e.g., low temperature) would, therefore, prolong the potential for odor problems associated with 2t,4c,7c-decatrienal and 2t,6c-nonadienal production. Nitrogen control in surface waters and removal of algal cells from intake water prior to oxidation would be the most likely methods for lessening the potential odor problems associated with *S. petersenii*.

APPENDIX A
ALGAL MEDIA

Table A.1

ASM-1

Compound	Final concentration, mg/L
$NaNO_3$	170
K_2HPO_4	17.4
Na_2HPO_4	14.2
$MgCl_2+6H_2O$	4.6
$MgSO_4+7H_2O$	49.2
$CaCl_2+2H_2O$	28.6
$FeCl_3+6H_2O$	1.08
H_3BO_3	2.48
$MnCl_2+4H_2O$	1.39
$ZnCl_2$	0.436
$CoCl_2+6H_2O$	0.019
$CuCl_2$	0.00011
$Na_2EDTA+2H_2O$	7.44
Distilled water	To final volume of 1 L

Source: Gorham et al. 1964

Table A.2

DY III

Compound	Final concentration, mg/L
$CaCl_2+2H_2O$	75
$NaSiO_3+9H_2O$	15
$MgSO_4+7H_2O$	50
NH_4NO_3	5
$NaNO_3$	20
KCl	3
$Na_2EDTA+2H_2O$	8
$FeCl_3+6H_2O$	2.4
$MnCl_2+4H_2O$	0.72
$ZnSO_4+7H_2O$	0.18
$NaMoO_4+2H_2O$	0.05
$CoCl_2+6H_2O$	0.03
H_3BO_3	4.56
Biotin	0.0005
Thiamin	0.2
B_{12}	0.0005
Na glycerophosphate	10
MES*	200
Distilled water	To final volume of 1 L

Source: Lehman 1976

*2-[N-Morpholino]ethane sulfonic acid

Table A.3

OCM

Compound	Final concentration, mg/L
K_2HPO_4	100
KH_2PO_4	75
$MgSO_4+7H_2O$	500
$Ca(NO_3)_2+4H_2O$	62.5
KNO_3	1
A5 + Co metal mix	1 mL
Fe-versenate	2 mL
Peptone*	5,000
Distilled water	To final volume of 1 L
A5 + Co metal mix	
H_3BO_3	2,860
$MnCl_2+4H_2O$	1,810
$ZnSO_4+7H_2O$	222
$Na_2MoO_4+2H_2O$	390
$CuSO_4+5H_2O$	79
$Co(NO_3)_2+6H_2O$	49.4
Distilled water	To final volume of 1 L
Fe versenate	
$FeSO_4+7H_2O$	5,000
Na_2EDTA	4,000
Distilled water	To final volume of 1 L

Source: Martek Biosciences Corp., Columbia, MD

*Added to OCM + peptone version

APPENDIX B
COMPOUNDS USED DURING COURSE OF EXTRACTION EXPERIMENTS AND FPA EVALUATIONS

Table B.1

Compounds used during course of extraction experiments and FPA evaluations

Compound	CAS#	Retention (min)	Source	Purity (%)
3-furfural	498-60-2	6.3	Aldrich†	99
Isovaleric acid	503-74-2	7.7	Aldrich	99
3-methylthio-1-propanol	505-10-2	11.5	Aldrich	98
Benzyl alcohol	100-51-6	13.3	Aldrich	99+
Chlorooctane	111-85-3	14.3	Chem. Services‡	99
Phenethyl alcohol	60-12-8	15.9	Aldrich	99+
2t,6c-nonadienal	557-48-2	17.2	Aldrich	95
Benzyl acetate	140-11-4	17.4	Aldrich	99+
2-MIB-d_3		18.3	Csiro§	
β-cyclocitral	432-25-7	19.2	Sigma**	70
Chlorodecane	1002-69-3	20.7	Chem. Services	98
1-indanone	83-33-0	21.0	Aldrich	99+
Geosmin-d_3		24.7	Csiro	
Chlorododecane	112-52-7	26.3	Pfaltz & Bauer††	98
2-phenylphenol	90-43-7	27.5	Aldrich	99+
Diphenylamine	122-39-4	30.2	Aldrich	99+
1-heptadecene	6765-39-5	31.8	Aldrich	99
n-Heptadecane	629-78-7	32.1	Aldrich	99
Myristic acid	544-63-8	33.6	Aldrich	99.5+
n-Octadecane	593-45-3	34.3	Aldrich	99
Chlorohexadecane	4860-03-1	35.9	Pfaltz & Bauer	95
n-Nonadecane	629-92-5	36.5	Aldrich	99
Chlorophene	120-32-1	36.6	Pfaltz & Bauer	95
Palmitic acid	57-10-3	37.9	Aldrich	99
n-Eicosane	112-95-8	38.5	Aldrich	99
Chlorooctadecane	3386-33-2	40.1	Chem. Services	98
Phytol*	7541-49-3	40.8	Aldrich	97
Linolenic acid	463-40-1	41.3	Aldrich	99
Squalene	111-02-4	54.8	Aldrich	97

* Contained two isomers

† Aldrich Chemical Company, Inc., Milwaukee, WI

‡ Chem Services, West Chester, PA

§ Csiro, Division of Water Resources, Griffith Australia

** Sigma Chemical Company, St. Louis, MO

†† Pfaltz & Bauer Inc., Waterbury, CT

APPENDIX C
STANDARDS

Table C.1

Standard solutions used to determine response factors

Compound	Retention (min)	Standard solution concentration 3a (ng/μL)	3b (ng/μL)	4a (ng/μL)	4b (ng/μL)
3-furfural	6.3	17.14	5.71		
Isovaleric acid	7.7			27.99	9.33
3-methylthio-1-propanol	11.5	34.11	11.37		
Benzyl alcohol	13.3			34.37	11.46
Chlorooctane	14.3	4.79	4.99	4.79	4.85
Phenethyl alcohol	15.9	34.29	11.43		
2t,6c-nonadienal	17.2			17.06	5.69
Benzyl acetate	17.4	12.94	4.31		
2-MIB-d_3	18.3	2.52	0.84		
β-cyclocitral	19.2			12.99	4.33
Chlorodecane	20.7	4.56	4.75	4.56	4.62
1-indanone	21.0			8.70	2.90
Geosmin-d_3	24.7			1.96	0.65
Chlorododecane	26.3	4.79	4.99	4.79	4.85
2-phenylphenol	27.5	12.90	4.30		
Diphenylamine	30.2			17.19	5.73
1-heptadecene	31.8	16.93	5.64		
Heptadecane	32.1			17.40	5.80
Myristic acid	33.6	68.79	22.93		
Octadecane	34.3			17.27	5.76
Chlorohexadecane	35.9	5.08	5.29	5.08	5.14
Nonadecane	36.5			16.67	5.56
Chlorophene	36.5	18.99	6.33		
Palmitic acid	37.9	108.99	36.33		
Eicosane	38.5			17.19	5.73
Chlorooctadecane	40.1	4.95	5.16	4.95	5.01
Phytol*	40.8			31.97	10.66
Linolenic acid	41.3	159.04	53.01		
Squalene	54.8	51.34	17.11		

*Contained two isomers, the later eluting isomer is presented here

Table C.2

Solutions used to determine CLLE-KD recovery efficiency

Compound	Retention (min)	Stock (μg/μL)	Recovery #1 (ng/μL)	Recovery #2 (ng/μL)
3-furfural	6.3	5.059	40	
Isovaleric acid	7.7	1.84		65.3
3-methylthio-1-propanol	11.5	5.072	79.6	
Benzyl alcohol	13.3	5.173		80.2
Phenethyl alcohol	15.9	5.11	80	
2t,6c-nonadienal	17.2	2.427		39.8
Benzyl acetate	17.4	9.68	30.2	
2-MIB-d_3	18.3	0.98	5.88	
β-cyclocitral	19.2	5		30.3
1-indanone	21.0	7.811		20.3
Geosmin-d_3	24.7	1.04		4.58
2-phenylphenol	27.5	5.673	30.1	
Diphenylamine	30.2	5.079		40.1
1-heptadecene	31.8	4.935	39.5	
Heptadecane	32.1	5.92		40.6
Myristic acid	33.6	10.288	160.5	
Octadecane	34.3	4.92		40.3
Nonadecane	36.5	5.227		38.9
Chlorophene	36.5	5.035	44.3	
Palmitic acid	37.9	10.64	254.3	
Eicosane	38.5	6.168		40.1
Phytol*	40.8	4.78		74.6
Linolenic acid	41.3	18.1	371.1	
Squalene	54.8	16.64	119.8	

*Contained two isomers, the later eluting isomer is presented here

Scheme I: Added 2.5, 5, 10, 15, 20, 25, 30, 40, 50, and 60 μL of each solution to 15-mL portions of sterile ASM-1 media; then extracted for 16 hours.

Scheme II: Added 30 μL of each solution to 15-mL portions of sterile ASM-1 media; extracted for 4, 8, 12, 16, 20, and 24 hours.

APPENDIX D
VALIDATION OF CLLE-KD METHOD

Table D.1

Results of Scheme I: effects of concentration on compound recovery

Compound	R-squared	Slope (KD_f)	Recovery* (percent)	Conc. range† (ng/mL)	Det. limit‡ (ng/mL)
3-furfural	0.994	0.875	87.5	6 - 160	10
Isovaleric acid	0.817	0.144	14.4	11 - 260	85
3-methylthio-1-propanol	0.921	1.607	160.7	13 - 320	70
Benzyl alcohol	0.990	1.103	110.3	50 - 320	15
Phenethyl alcohol	0.976	1.661	166.1	13 - 320	23
2t,6c-nonadienal	0.966	0.959	95.9	6 - 160	6
Benzyl acetate	0.982	0.970	97.0	5 - 120	15
2-MIB-d_3	0.946	1.162	116.2	0.9 - 25	5
β-cyclocitral	0.966	0.910	91.0	5 - 120	4
1-indanone	0.743	0.737	73.7	3 - 80	30
Geosmin-d_3	0.859	0.959	95.9	0.7 - 20	3.6
2-phenylphenol	0.897	1.773	177.3	5 - 120	30
Diphenylamine	0.761	0.824	82.4	6 - 160	8
1-heptadecene	0.993	0.778	77.8	6 - 160	12
n-Heptadecane	0.998	0.758	75.8	6 - 160	10
Myristic acid	0.882	1.849	184.9	25 - 650	150
n-Octadecane	0.995	0.759	75.9	6 - 160	6
n-Nonadecane	0.983	0.843	84.3	6 - 160	4
Chlorophene	0.828	1.500	150.0	7 - 180	55
Palmitic acid	0.825	1.705	170.5	40 - 1,020	300
n-Eicosane	0.984	0.845	84.5	6 - 160	4
Phytol§	0.962	0.869	86.9	12 - 300	45
Linolenic acid	0.865	2.092	209.2	60 - 1,500	350
Squalene	0.953	0.884	88.4	20 - 480	60

* Recoveries are relative to the n-chloroalkanes used as internal standards

† 15-mL samples were extracted; 10 concentrations of each compound were evaluated

‡ Method by Sharaf (1986)

§ The later eluting isomer is represented here.

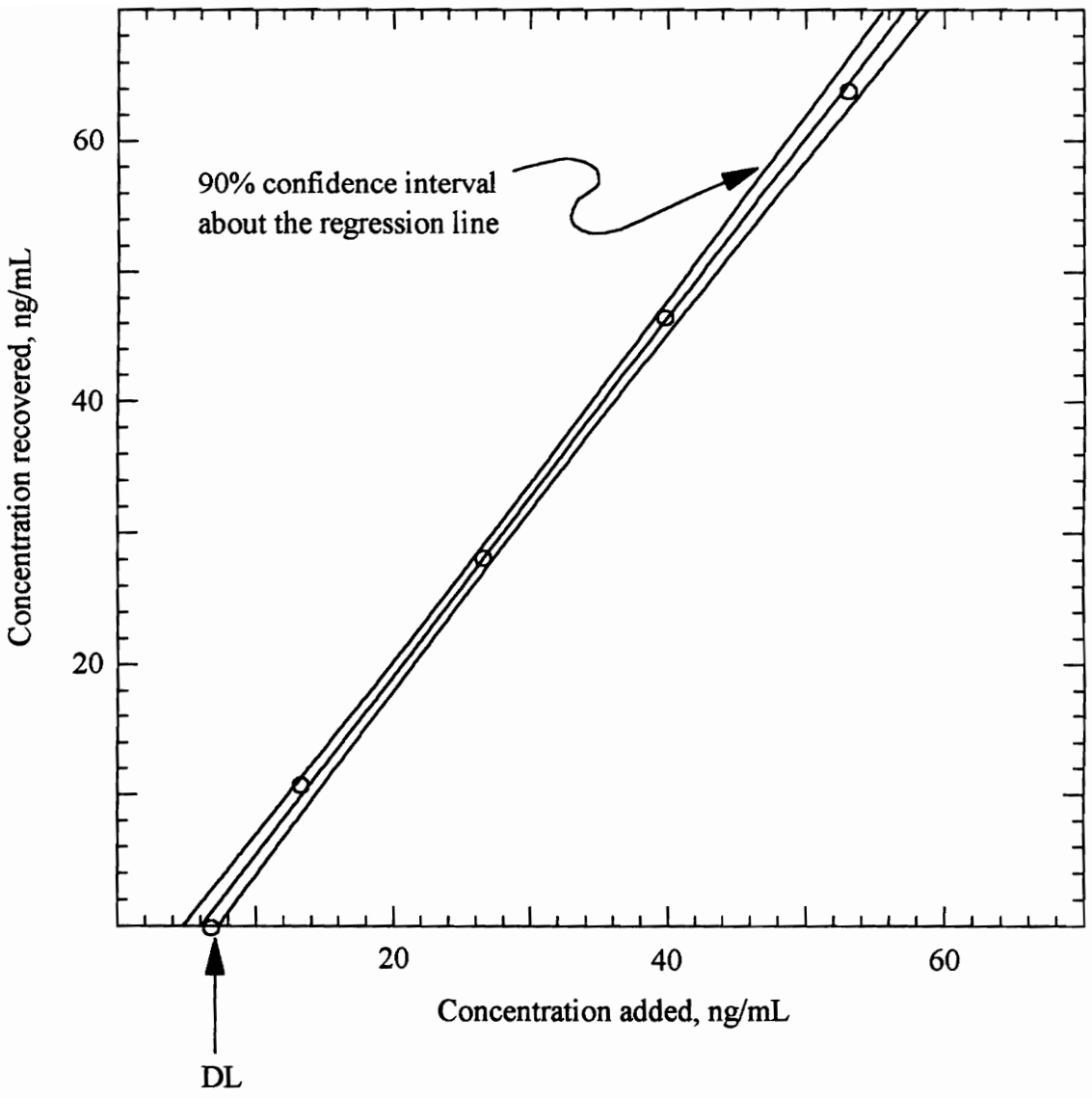


Figure D.1 Determination of 2t,6c-nonadienal detection limit (DL), using the method described by Sharaf (1986)

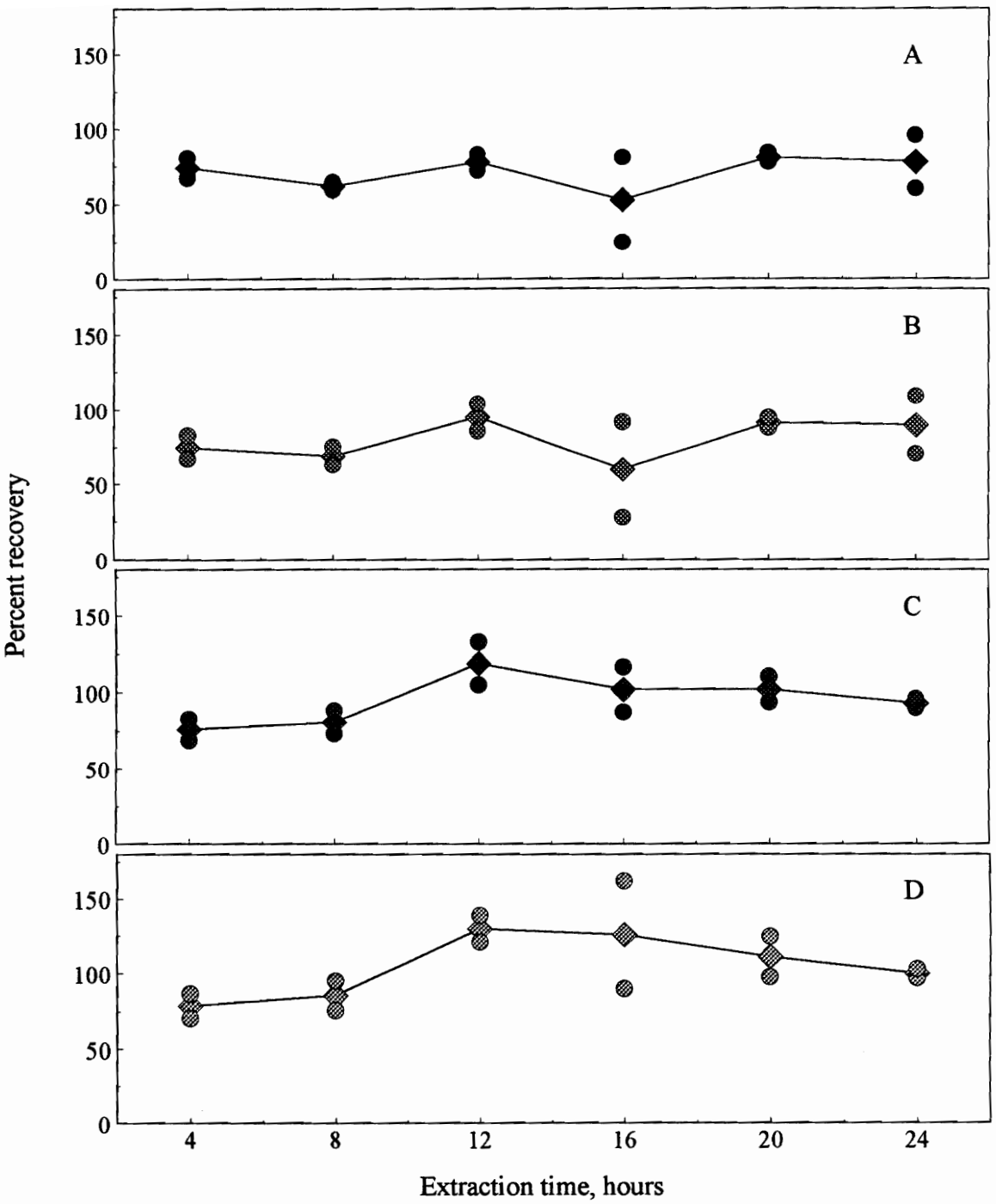


Figure D.2 Recoveries of four long straight-chain hydrocarbons from ASM-1 medium, (A) n-heptadecane, (B) n-octadecane, (C) n-nonadecane, and (D) n-eicosane, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

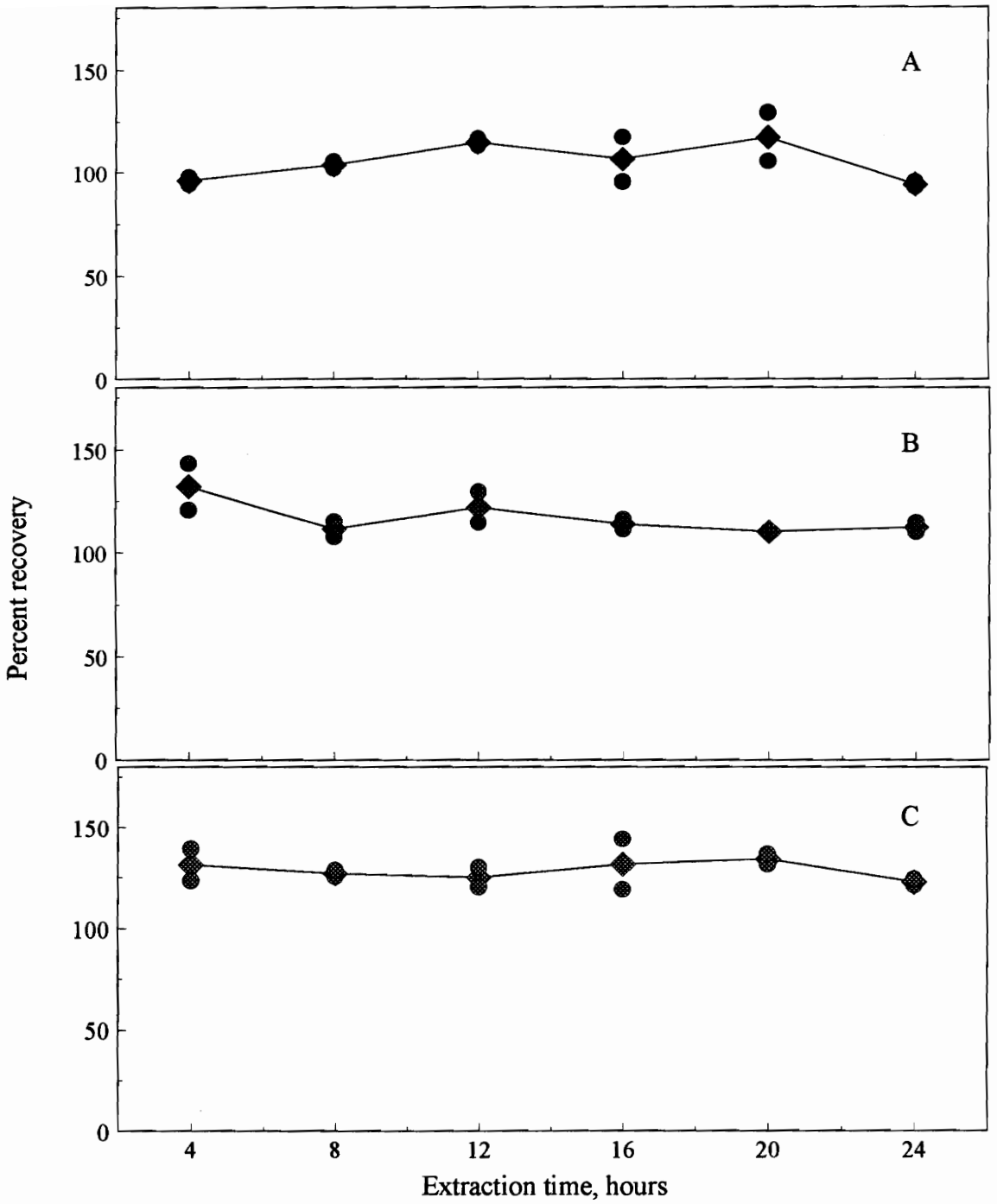


Figure D.3 Recoveries of (A) geosmin, (B) 2-methylisoborneol, and (C) 2t,6c-nonadienal from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

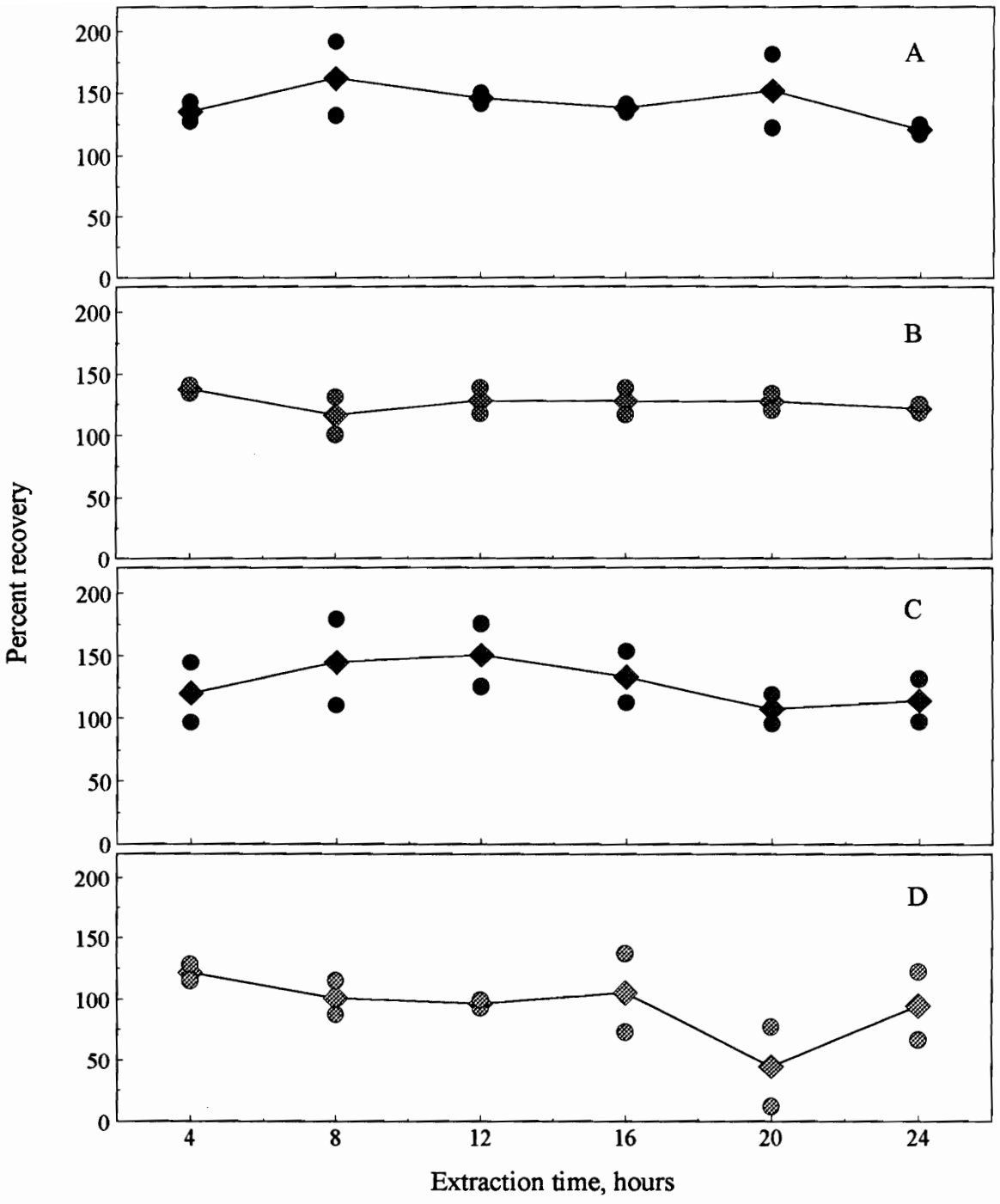


Figure D.4 Recoveries of (A) indanone, (B) phenethyl alcohol, (C) squalene, and (D) phytol from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

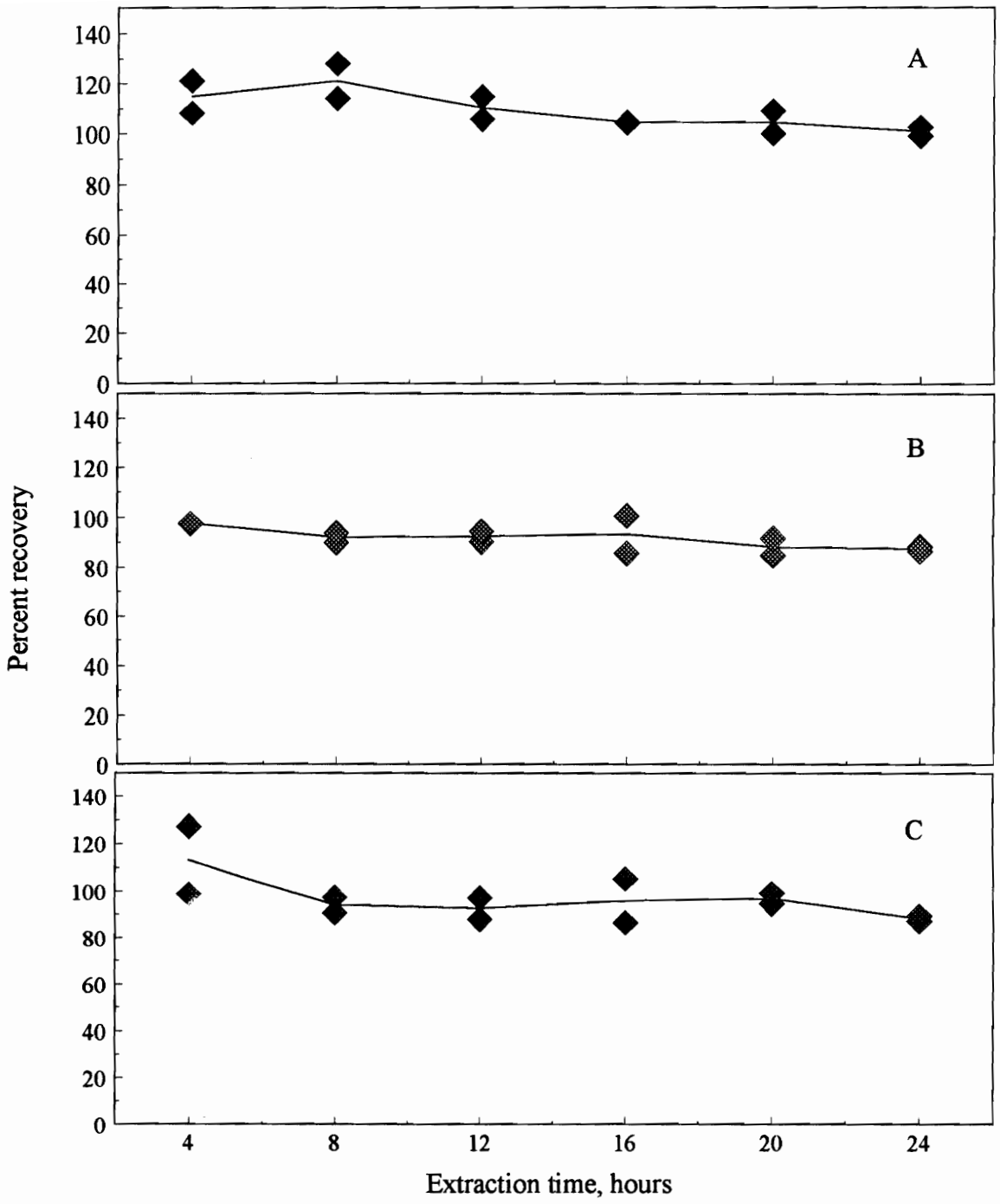


Figure D.5 Recoveries of (A) benzyl alcohol, (B) benzyl acetate, and (C) *B*-cyclocitral from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

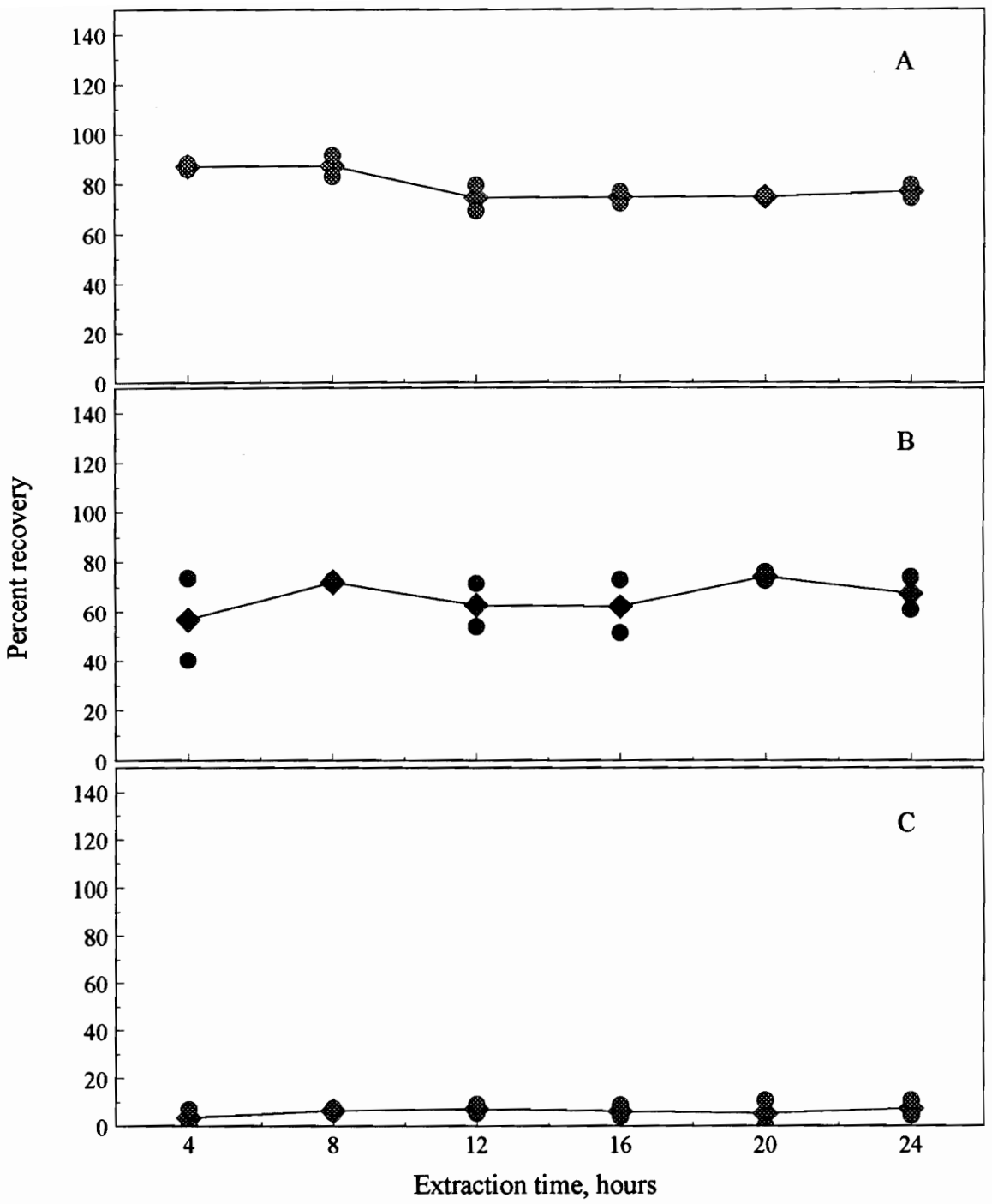


Figure D.6 Recoveries of (A) 3-furfural, (B) 1-heptadecene, and (C) isovaleric acid from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

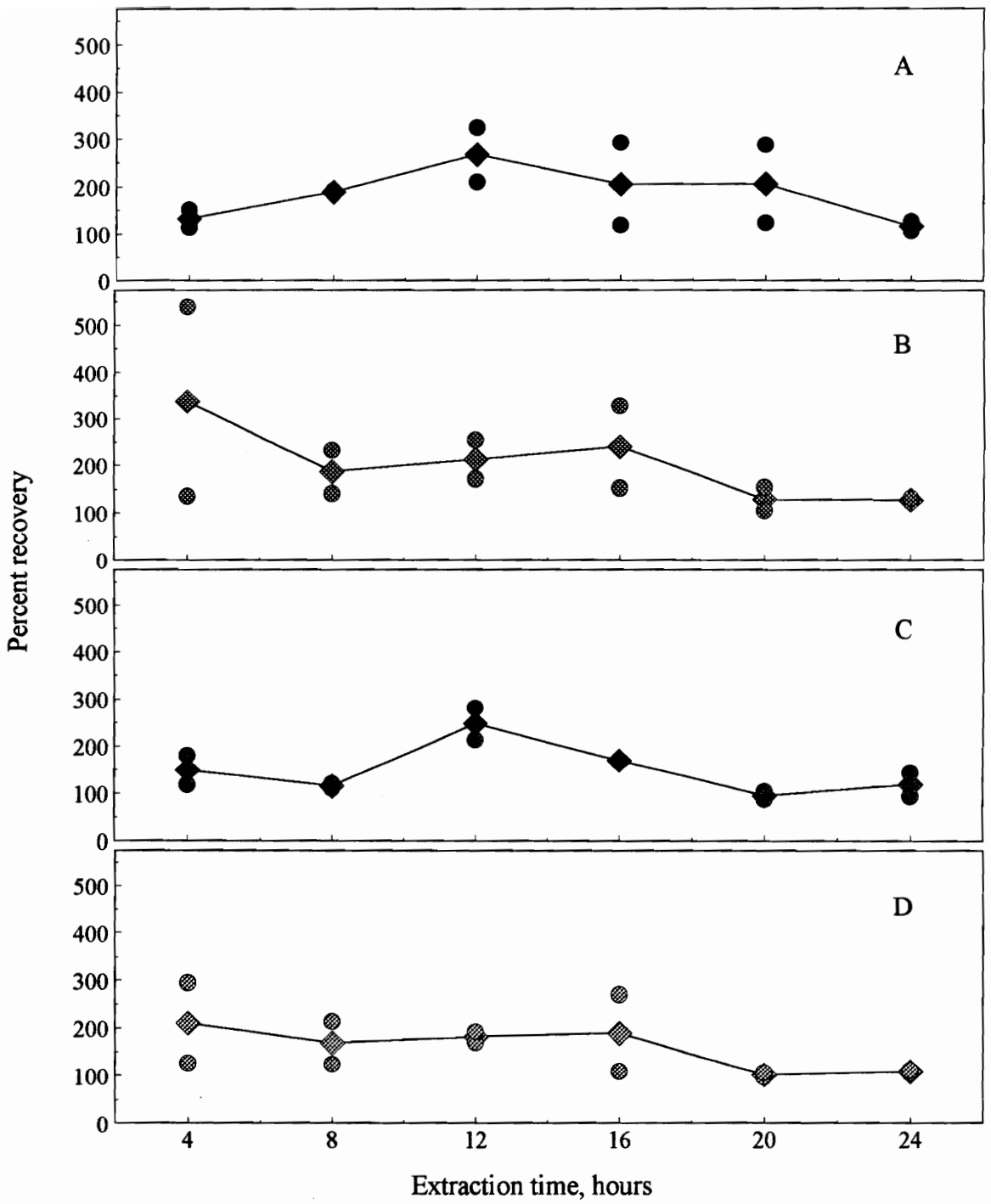


Figure D.7 Recoveries of (A) diphenylamine, (B) chlorophene, (C) 3-methylthio-1-propanol, and (D) 2-phenylphenol from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

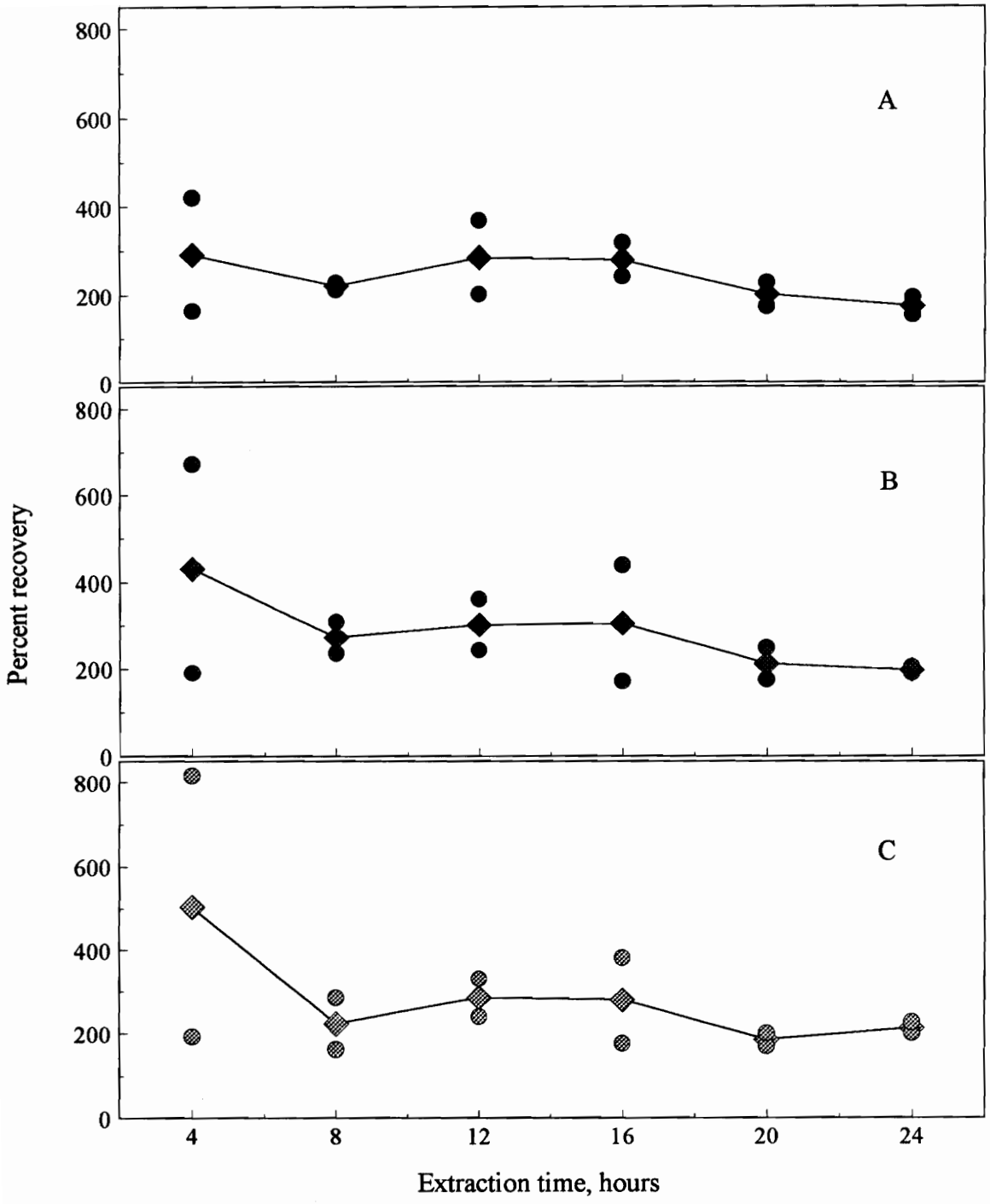


Figure D.8 Recoveries of (A) myristic, (B) palmitic, and (C) linolenic acids from ASM-1 medium, as functions of extraction time. (The dots represent actual data points, the diamonds represent the means.)

APPENDIX E
MASS SPECTRA AND GC-MS CHROMATOGRAMS OF COMPOUNDS

(Note: The ion scan range was from 40 to 400 amu; however, neither molecular ions nor ion fragments greater than 290 m/z were detected during analyses of the algal extracts.)

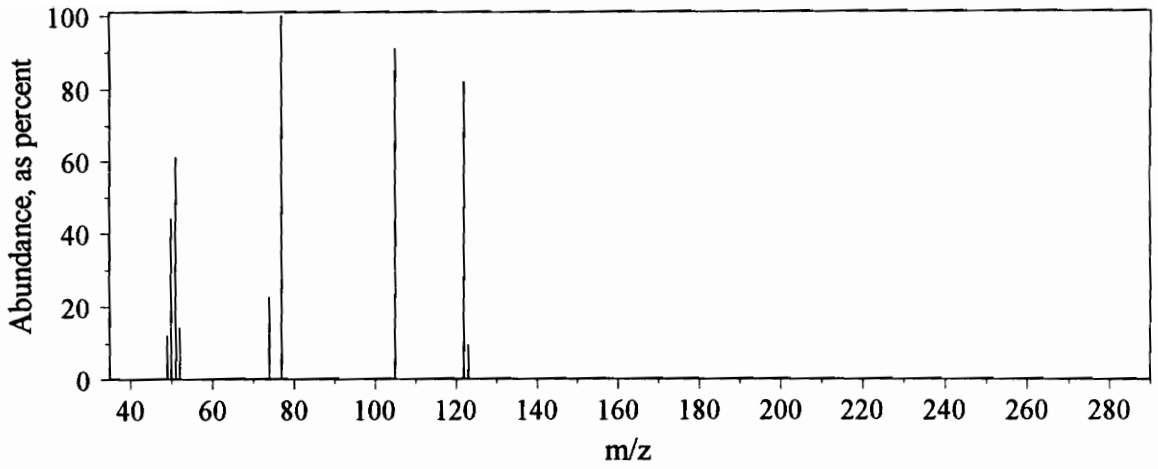


Figure E.1 Benzoate (a.k.a. benzoic acid, mol. wt. 122)

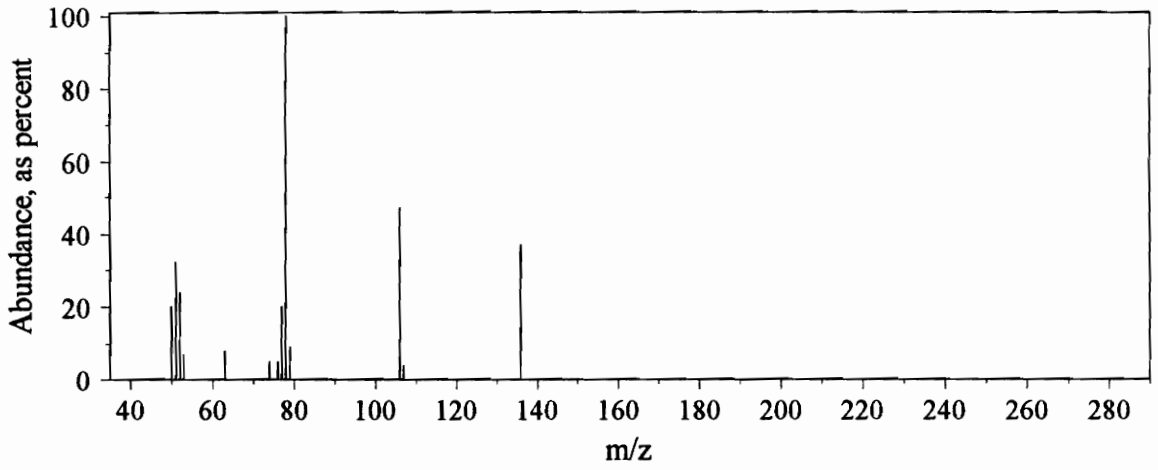


Figure E.2 4H-1,3-benzodioxin (mol. wt. 136)

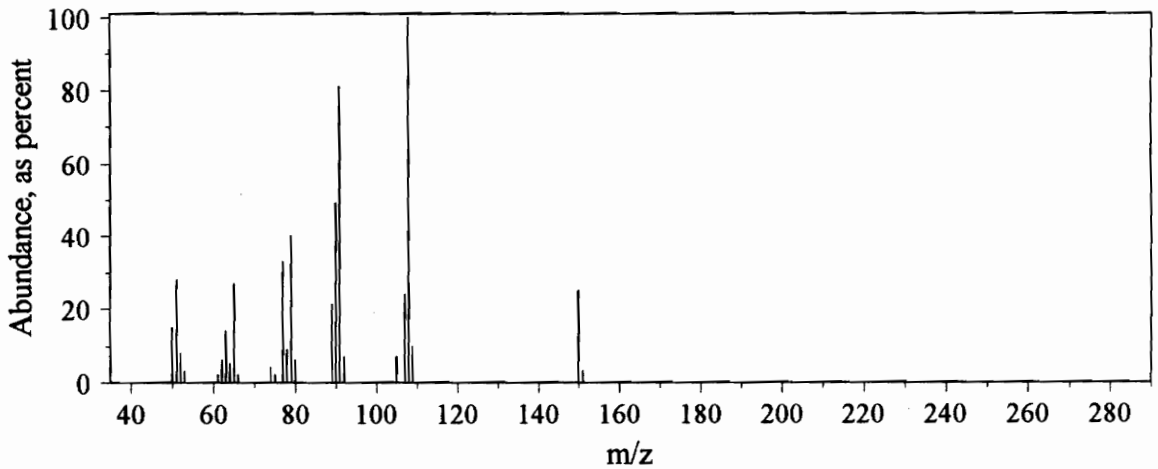


Figure E.3 Benzylacetate (a.k.a. acetic acid, phenylmethyl ester; mol. wt. 150)

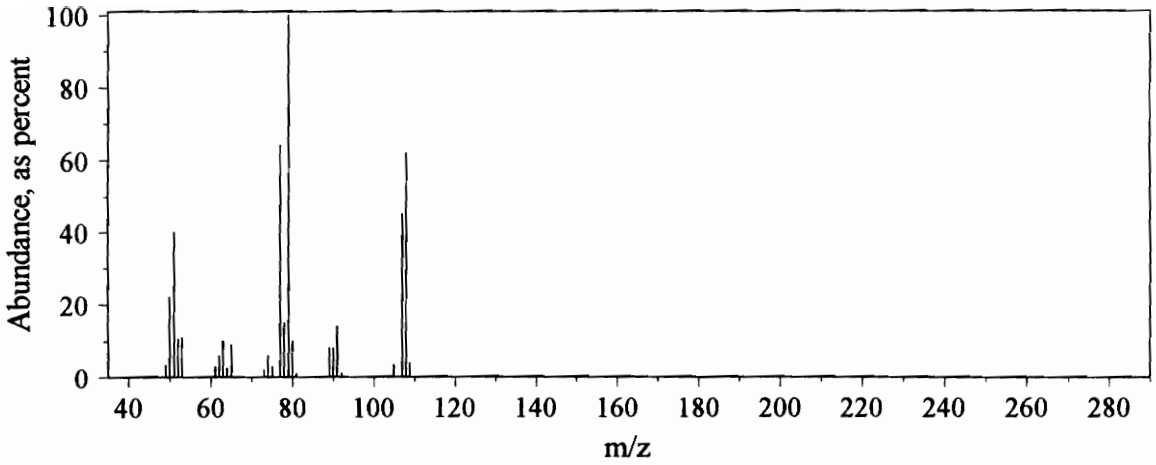


Figure E.4 Benzyl alcohol (a.k.a. benzene methanol, mol. wt. 108)

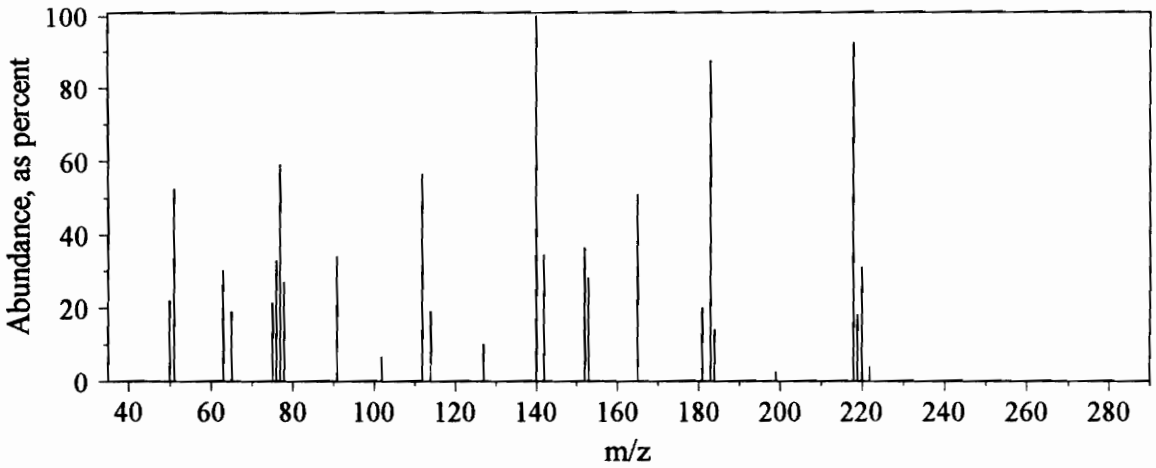


Figure E.5 Chlorophene (a.k.a. o-benzyl-p-chlorophenol, mol. wt. 218)

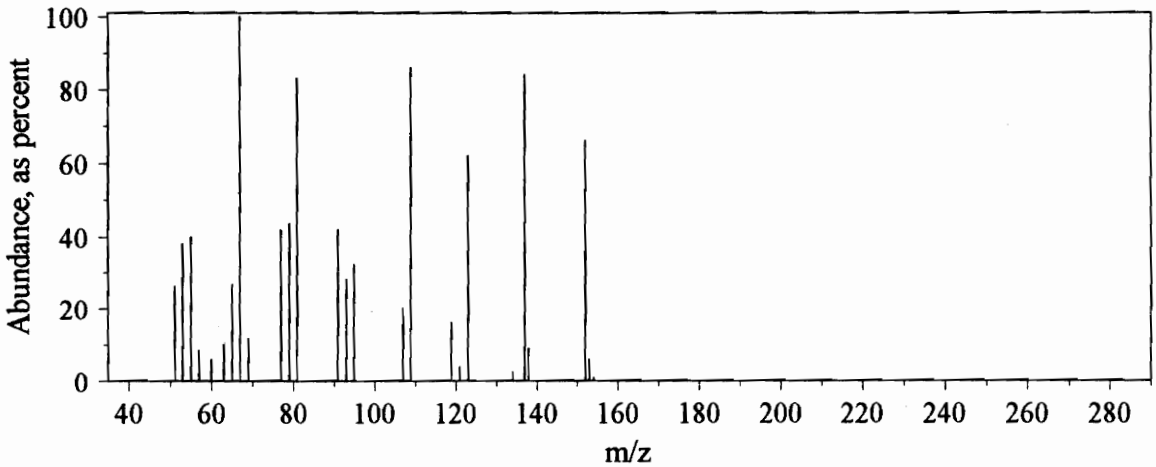


Figure E.6 *B*-cyclocitral (a.k.a. 2,6,6-trimethyl-1-cyclohexene-1-al, mol. wt. 152)

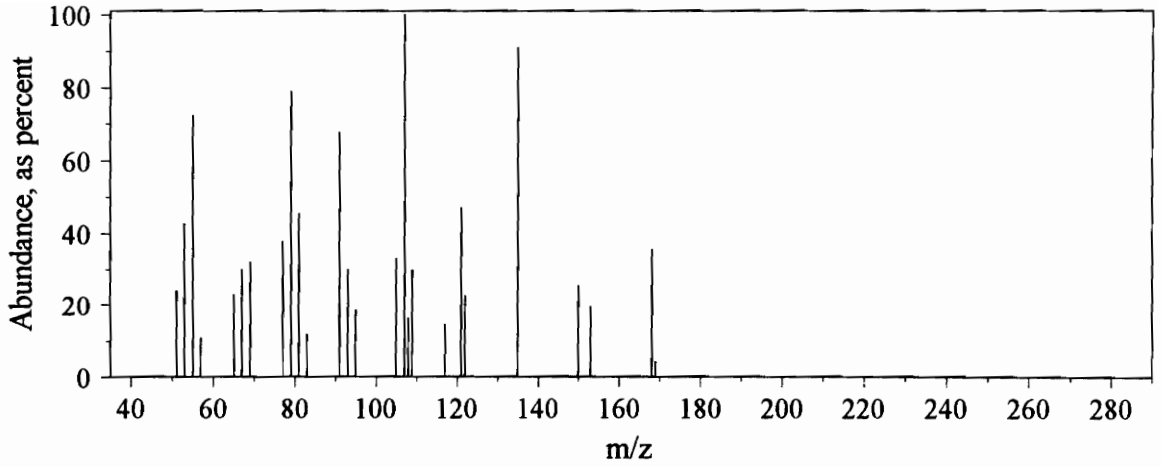


Figure E.7 Hydroxy-*B*-cyclocitral (a.k.a. 2,6,6-trimethyl-1-cyclohexene-4-ol-1-al, mol. wt. 168)

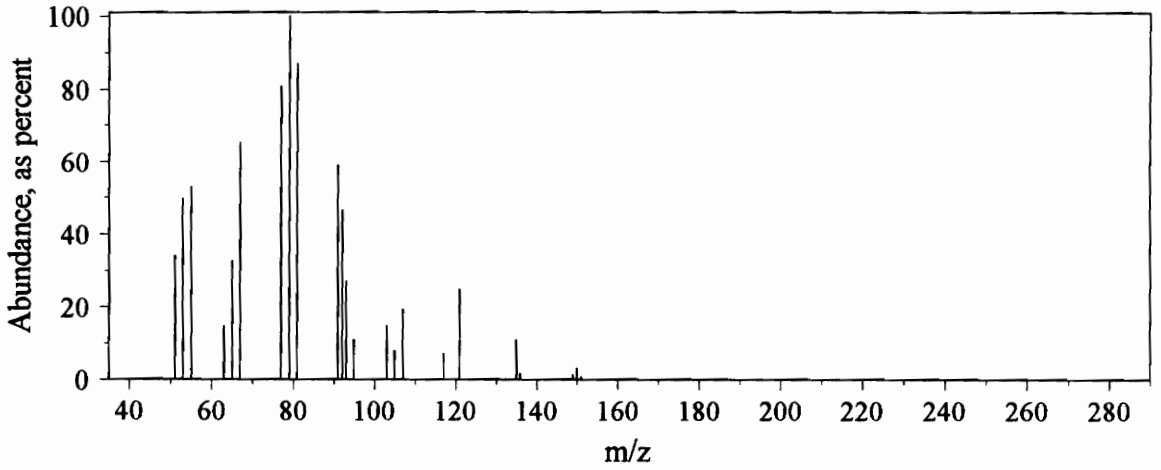


Figure E.8 2t,4c,7c-decatrienal (mol. wt. 150)

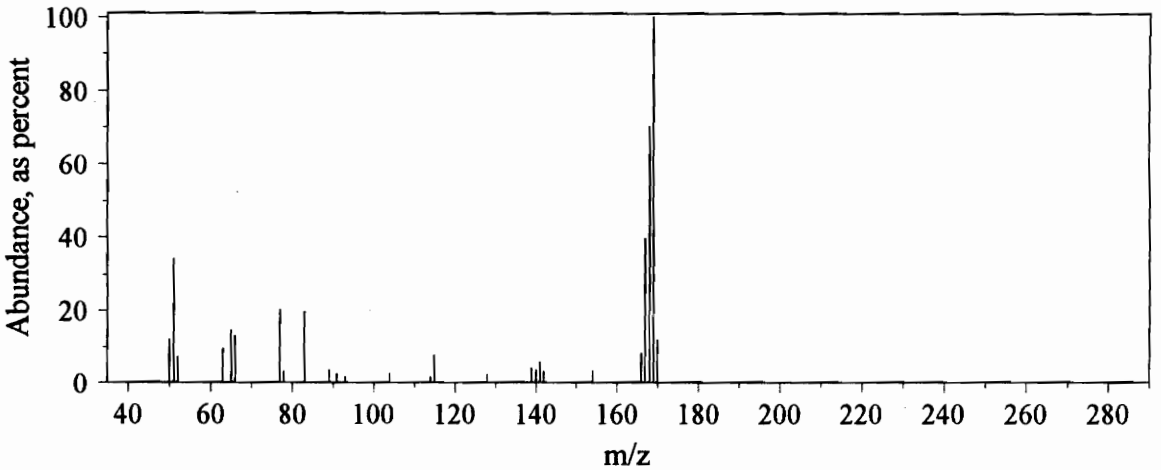


Figure E.9 Diphenylamine (mol. wt. 169)

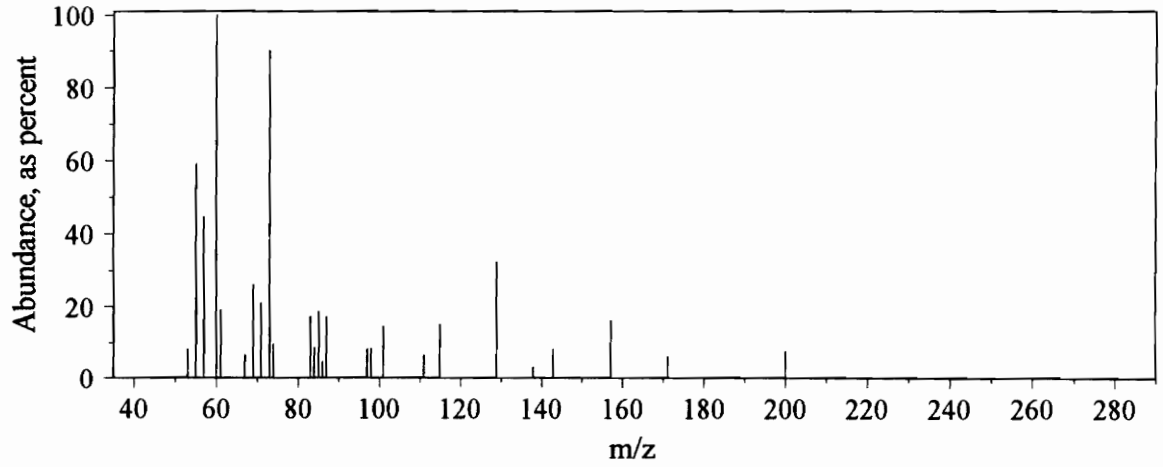


Figure E.10 Dodecanoate (a.k.a. dodecanoic acid, mol. wt. 200)

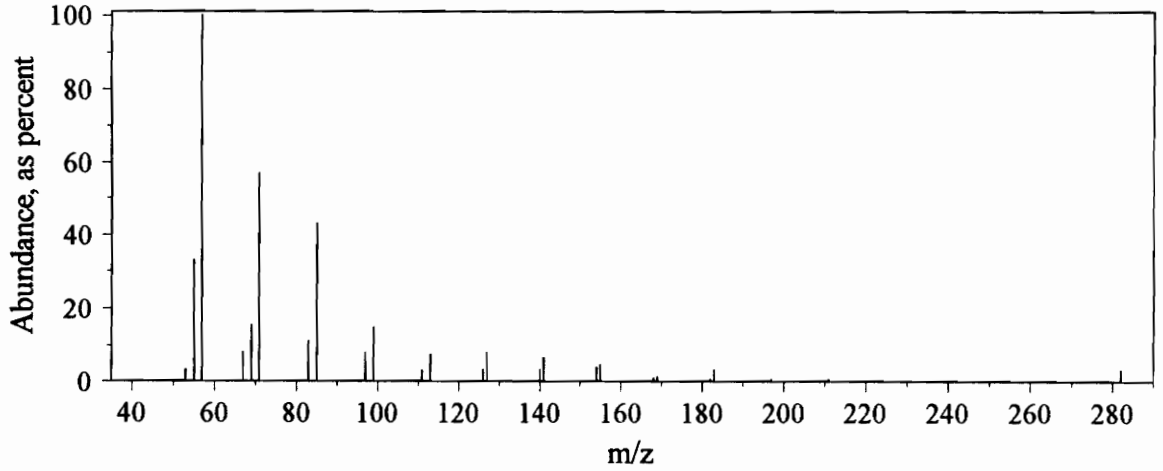


Figure E.11 n-Eicosane (mol. wt. 282)

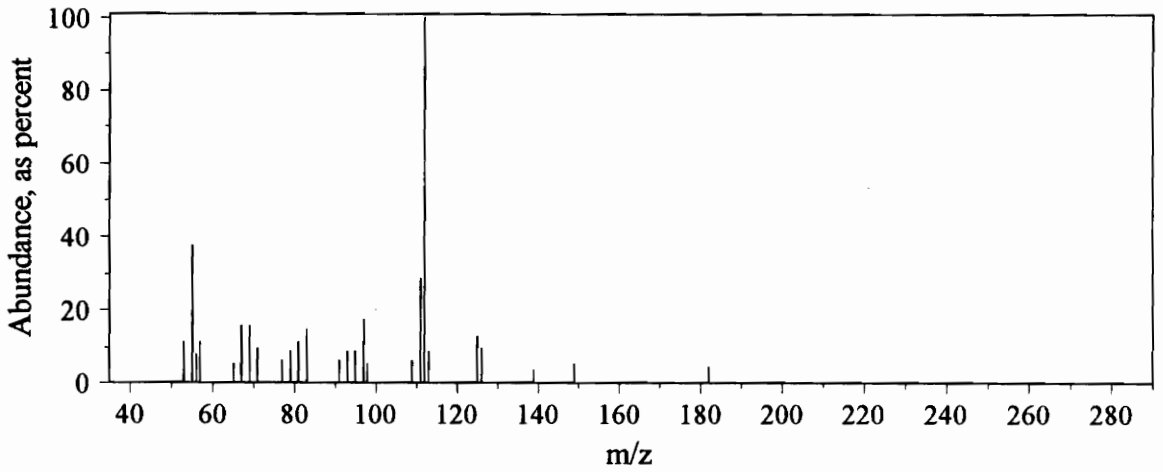


Figure E.12 Geosmin (mol. wt. 182)

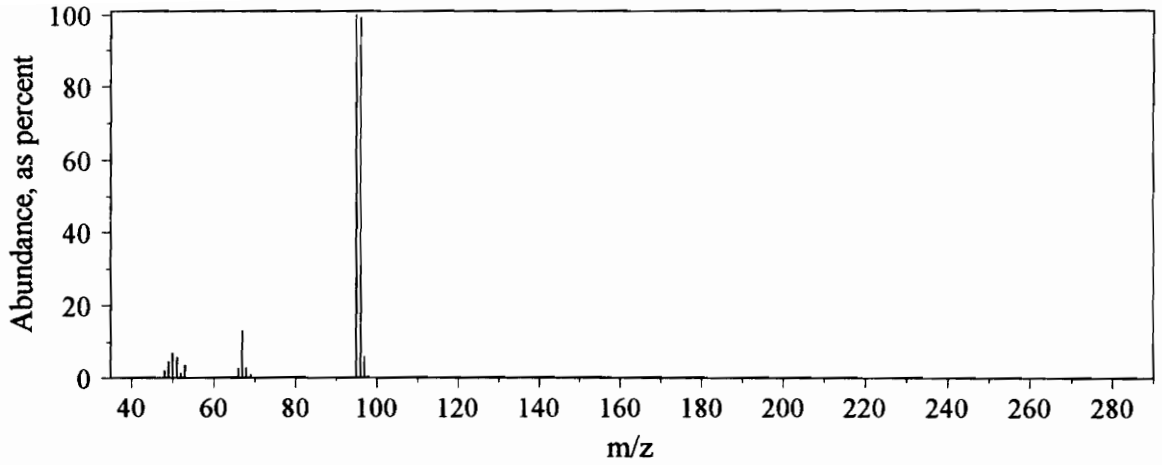


Figure E.13 2-Furfural (mol. wt. 96)

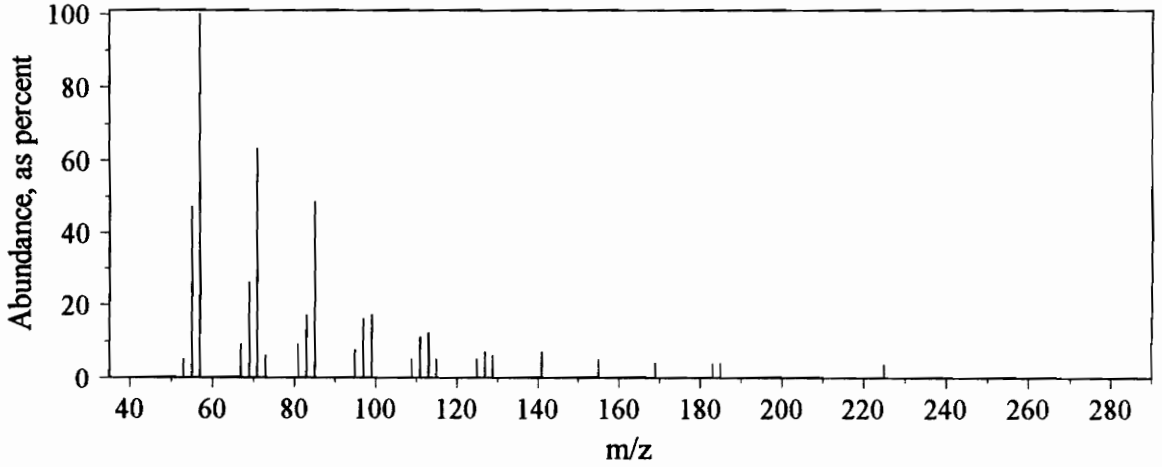


Figure E.14 n-Heneicosane (mol. wt. 296)

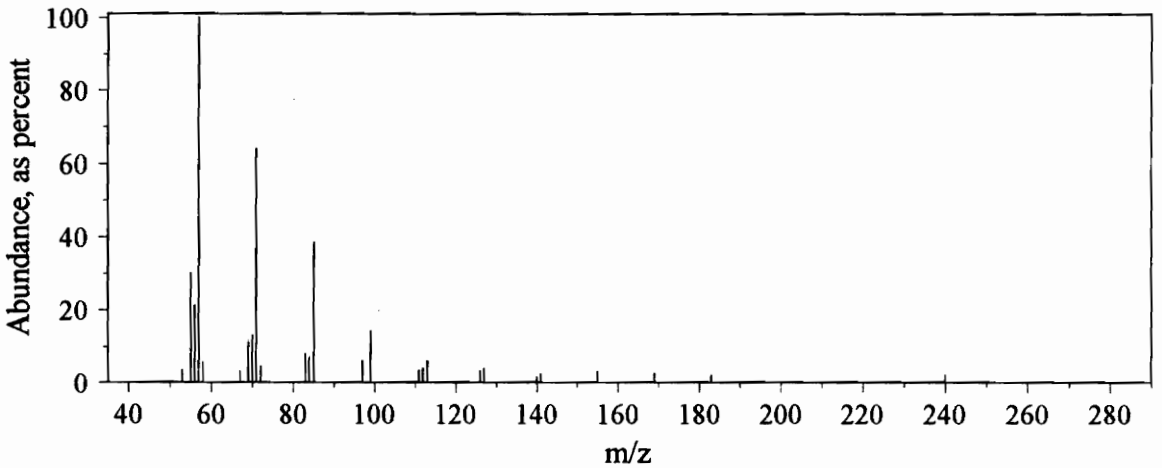


Figure E.15 n-Heptadecane (mol. wt. 240)

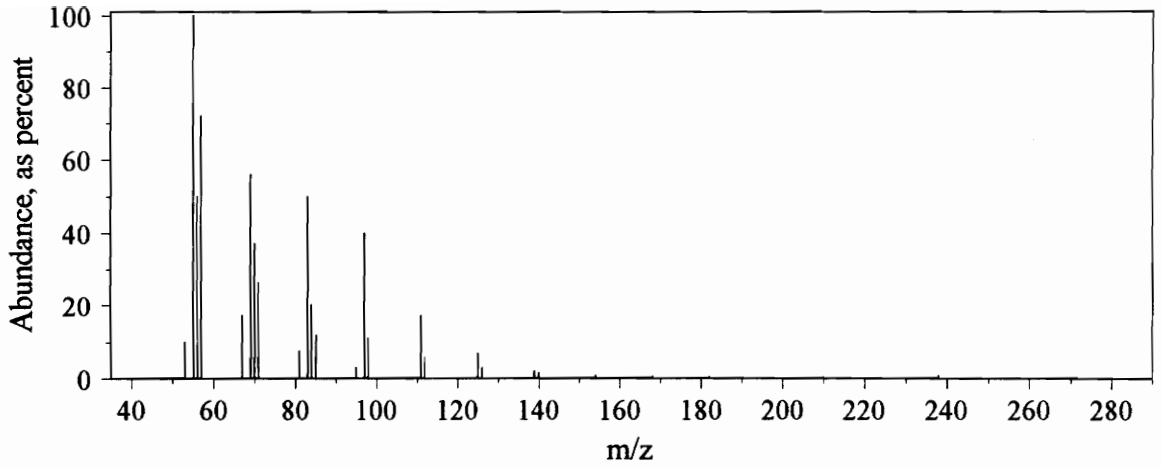


Figure E.16 Heptadecene (mol. wt. 238)

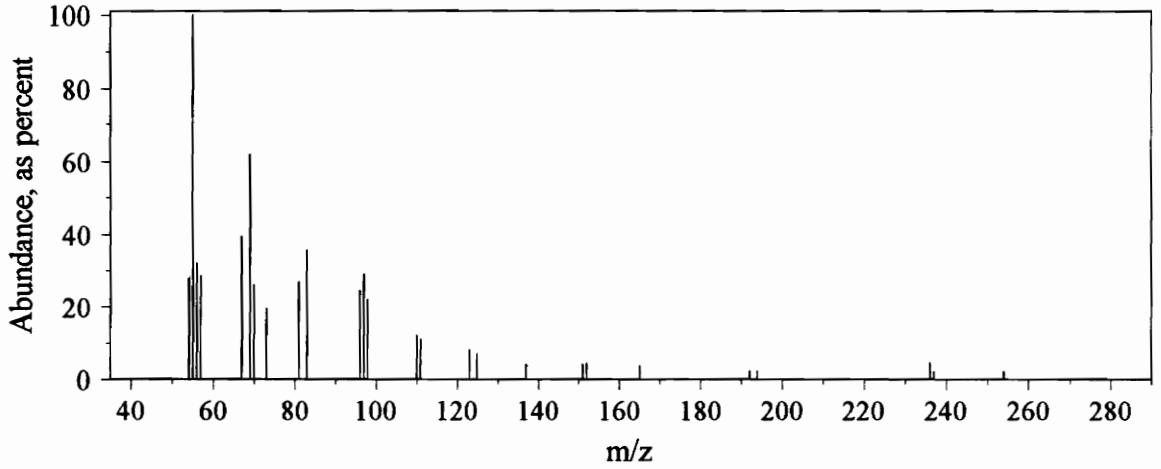


Figure E.17 Hexadecenoate (mol. wt. 254)

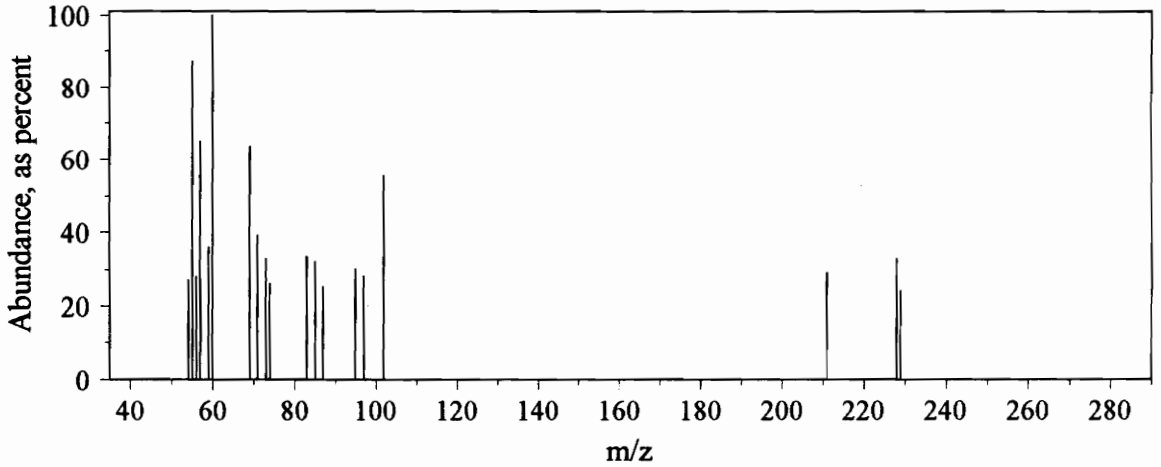


Figure E.18 Isopropyl myristate (mol. wt. 270)

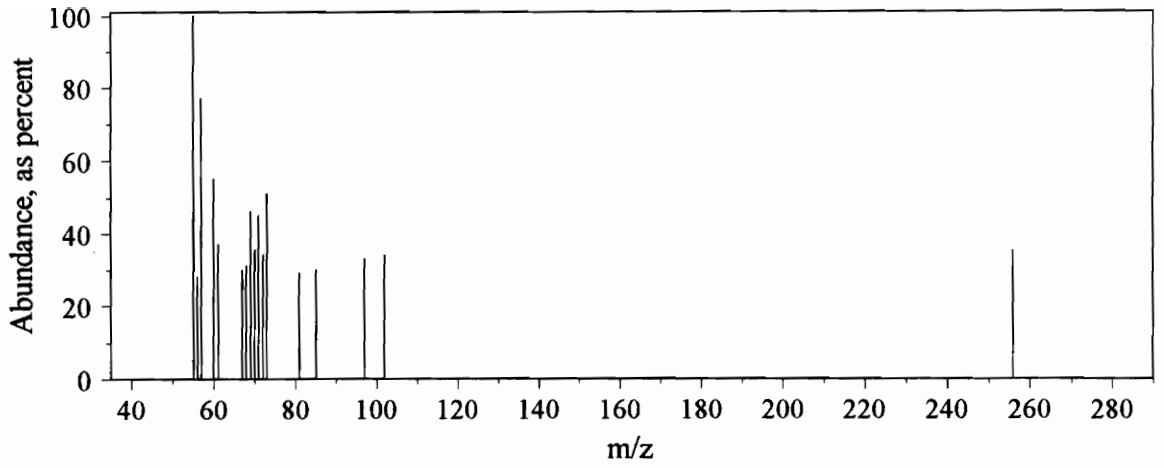


Figure E.19 Isopropyl palmitate (mol. wt. 298)

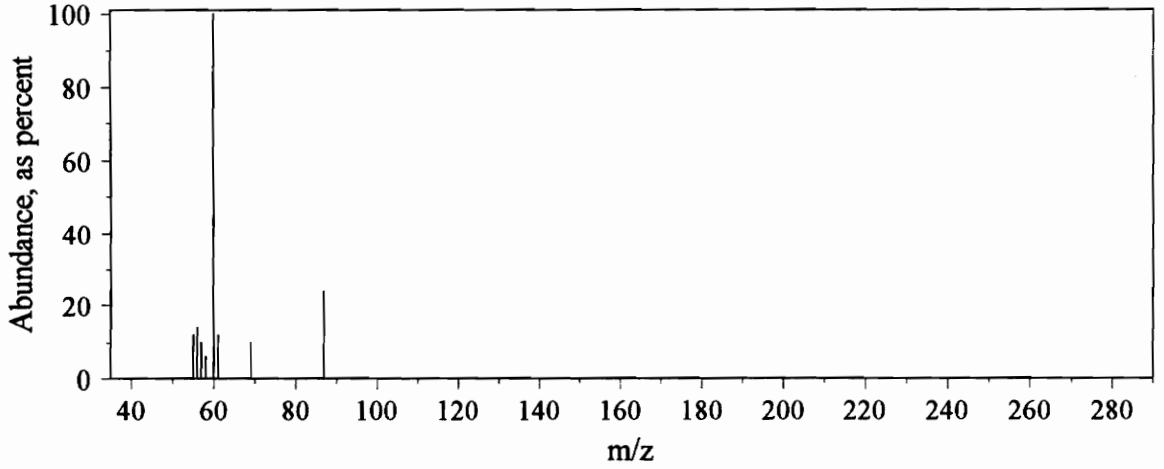


Figure E.20 Isovaleric acid (a.k.a. 3-methyl butyrate, mol. wt. 102)

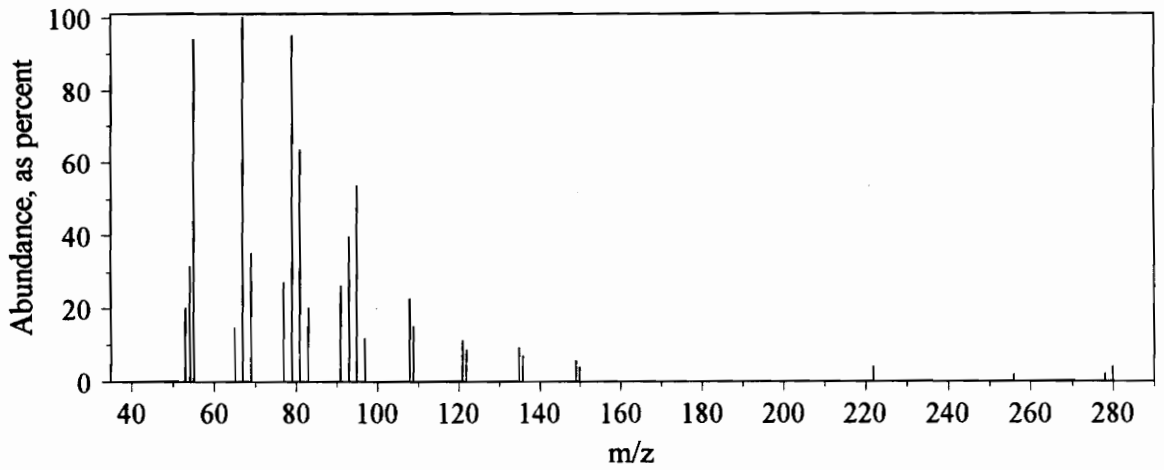


Figure E.21 Linoleic acid (a.k.a. 9,12-octadecadienoate, mol. wt. 280)

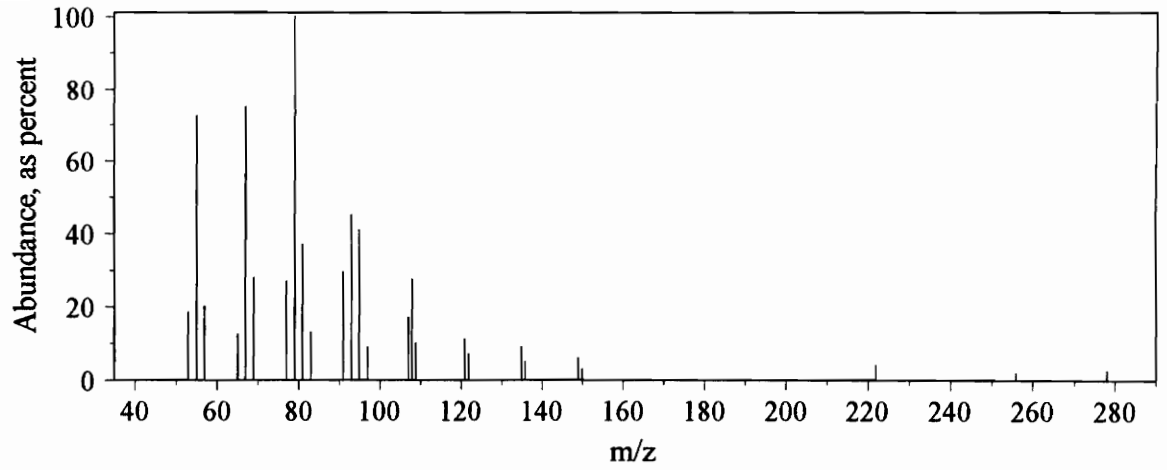


Figure E.22 Linolenic acid (a.k.a. 9,12,15-octadecatrienoate, mol. wt. 278)

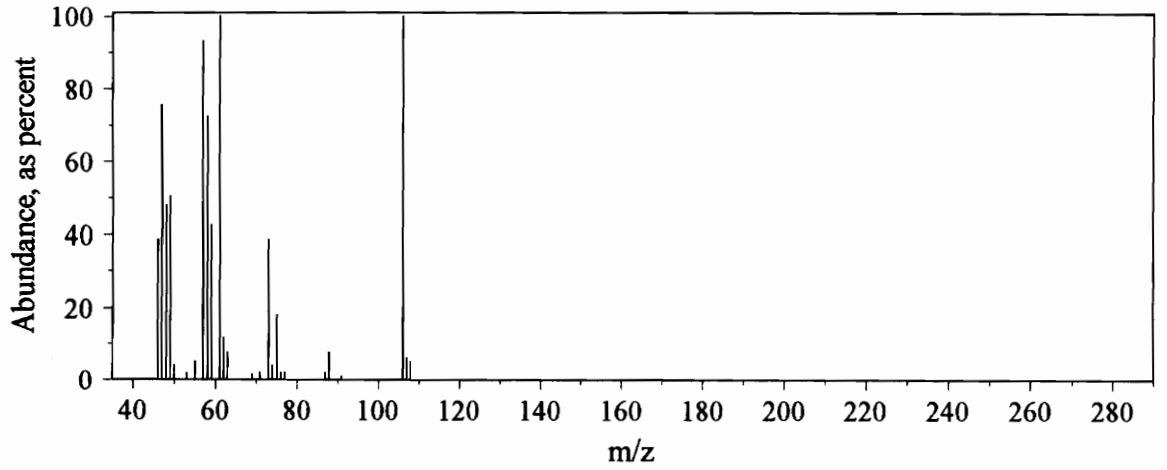


Figure E.23 3-Methylthio-1-propanol (mol. wt. 106)

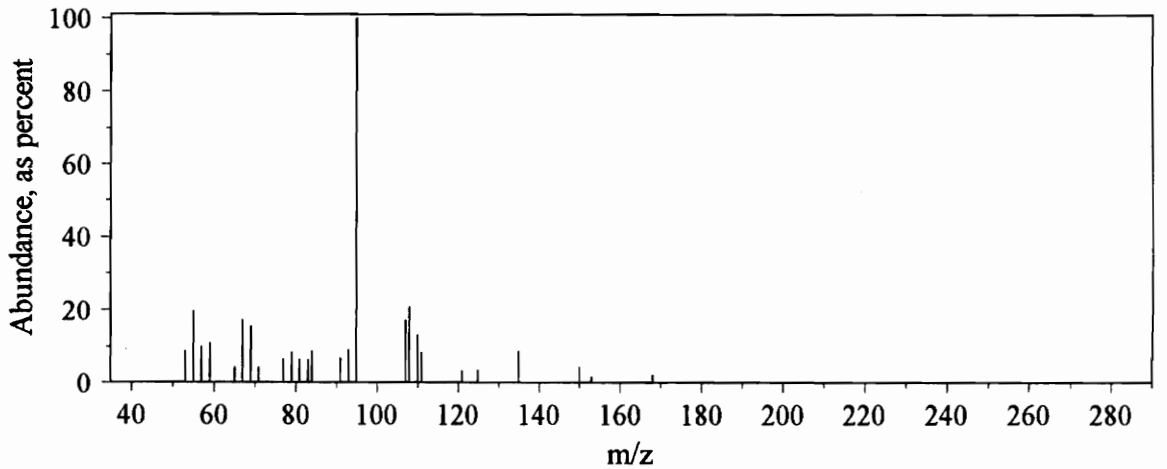


Figure E.24 MIB (2-methylisoborneol, mol. wt. 168)

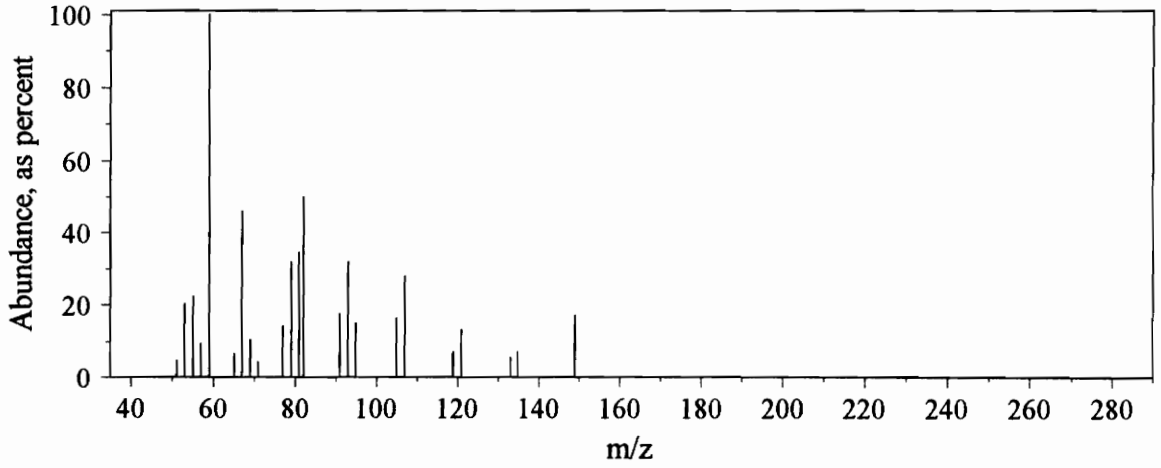


Figure E.25 Myrcenol (mol. wt. 154)

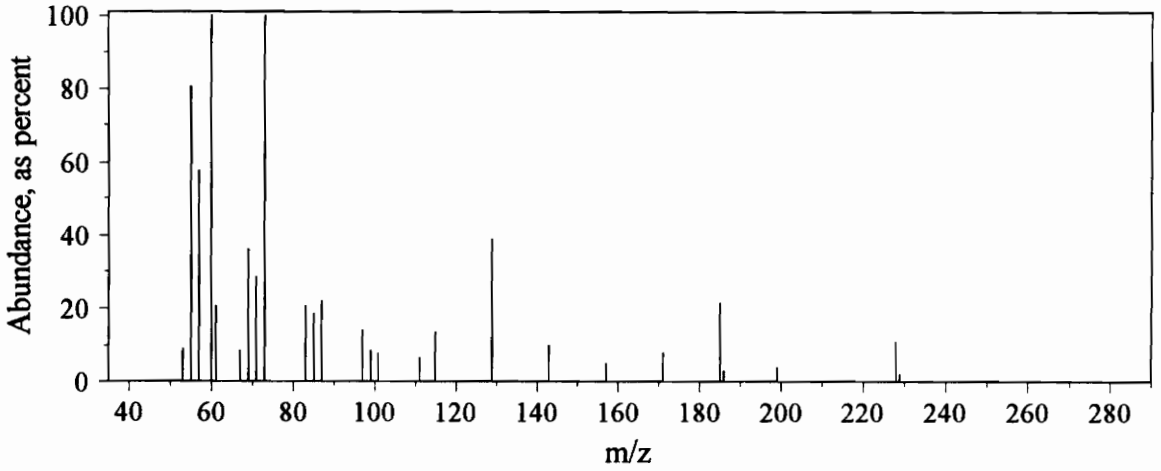


Figure E.26 Myristic acid (a.k.a. tetradecanoate, mol. wt. 228)

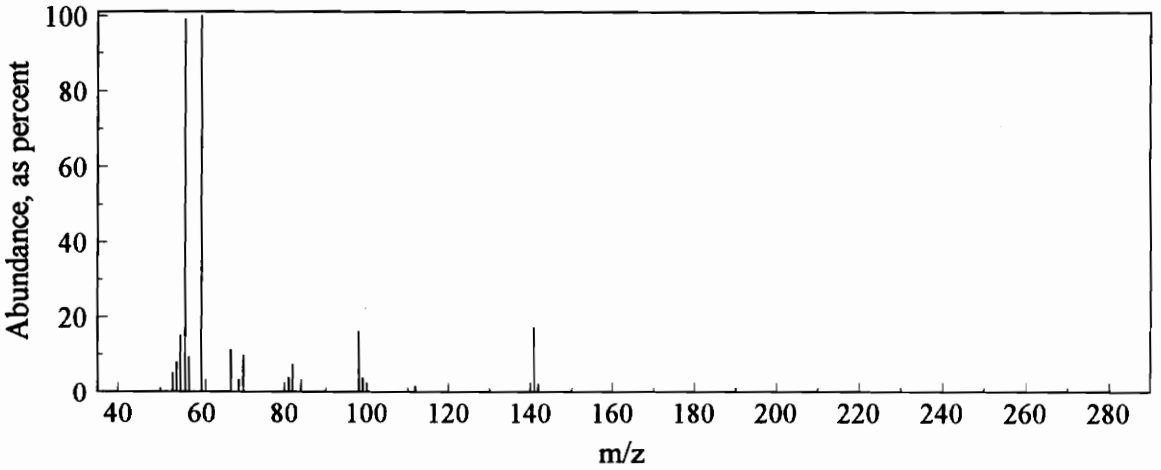


Figure E.27 N-cyclohexyl-acetamide (mol. wt. 141)

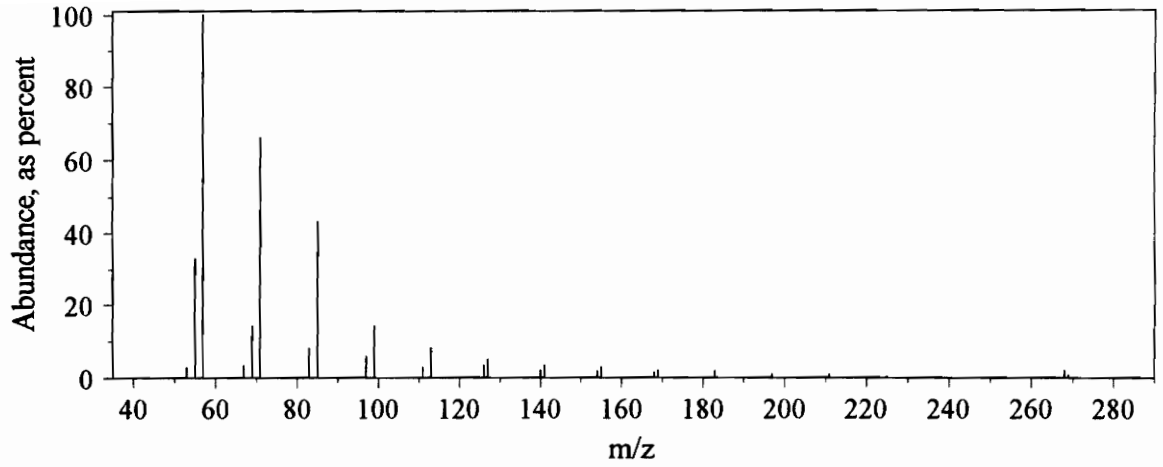


Figure E.28 n-Nonadecane (mol. wt. 268)

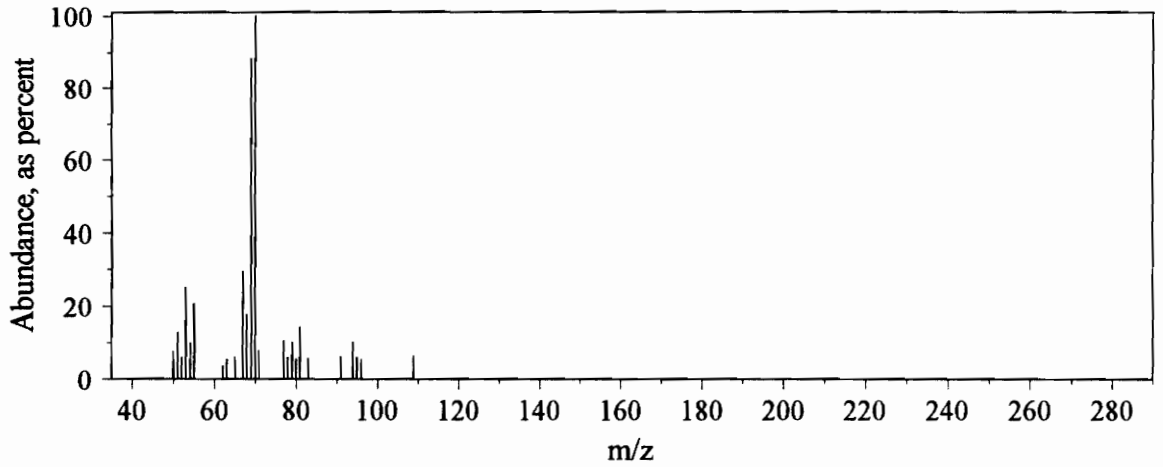


Figure. E.29 2t,6c-nonadienal (mol. wt. 138)

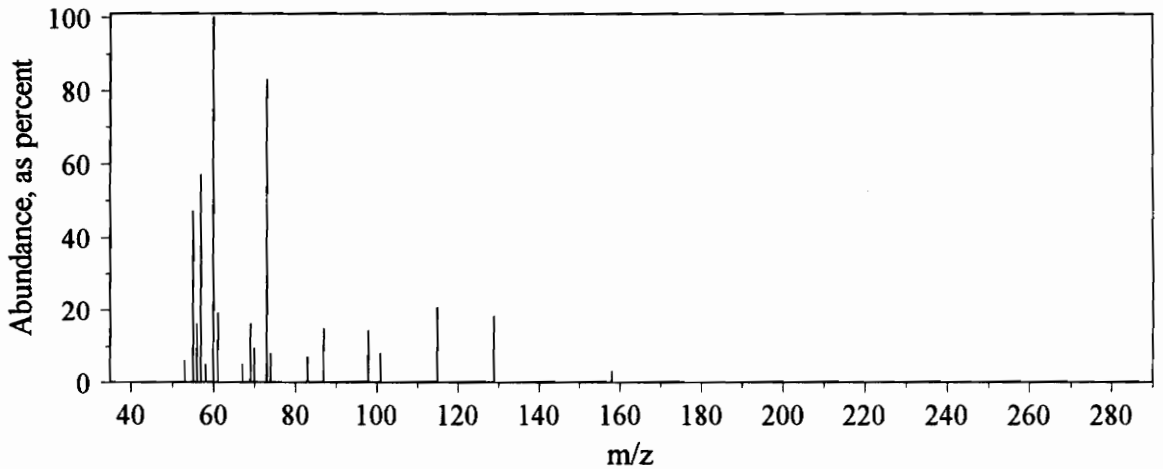


Figure E.30 Nonanoate (a.k.a. nonanoic acid, mol. wt. 158)

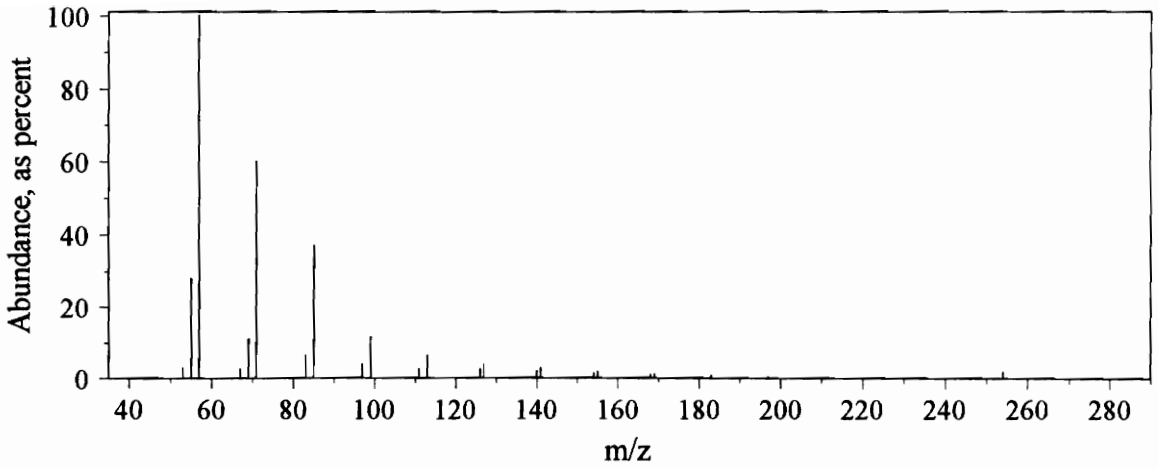


Figure E.31 n- Octadecane (mol. wt. 254)

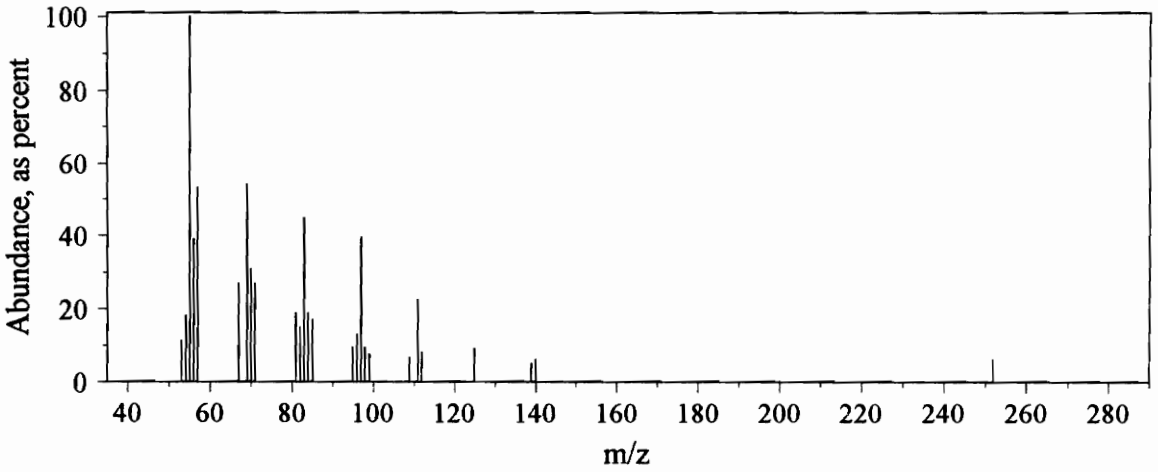


Figure E.32 1-Octadecene (mol. wt. 252)

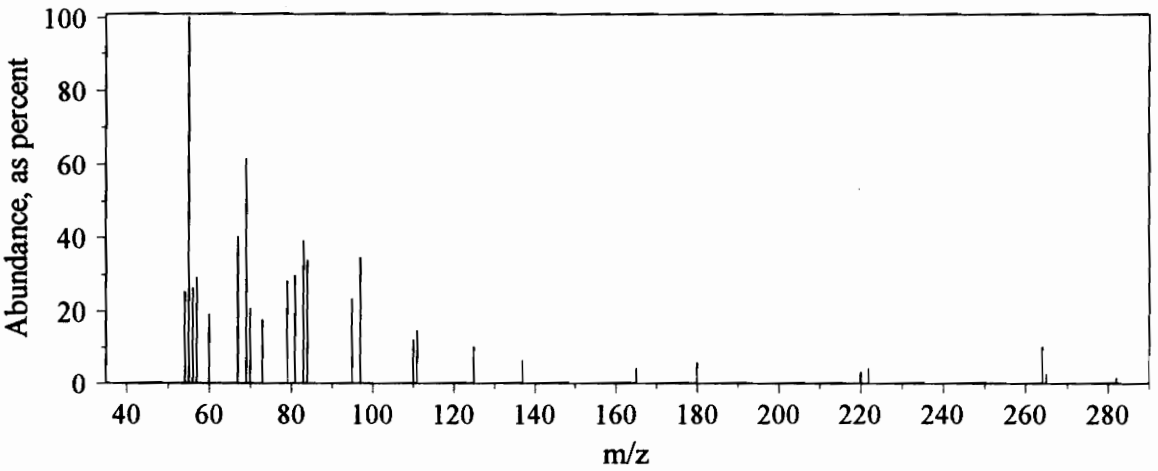


Figure E.33 Oleic acid (a.k.a. 9-octadecenoate, mol. wt. 282)

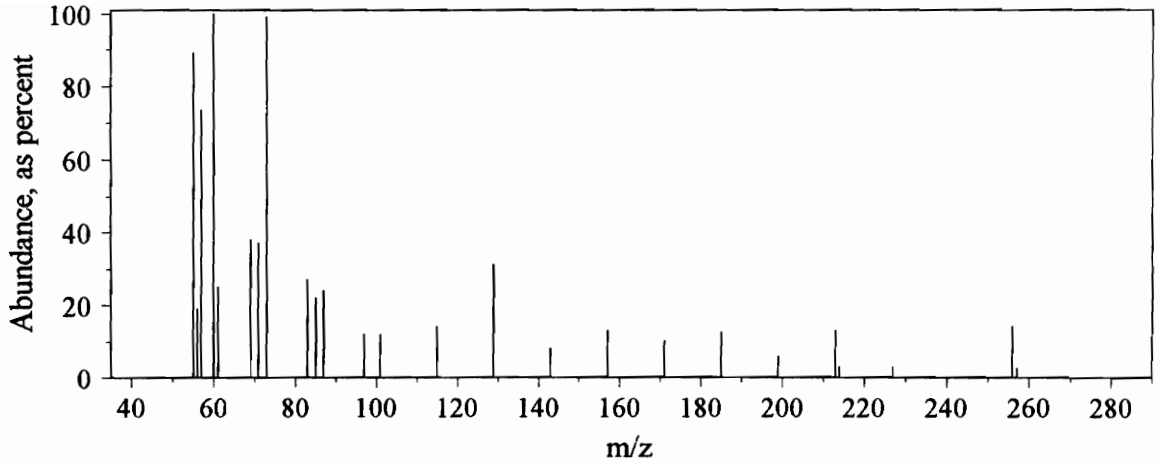


Figure E.34 Palmitic acid (a.k.a. hexadecanoate, mol. wt. 256)

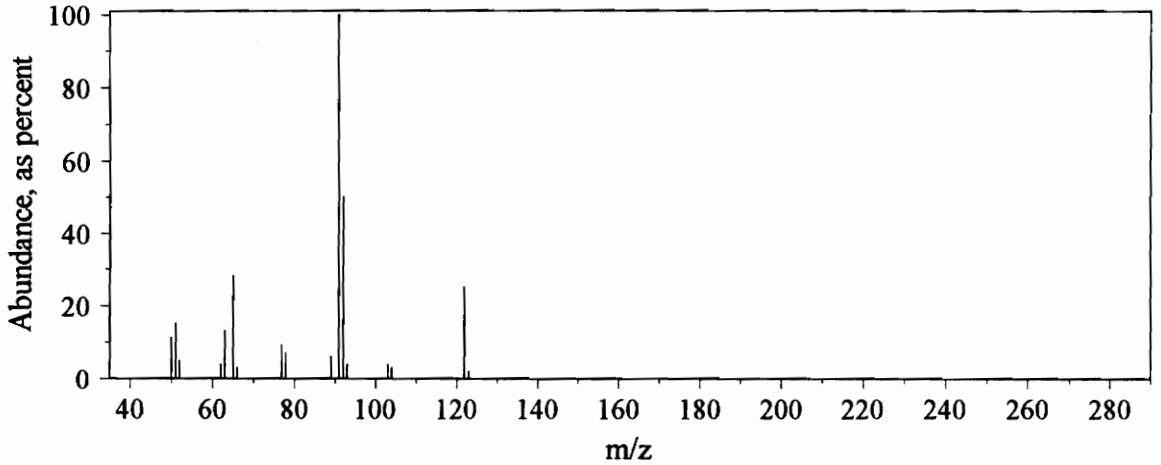


Figure E.35 Phenethyl alcohol (a.k.a benzeneethanol, mol. wt. 122)

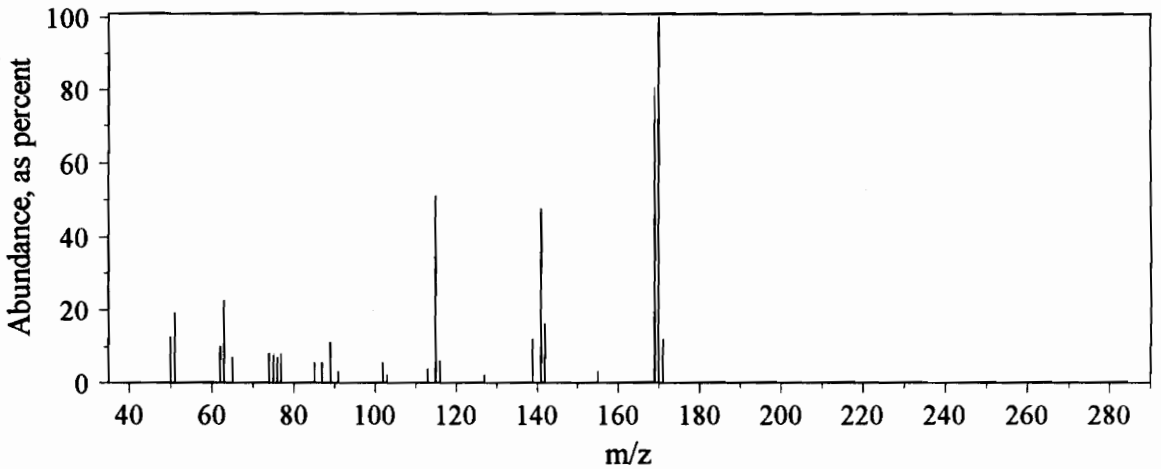


Figure E.36 2-Phenylphenol (a.k.a. o-hydroxybiphenyl, mol. wt. 170)

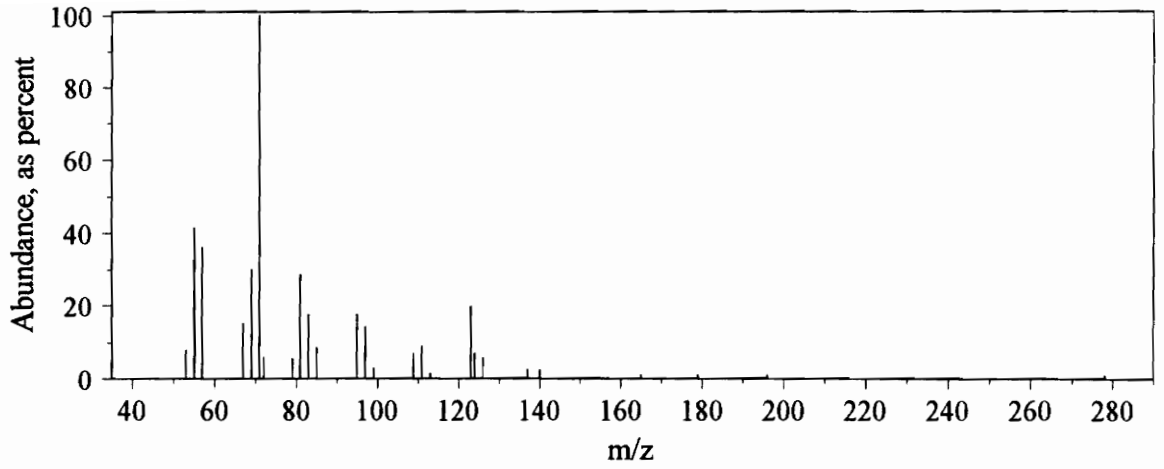


Figure E.37 Phytol (a.k.a. 3,7,11,15-tetramethyl-2-hexadecen-1-ol, mol. wt. 296)

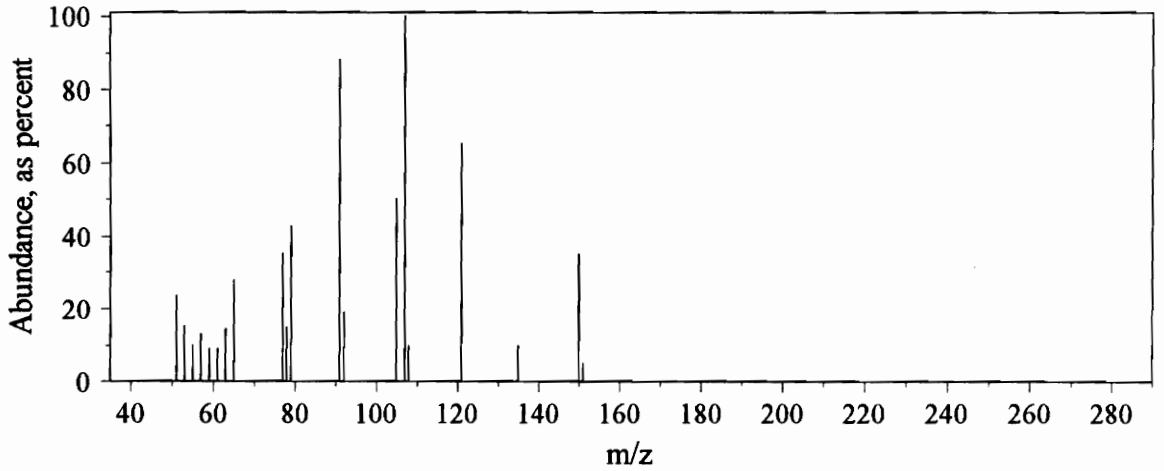


Figure E.38 Safranal (a.k.a. dehydro-*B*-cyclocitral, mol. wt. 150)

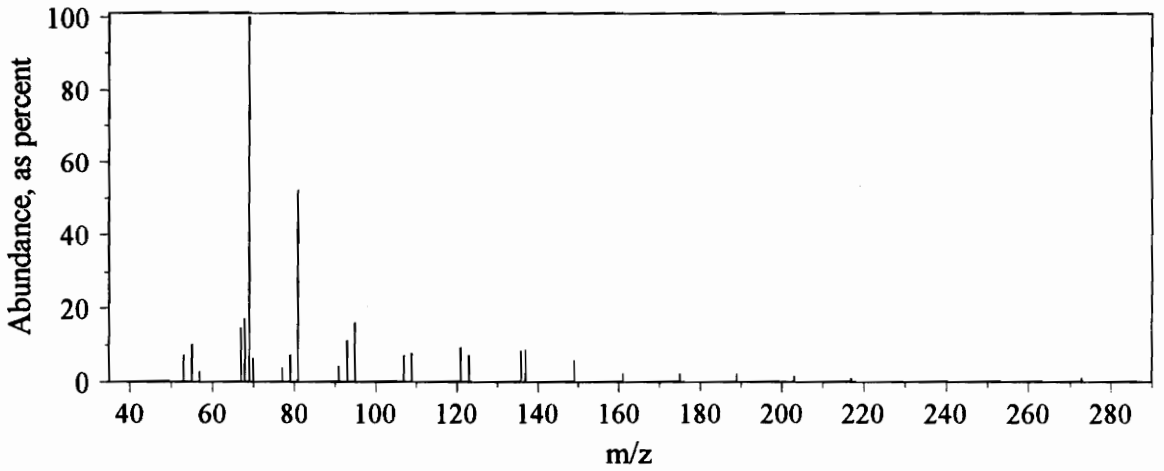


Figure E.39 Squalene (a.k.a. 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, mol. wt. 410)

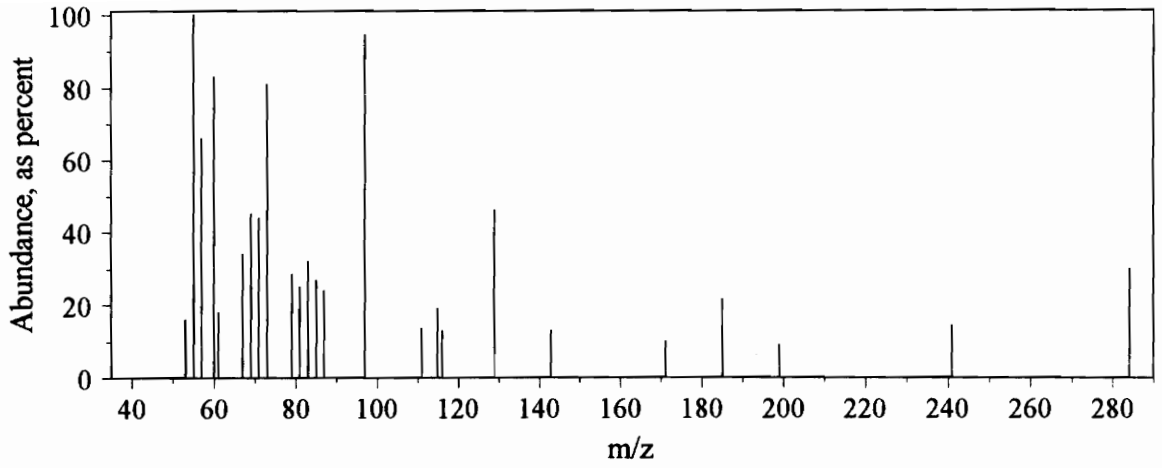


Figure E.40 Stearic acid (a.k.a. octadecanoate, mol. wt. 284)

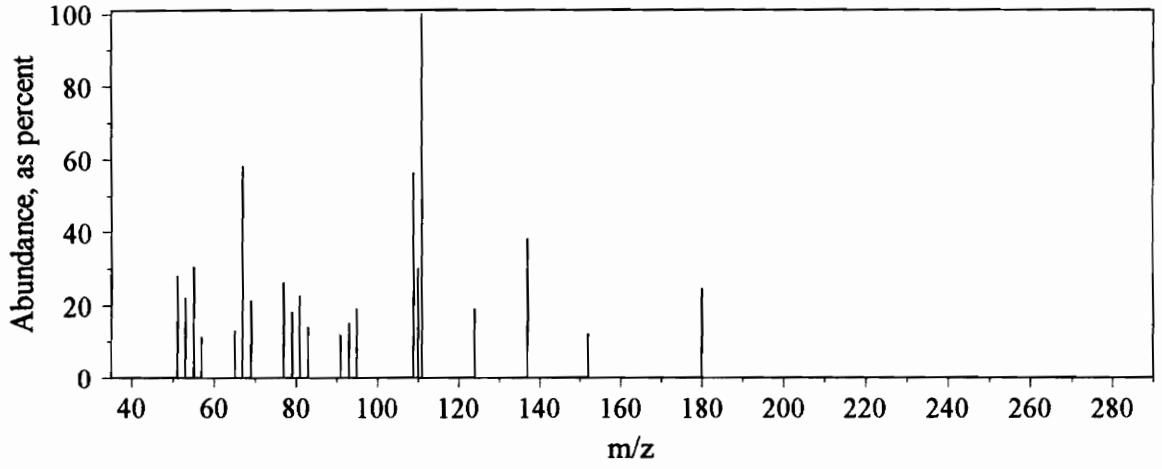


Figure E.41 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (mol. wt. 180)

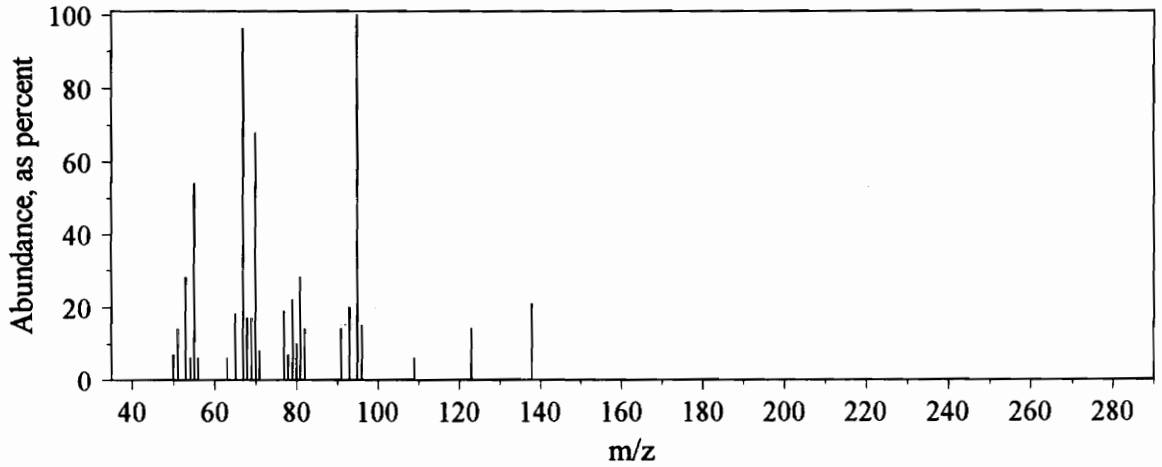


Figure E.42 Unknown @ 10.8 min (*B*-cyclocitral related?)

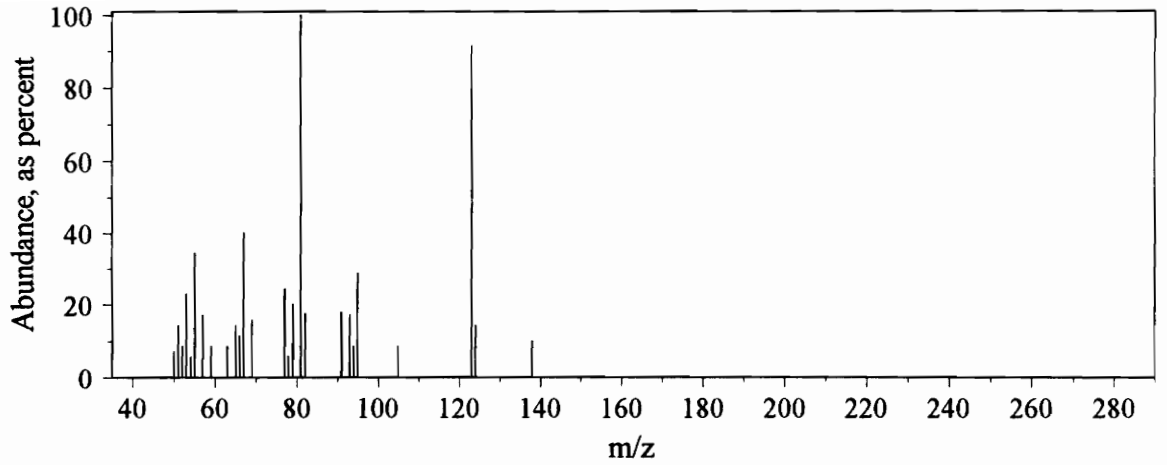


Figure E.43 Unknown @ 13 min (*B*-cyclocitral related?)

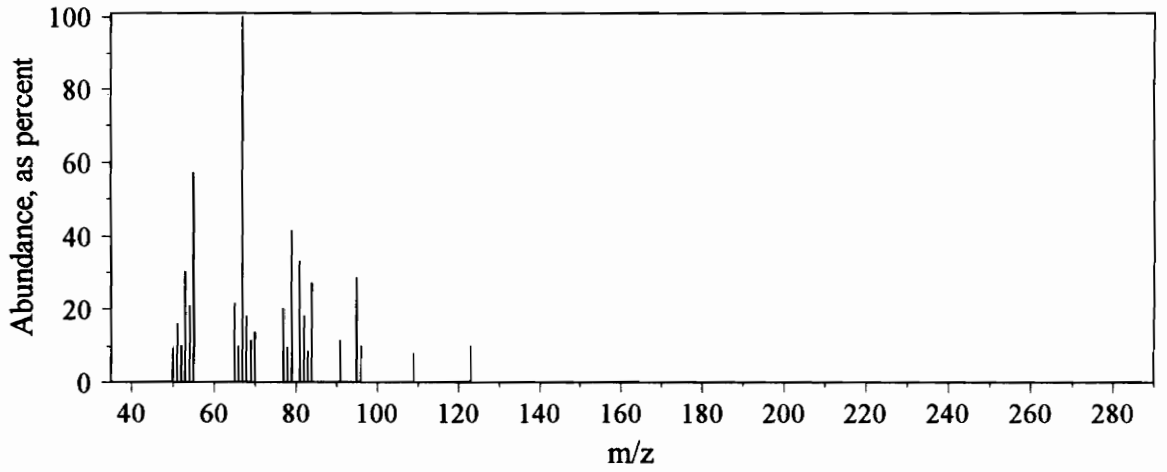


Figure E.44 Unknown @ 15.4 min

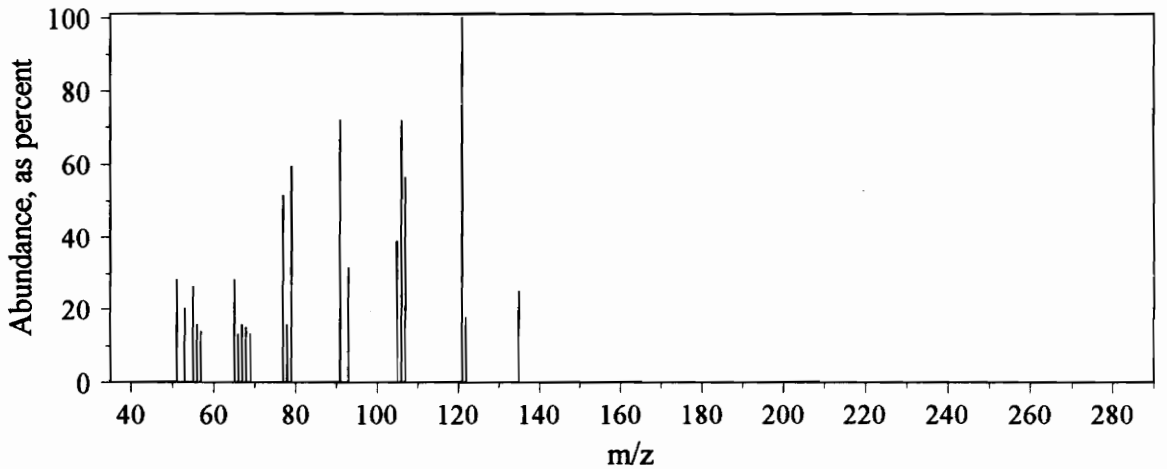


Figure E.45 Unknown @ 15.7 min (*B*-cyclocitral related?)

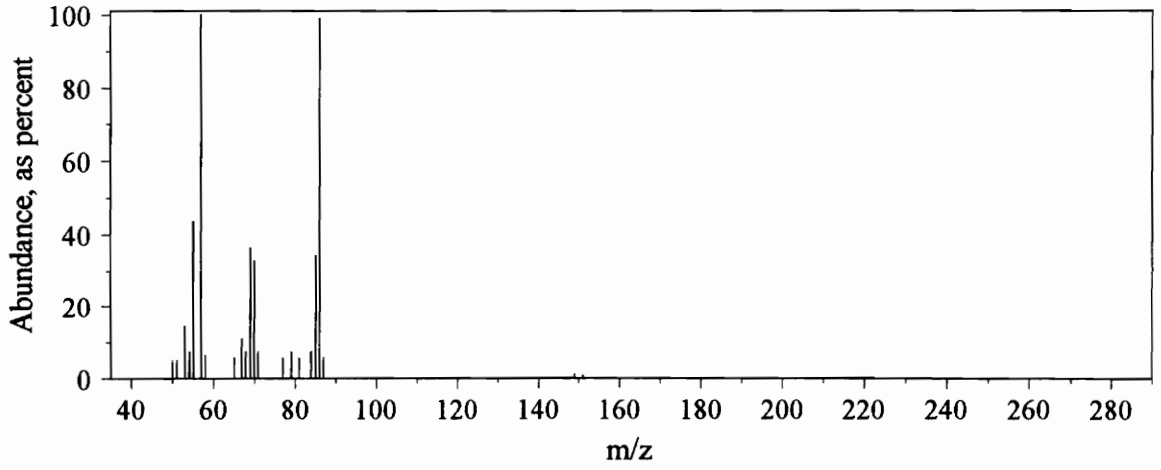


Figure E.46 Unknown @ 22.7 min

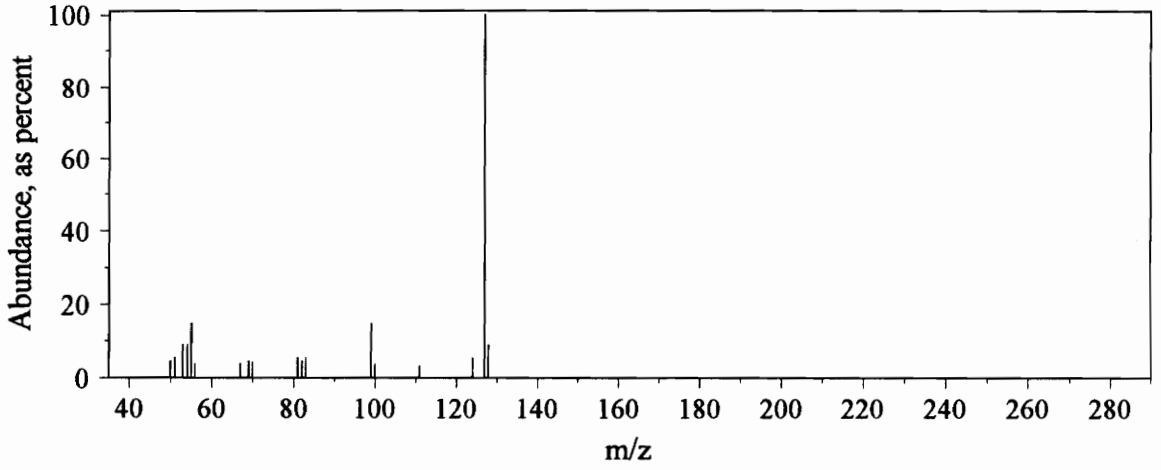


Figure E.47 Unknown @ 28.8 min

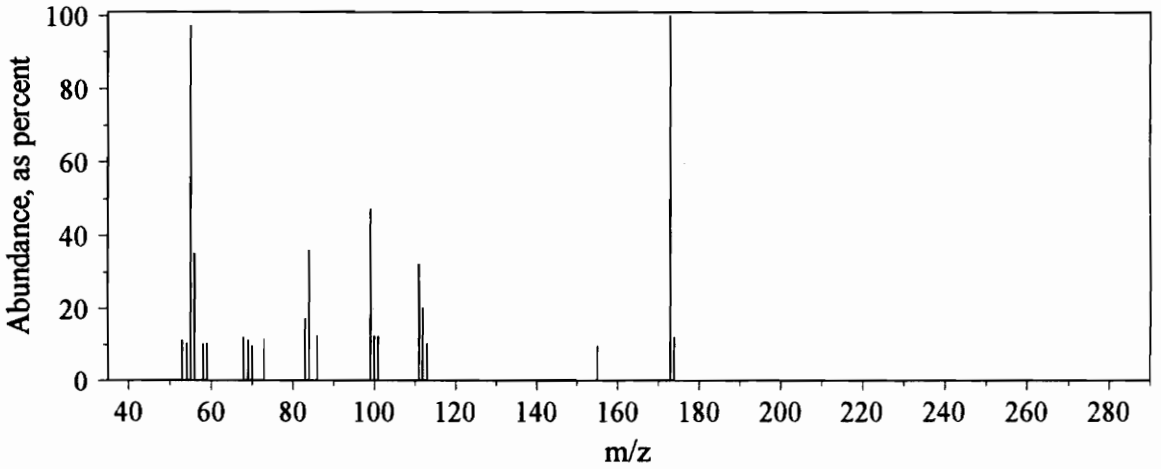


Figure E.48 Unknown @ 30.5 min

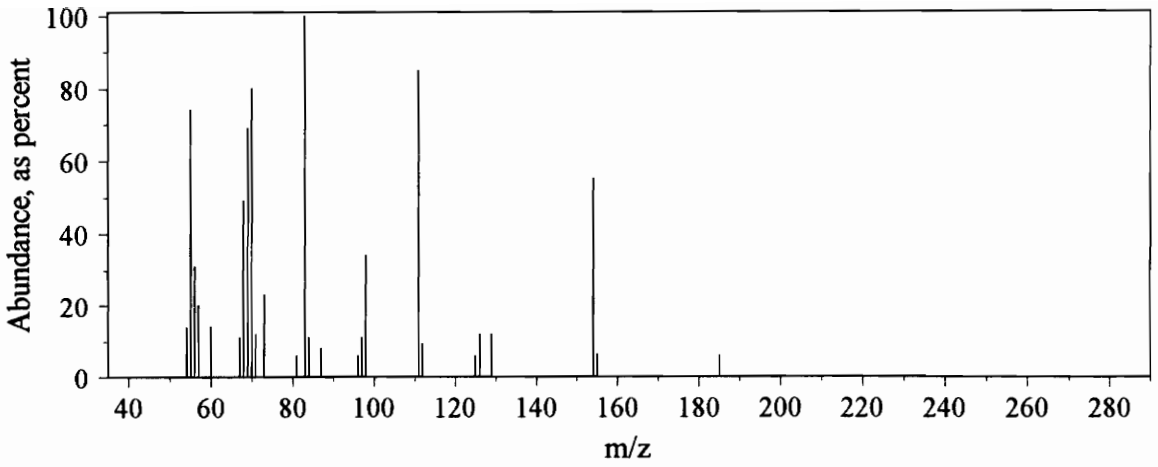


Figure E.49 Unknown @ 33.3 min

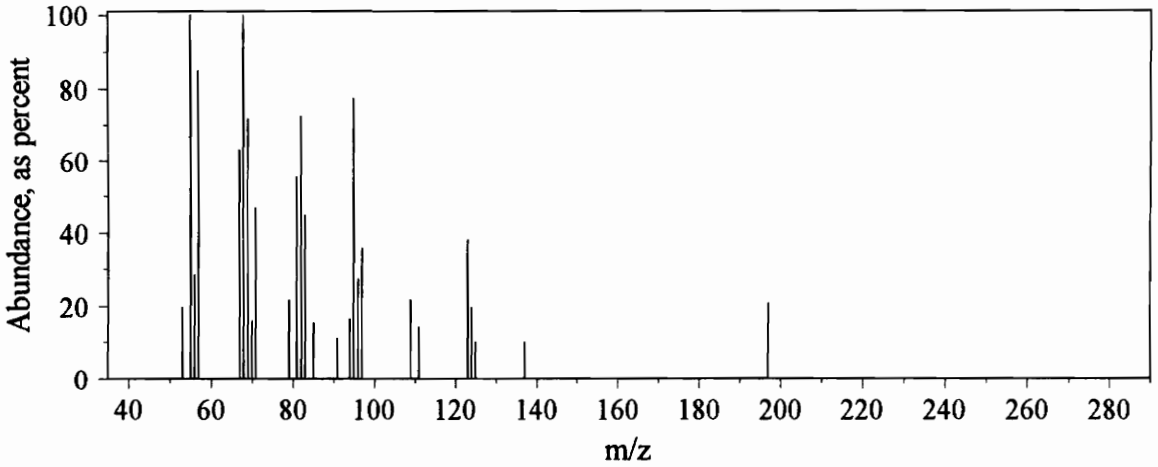


Figure E.50 Unknown @ 35.1 min

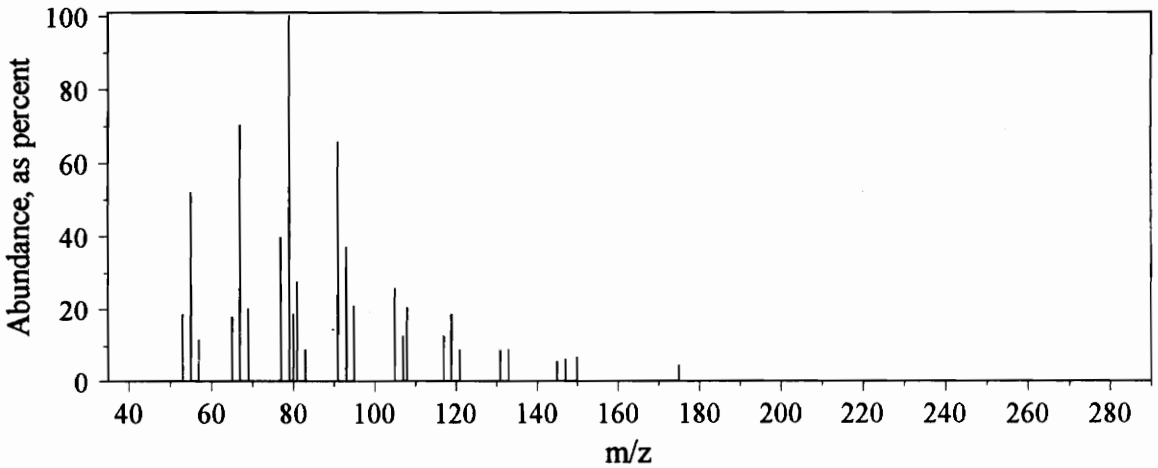


Figure E.51 Unknown @ 39.3 min (isomer of Unknown @ 39.5 min)

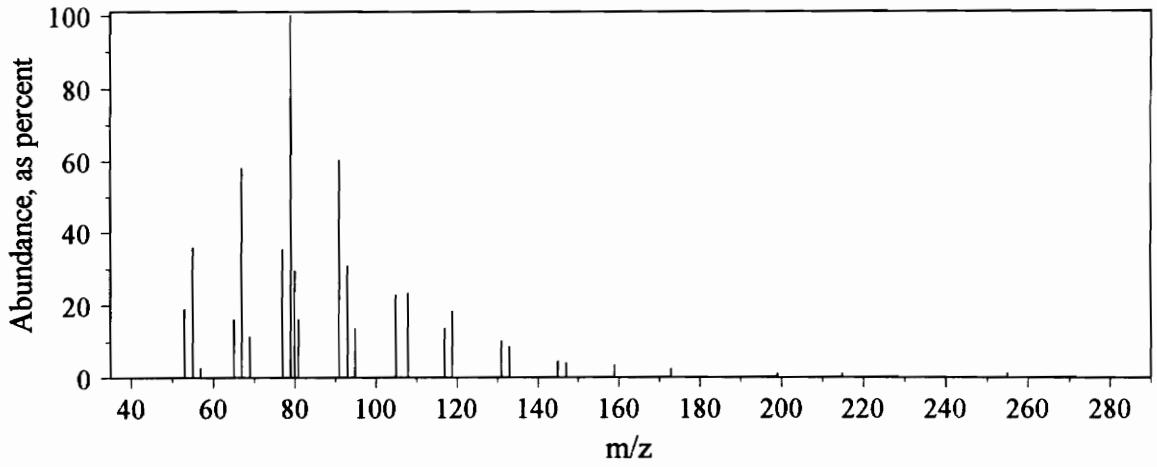
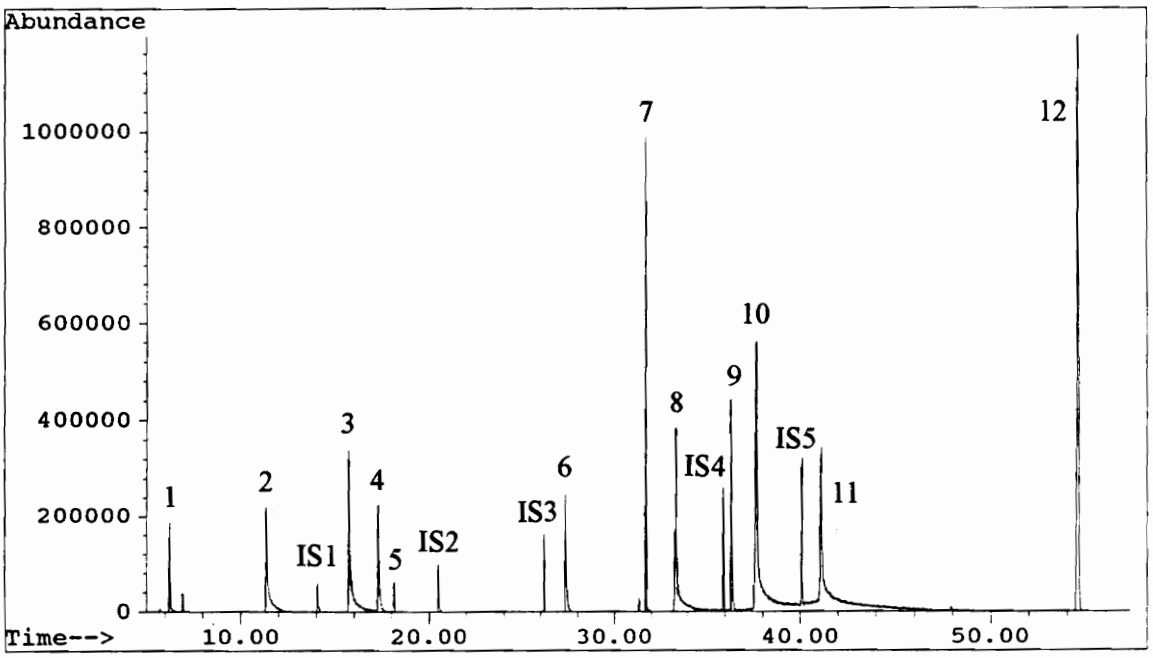


Figure E.52 Unknown @ 39.5 min



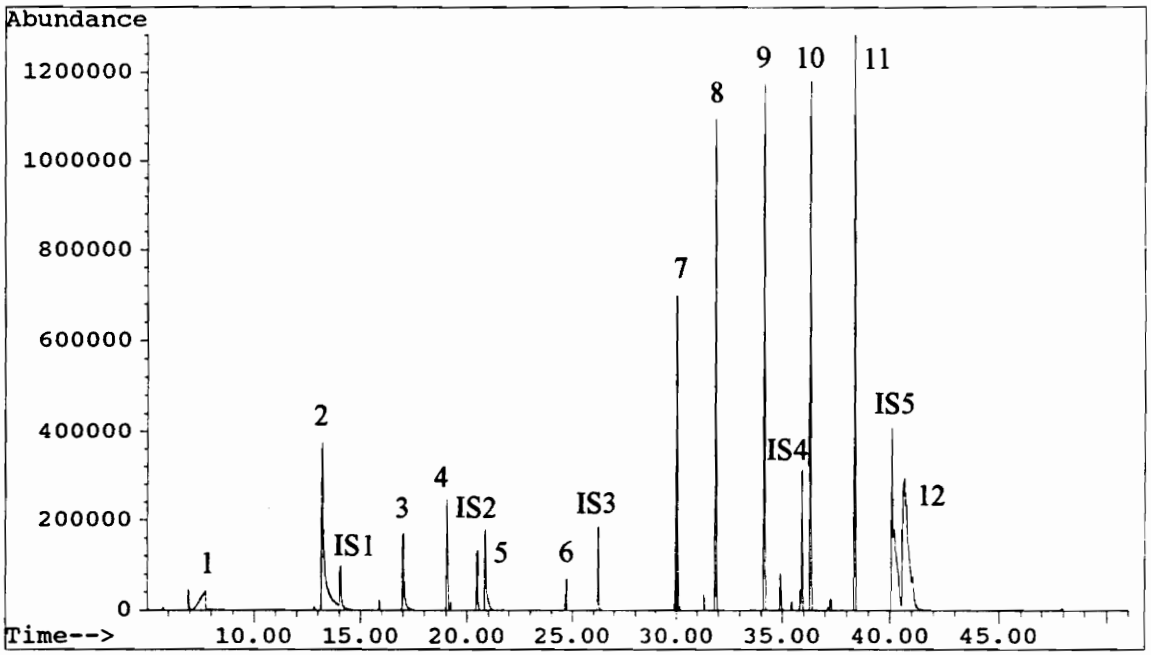
Internal Standards

IS1 chlorooctane
IS2 chlorodecane
IS3 chlorododecane
IS4 chlorohexadecane
IS5 chlorooctadecane

Compound Standards

1 3-furfural
2 3-methylthio-1-propanol
3 phenethyl alcohol
4 benzylacetate
5 2-MIB-d3
6 2-phenylphenol
7 1-heptadecene
8 myristic acid
9 chlorophene
10 palmitic acid
11 linolenic acid
12 squalene

Figure E.53 GC-MS chromatogram of a 3-uL aliquot of standard solution "3a" (see Table C.1).



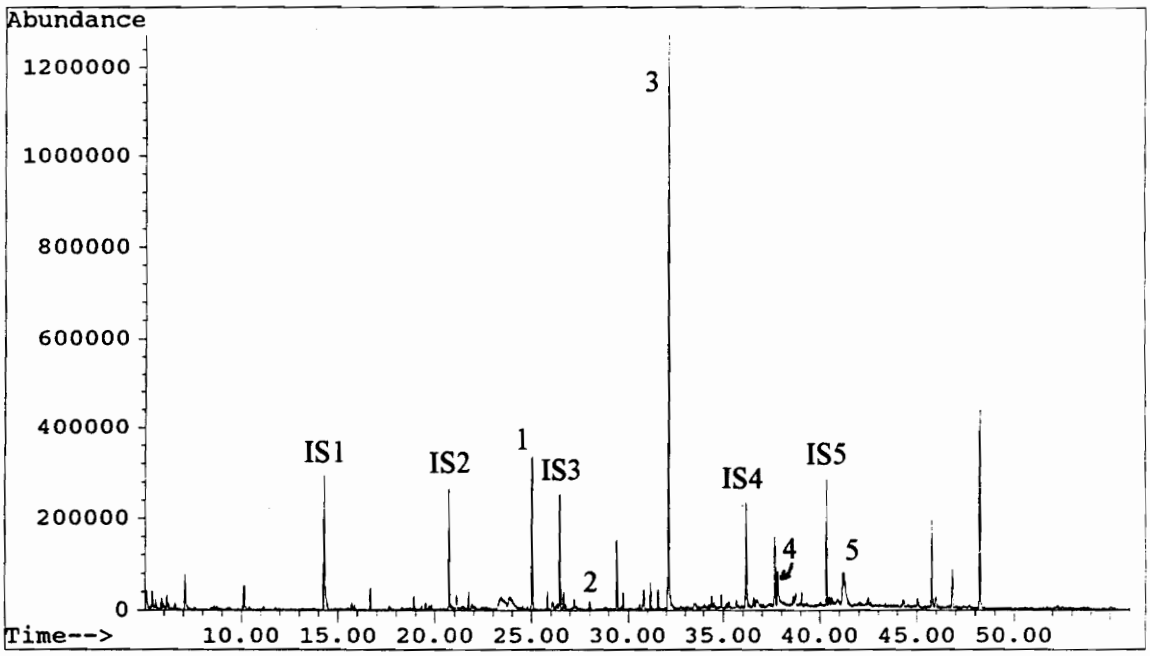
Internal Standards

IS1 chlorooctane
IS2 chlorodecane
IS3 chlorododecane
IS4 chlorohexadecane
IS5 chlorooctadecane

Compound Standards

1 isovaleric acid
2 benzyl alcohol
3 2t,6c-nonadienal
4 *B*-cyclocitral
5 1-indanone
6 geosmin-d3
7 diphenylamine
8 heptadecane
9 octadecane
10 nonadecane
11 eicosane
12 phytol

Figure E.54 GC-MS chromatogram of a 3-uL aliquot of standard solution "4a" (see Table C.1).



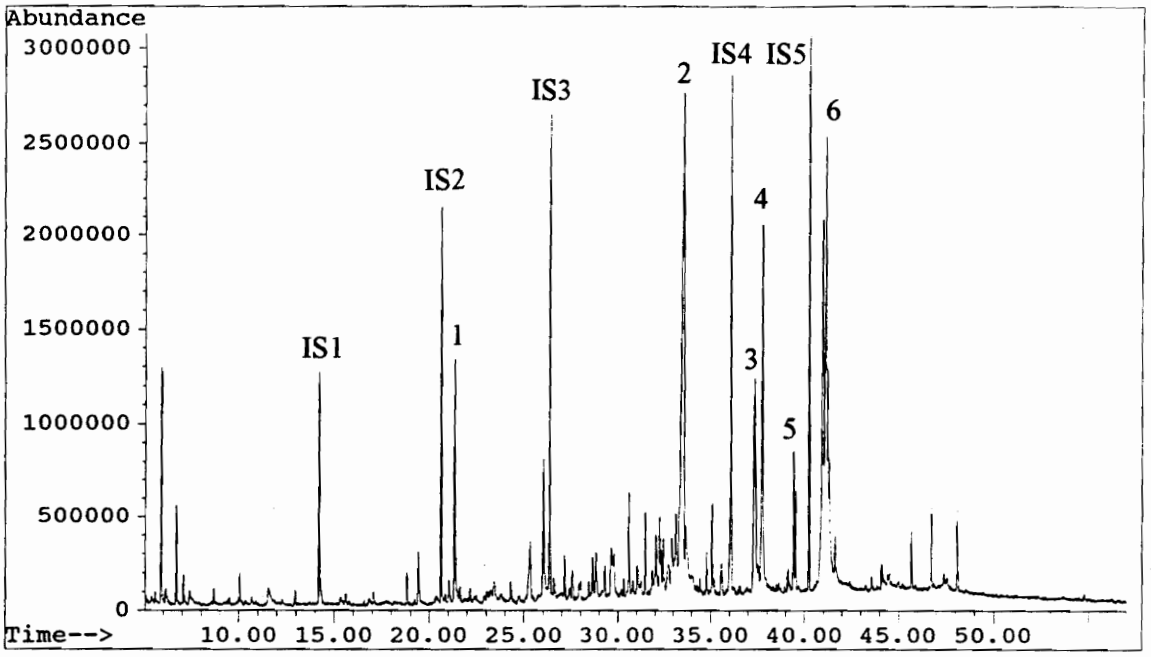
Internal Standards

IS1 chlorooctane
IS2 chlorodecane
IS3 chlorododecane
IS4 chlorohexadecane
IS5 chlorooctadecane

Compounds

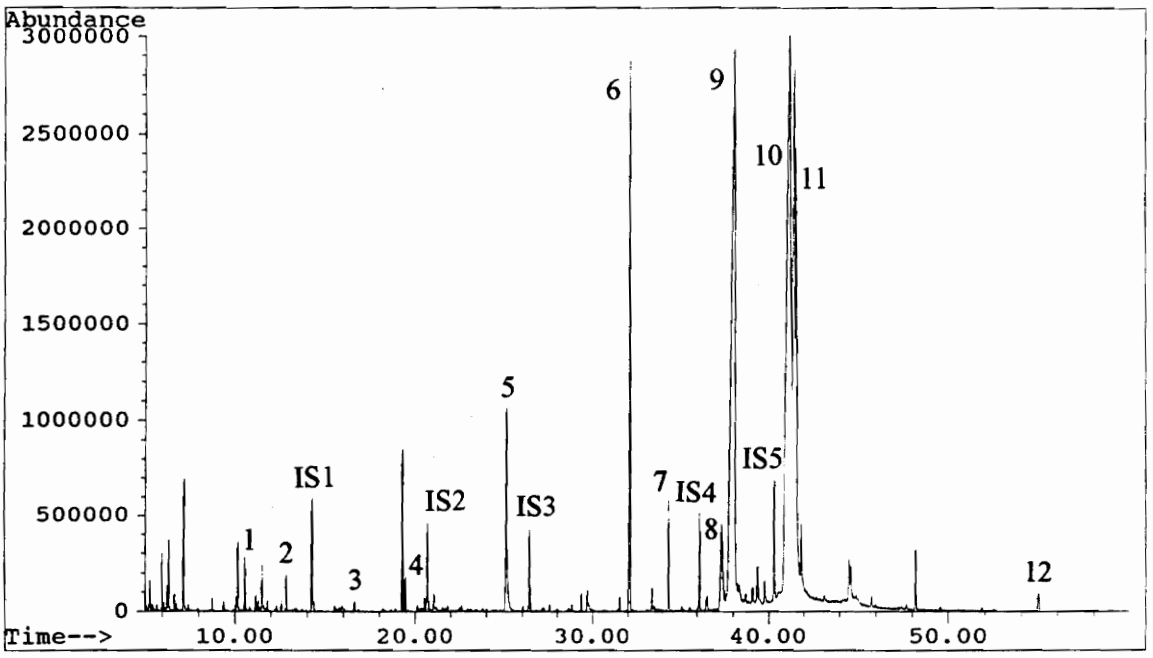
1 geosmin
2 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone
3 heptadecane
4 palmitic acid
5 phytol

Figure E.55 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 28-day old culture of *Anabaena laxa.*



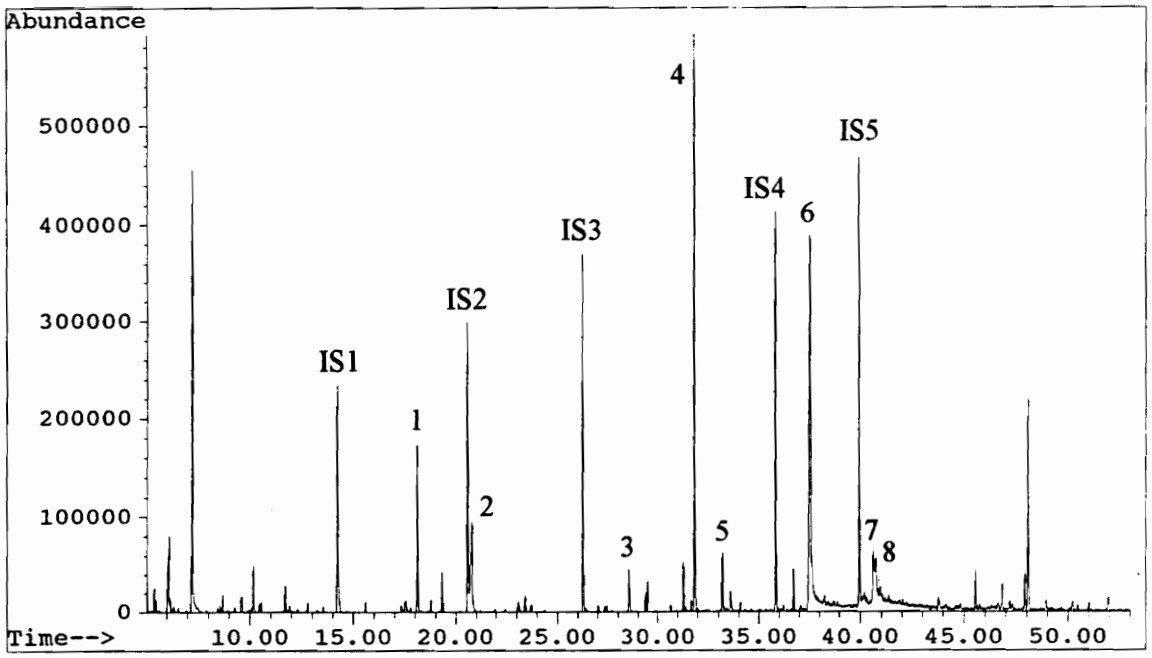
Internal Standards		Compounds	
IS1	chlorooctane	1	2t,4c,7c-decatrienal
IS2	chlorodecane	2	myristic acid
IS3	chlorododecane	3	hexadecenoate
IS4	chlorohexadecane	4	palmitic acid
IS5	chlorooctadecane	5	unknowns @ 39.3 and 39.5 min
		6	18C fatty acids

Figure E.56 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 23-day old culture of *Dinobryon cylindricum*.



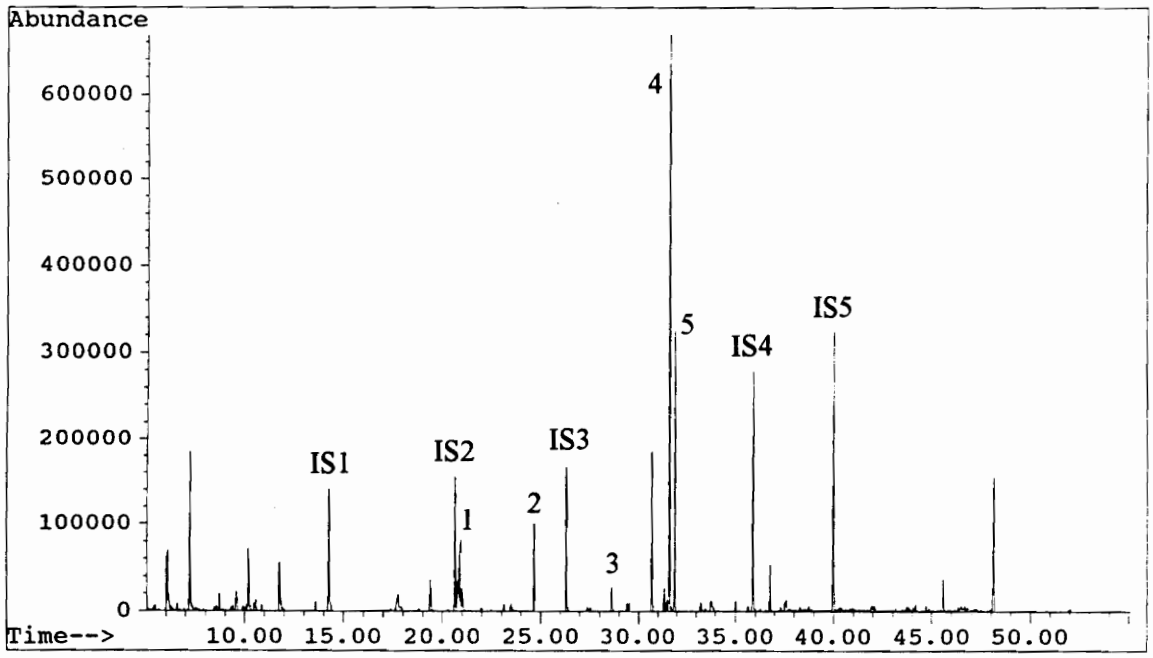
Internal Standards	Compounds
IS1 chlorooctane	1 unknown @ 10.8 min
IS2 chlorodecane	2 unknown @ 13 min
IS3 chlorododecane	3 phenethyl alcohol
IS4 chlorohexadecane	4 *B*-cyclocitral
IS5 chlorooctadecane	5 hydroxy-*B*-cyclocitral
	6 heptadecane
	7 octadecane
	8 hexadecenoate
	9 palmitic acid
	10 phytol
	11 18C fatty acids
	12 squalene

Figure E.57 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 40-day old culture of *Microcystis aeruginosa*.



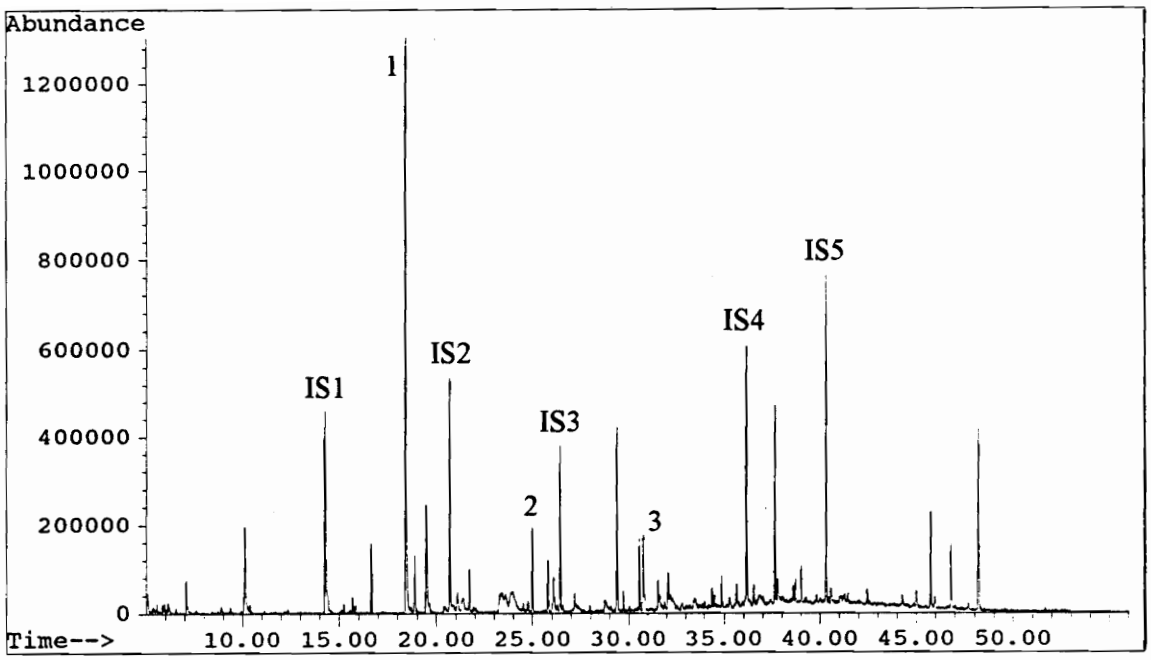
Internal Standards	Compounds
IS1 chlorooctane	1 MIB
IS2 chlorodecane	2 nonanoate
IS3 chlorododecane	3 dodecanoate
IS4 chlorohexadecane	4 heptadecane
IS5 chlorooctadecane	5 myristic acid
	6 palmitic acid
	7 phytol
	8 linoleic acid

Figure E.58 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 20-day old culture of *Oscillatoria* sp.



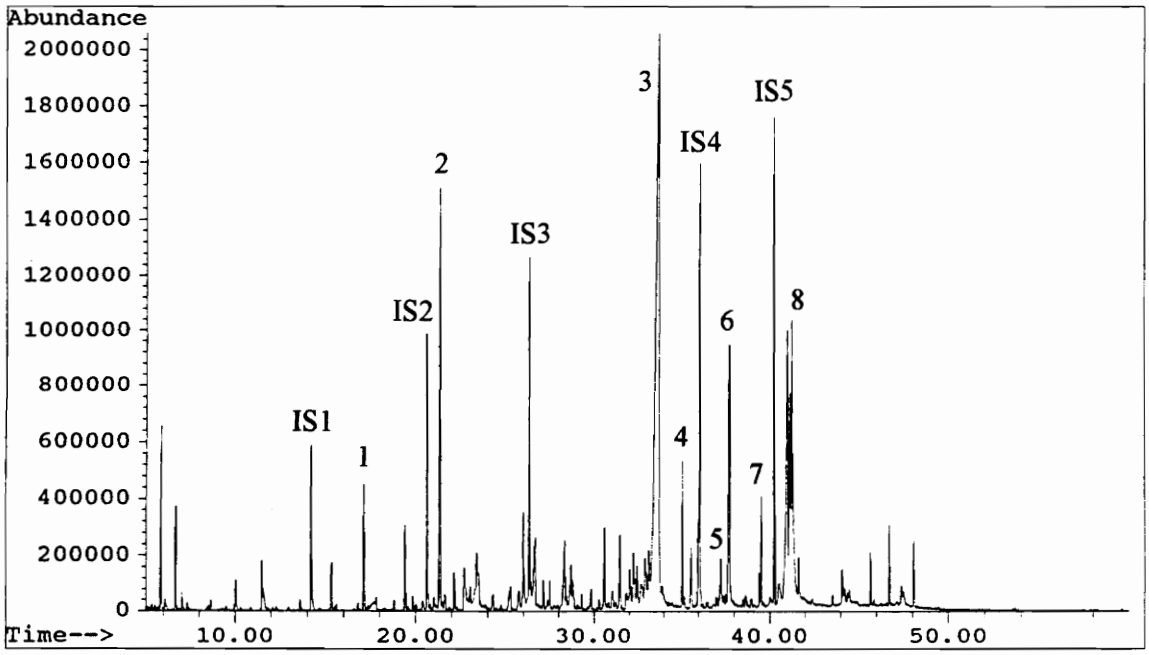
Internal Standards		Compounds	
IS1	chlorooctane	1	nonanoate
IS2	chlorodecane	2	geosmin
IS3	chlorododecane	3	dodecanoate
IS4	chlorohexadecane	4	heptadecene
IS5	chlorooctadecane	5	heptadecane

Figure E.59 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 39-day old culture of *Oscillatoria tenuis*.



Internal Standards	Compounds
IS1 chlorooctane	1 MIB
IS2 chlorodecane	2 geosmin
IS3 chlorododecane	3 myrcenol
IS4 chlorohexadecane	
IS5 chlorooctadecane	

Figure E.60 GC-MS chromatogram of a 3-uL aliquot of the cell-free media extract obtained from a 40-day old culture of *Phormidium* sp.



Internal Standards

IS1 chlorooctane
IS2 chlorodecane
IS3 chlorododecane
IS4 chlorohexadecane
IS5 chlorooctadecane

Compounds

1 2t,6c-nonadienal
2 2t,4c,7c-decatrienal
3 myristic acid
4 unknown @ 35.1 min
5 hexadecenoate
6 palmitic acid
7 unknown @ 39.5 min
8 18C fatty acids

Figure E.61 GC-MS chromatogram of a 3-uL aliquot of the cell extract obtained from a 17-day old culture of *Synura petersenii*.

APPENDIX F
DATA OBTAINED FROM NUTRIENT AND ENVIRONMENTAL TREATMENT EXPERIMENTS

Table F.1

Anabaena laxa data

Treatment	Age (days)	Density (cells/mL)	Temp. (°C)	Light ($\mu E/m^2/s$)	NO_3-N (mg/L)	PO_4-P (mg/L)
low light	0	50,300	25	7	31.86	5.24
low light	3	69,000	25	7	(29.5)*	(6.1)
low light	8	116,900	25	7	26.53	7.34
low light	14	354,200	25	7	(27.5)	(6.1)
low light	18	528,500	25	7	28.60	5.47
low light	28	1,837,600	25	7	27.75	5.34
low light	40	9,583,000	25	7	19.09	3.86
low temp	0	11,800	15	28	31.08	5.77
low temp	3	123,600	15	28	(33.0)	(5.7)
low temp	8	649,400	15	28	35.35	5.62
low temp	14	1,862,600	15	28	(29.5)	(4.6)
low temp	19	6,229,900	15	28	26.09	4.02
low temp	28	11,594,300	15	28	18.94	0.78
low temp	40	14,303,600	15	28	13.97	2.24
low P	0	24,500	25	36	26.42	0.36
low P	3	36,500	25	36	(28.0)	(0.35)
low P	8	1,188,600	25	36	29.12	0.34
low P	14	2,975,200	25	36	(29.5)	(0.25)
low P	19	1,242,400	25	36	30.55	0.19
low P	28	570,700	25	36	27.70	0.15
low P	40	1,190,000	25	36	27.96	0.11
mid N	0	34,100	25	32	5.36	5.53
mid N	3	195,400	25	32	(4.5)	(5.3)
mid N	8	2,004,100	25	32	3.35	4.96
mid N	14	4,990,300	25	32	(1.5)	(4.1)
mid N	18	4,979,300	25	32	1.01	3.62
mid N	28	4,667,400	25	32	0.47	2.57
mid N	40	7,848,400	25	32	0.37	2.73
low N	0	26,800	25	32	2.84	4.20
low N	3	86,300	25	32	(2.25)	(4.5)
low N	8	3,308,700	25	32	1.60	4.86
low N	14	5,787,600	25	32	(0.9)	(4.6)
low N	17	8,102,700	25	32	0.31	4.41
low N	28	13,647,300	25	32	0.16	3.63
low N	40	19,094,200	25	32	0.25	3.11
ASM-1	0	46,000	25	32	36.1	6.28
ASM-1	3	258,900	25	32	(35.5)	(5.8)
ASM-1	7	1,491,900	25	32	34.8	5.24
ASM-1	14	3,192,000	25	32	(26.0)	(4.4)
ASM-1	19	3,518,100	25	32	22.04	4.02
ASM-1	28	6,781,100	25	32	18.36	3.88
ASM-1	40	9,945,800	25	32	6.71	3.49

*Brackets ( ) indicate interpolated values

(continued)

Table F.1 (continued)

Compound production (ng/10,000 cells)			Compound concentration (ng/mL)	
Intracellular geosmin	Extracellular geosmin	Total geosmin	Intracellular geosmin	Total geosmin
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.037	0	0.037	1.93	1.93
0.045	0.023	0.067	8.17	12.37
0.032	0.010	0.042	30.8	40.09
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.047	0	0.047	8.74	8.74
0.072	0.002	0.074	45.01	45.95
0.066	0.006	0.072	76.84	83.82
0.059	0.012	0.072	84.52	102.26
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.004	0.009	0.013	1.28	3.81
0.013	0.106	0.119	1.62	14.76
0.024	0.198	0.222	1.38	12.69
0	0.096	0.096	0	11.41
0	0	0	0	0
0	0	0	0	0
0.045	0	0.045	9.09	9.09
0.111	0.014	0.125	55.26	62.22
0.132	0.033	0.165	65.84	82.22
0.042	0.081	0.123	19.71	57.41
0.049	0.064	0.113	38.37	88.89
0	0	0	0	0
0	0	0	0	0
0.019	0	0.019	6.22	6.22
0.078	0.006	0.084	45.14	48.46
0.044	0.004	0.048	35.76	38.95
0.123	0.018	0.141	167.84	192.37
0.064	0.011	0.076	123.04	144.41
0	0	0	0	0
0	0	0	0	0
0.019	0	0.019	2.84	2.84
0.024	0.003	0.027	7.71	8.66
0.025	0.012	0.037	8.71	13.08
0.016	0.013	0.029	10.62	19.38
0.009	0.010	0.019	8.87	18.36

Table F.2

Phormidium sp. data

Treatment	Age (days)	Density (cells/mL)	Temp. (°C)	Light ($\mu E/m^2/s$)	NO_3-N (mg/L)	PO_4-P (mg/L)
high light	0	4,200	25	28	34.23	4.98
high light	3	14,600	25	28	(31.5)*	(6.1)
high light	8	51,500	25	28	26.74	7.67
high light	14	153,600	25	28	(24.0)	(5.9)
high light	18	155,000	25	28	21.95	4.93
high light	28	259,300	25	28	19.42	4.82
high light	40	628,100	25	28	11.92	4.54
low temp	0	10,600	15	8	30.92	5.59
low temp	3	20,200	15	8	(33.0)	(5.4)
low temp	8	22,800	15	8	35.81	5.26
low temp	14	104,000	15	8	(33.0)	(5.0)
low temp	19	155,800	15	8	31.22	4.83
low temp	28	307,100	15	8	31.13	5.05
low temp	40	570,300	15	8	21.99	2.72
low P	0	2,800	25	9	26.80	0.42
low P	3	18,200	25	9	(27.5)	(0.4)
low P	8	16,300	25	9	31.27	0.36
low P	14	45,200	25	9	(31.0)	(0.25)
low P	19	82,600	25	9	30.53	0.19
low P	28	320,900	25	9	28.95	0.12
low P	40	97,300	25	9	26.88	0.07
mid N	0	2,800	25	8	5.10	5.35
mid N	3	10,000	25	8	(5.5)	(5.25)
mid N	8	25,900	25	8	6.28	5.22
mid N	14	32,700	25	8	(6.1)	(5.2)
mid N	18	70,200	25	8	6.01	5.20
mid N	28	118,000	25	8	4.15	4.61
mid N	40	317,400	25	8	2.14	5.88
low N	0	7,400	25	8	2.51	4.18
low N	3	14,400	25	8	(2.6)	(4.4)
low N	8	38,500	25	8	2.66	4.64
low N	14	45,100	25	8	(2.0)	(4.75)
low N	17	42,100	25	8	1.83	4.85
low N	28	62,800	25	8	1.10	4.95
low N	40	167,400	25	8	0.29	4.61
ASM-1	0	3,800	25	8	32.39	5.50
ASM-1	3	28,600	25	8	(31.5)	(5.7)
ASM-1	7	95,500	25	8	30.26	5.89
ASM-1	14	214,600	25	8	(31.0)	(5.6)
ASM-1	19	530,000	25	8	31.66	5.36
ASM-1	28	970,200	25	8	30.33	7.21
ASM-1	40	3,219,400	25	8	7.34	3.23

*Brackets ( ) interpolated estimated values

(continued)

Table F.2 (continued)

Compound production (ng/10,000 cells)			Compound concentration (ng/mL)	
Intracellular MIB	Extracellular MIB	Total MIB	Extracellular MIB	Total MIB
0	5.571	5.571	2.34	2.34
0	1.363	1.363	1.99	1.99
0.194	2.320	2.515	11.95	12.95
0.298	1.971	2.269	30.27	34.85
0.546	3.705	4.251	57.43	65.89
0.570	3.522	4.093	91.33	106.12
0.174	1.254	1.428	78.79	89.72
0	2.132	2.132	2.26	2.26
0	0.668	0.668	1.35	1.35
0	0.614	0.614	1.40	1.40
0.090	0.789	0.879	8.20	9.14
0.521	0.699	1.220	10.89	19.01
0.515	0.906	1.420	27.81	43.62
0.542	1.479	2.022	84.36	115.29
0	0	0	0	0
0	0	0	0	0
0	0.699	0.699	1.14	1.14
0	1.228	1.228	5.55	5.55
0	1.289	1.289	10.65	10.65
0.084	0.937	1.021	30.06	32.75
0.290	4.408	4.698	42.89	45.71
0	8.500	8.500	2.38	2.38
0	0	0	0	0
0.081	0.575	0.656	1.49	1.70
0	1.654	1.654	5.41	5.41
0	1.331	1.331	9.34	9.34
0.205	2.140	2.345	25.25	27.67
0.333	1.428	1.761	45.33	55.90
0	1.743	1.743	1.29	1.29
0	1.021	1.021	1.47	1.47
0	1.561	1.561	6.01	6.01
0.718	2.816	3.534	12.70	15.94
0.736	4.017	4.753	16.91	20.01
0.819	3.269	4.088	20.53	25.67
0.651	2.497	3.148	41.80	52.69
0	0	0	0	0
0	0	0	0	0
0.058	0.338	0.396	3.23	3.78
0.056	0.782	0.839	16.79	18.00
0.112	0.463	0.574	24.52	30.43
0.127	0.715	0.842	69.36	81.69
0.089	0.321	0.410	103.25	131.89

Table F.3

Nonparametric results for *Phormidium* sp.

Treatment	Rank sum for population cell density	Rank sum for total MIB production
high light (HL)	29.0	30.0
low temperature (LT)	30.0	17.0
low phosphorus (LP)	18.5	20.0
mid nitrogen (MN)	13.5	20.0
low nitrogen (LN)	17.0	32.0
regular DY III (Reg)	39.0	7.0
Friedman's S	19.347	19.810
P	0.002	0.001
	Multiple comparisons†	
Treatments: (for k = 6, n = 7, and α = 0.049)*	MN LN LP HL LT Reg	Reg LT LP MN HL LN
Conclusion:	MN, LN, LP ≠ Reg	Reg ≠ HL, LN

* k = 6, n = 6, and α = 0.040 for total MIB production, because T_0 = 0 days was not used

† The lines beneath the treatments indicate that the attained values were not significantly different

Table F.4

Phormidium sp. population density regression results

Dependent variable cell density (log cells/mL)	R-squared = 0.911	Adjusted R-squared = 0.889	Standard error of estimate = 0.197
Model variables	Coefficient	T	P (2 tail)
constant	2.88	4.815	< 0.001
age	0.072	6.769	< 0.001
light intensity	-0.024	-3.686	< 0.001
temperature	0.045	4.010	< 0.001
age * age	-7.99×10^{-4}	-3.359	0.002
initial NO_3-N	0.031	5.351	< 0.001
initial PO_4-P	-0.027	-0.264	0.794
N to P ratio	-0.012	-1.347	0.189
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	11.1	7	< 0.001
residual	1.08	28	

Table F.5

Phormidium sp. intracellular MIB regression results

Dependent variable intracellular MIB (ng/10,000 cells)	R-squared = 0.667	Adjusted R-squared = 0.584	Standard error of estimate = 0.168
Model variables	Coefficient	T	P (2 tail)
constant	2.11	4.136	< 0.001
age	0.031	3.390	0.002
light intensity	0.003	0.583	0.565
temperature	-0.023	-2.422	0.022
age * age	-4.73×10^{-4}	-2.330	0.027
initial NO_3-N	0.006	1.234	0.227
initial PO_4-P	-0.335	-3.771	< 0.001
N to P ratio	-0.029	-3.832	< 0.001
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	1.58	7	< 0.001
residual	0.79	28	

Table F.6

Phormidium sp. extracellular MIB regression results

Dependent variable extracellular MIB (ng/10,000 cells)	R-squared = 0.633	Adjusted R-squared = 0.541	Standard error of estimate = 0.792
Model variables	Coefficient	T	P (2 tail)
constant	6.60	2.745	0.010
age	0.124	2.917	0.007
light intensity	0.071	2.704	0.012
temperature	-0.047	-1.049	0.303
age * age	-0.002	-2.170	0.039
initial NO_3-N	-0.006	-0.270	0.789
initial PO_4-P	-1.116	-2.662	0.013
N to P ratio	-0.082	-2.311	0.028
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	30.3	7	< 0.001
residual	17.6	28	

Table F.7

Phormidium sp. total MIB production regression results

Dependent variable total MIB (ng/10,000 cells)	R-squared = 0.666	Adjusted R-squared = 0.582	Standard error of estimate = 0.880
Model variables	Coefficient	T	P (2 tail)
constant	8.71	3.257	0.003
age	0.155	3.270	0.003
light intensity	0.074	2.543	0.017
temperature	-0.070	-1.405	0.171
age * age	-0.003	-2.396	0.023
initial NO_3-N	-1.90×10^{-4}	-0.007	0.994
initial PO_4-P	-1.45	-3.113	0.004
N to P ratio	-0.111	-2.809	0.009
	Analysis of variance		
Source	Sum-of-squares	Degrees of freedom	P
regression	43.3	7	< 0.001
residual	21.7	28	

Table F.8

Synura petersenii data

Treatment	Age (days)	Density (cells/mL)	Temp. (°C)	Light ($\mu E/m^2/s$)	NO_3-N (mg/L)	NH_3-N (mg/L)	AHP (mg/L)
high light	0	2,500	20	45	2.93	0.75	0.98
high light	3	2,800	20	45	(2.8)*	(0.6)	(1.06)
high light	8	30,400	20	45	2.63	0.41	1.16
high light	14	201,600	20	45	(1.5)	(0.18)	(0.8)
high light	18	367,000	20	45	0.80	0.06	0.62
low temp	0	2,800	10	8	2.47	0.71	0.85
low temp	3	3,500	10	8	(2.6)	(0.71)	(0.87)
low temp	8	4,600	10	8	2.79	0.72	0.90
low temp	14	13,400	10	8	(2.0)	(0.6)	(0.6)
low temp	18	28,600	10	8	1.72	0.54	0.44
low temp	28	102,500	10	8	2.33	<0.05	0.57
low temp	40	111,400	10	8	1.50	<0.05	0.66
low AHP	0	1,700	20	8	3.83	0.90	0.55
low AHP	4	15,300	20	8	(3.3)	(0.65)	(0.4)
low AHP	7	34,500	20	8	2.81	0.49	0.27
low AHP	14	117,800	20	8	(2.1)	(0.15)	(0.14)
low AHP	17	214,300	20	8	1.96	<0.05	0.11
low AHP	27	371,900	20	8	0.85	<0.05	0.11
low AHP	41	329,000	20	8	0.22	<0.05	0.12
mid N	0	4,400	20	8	1.53	0.45	0.84
mid N	3	10,300	20	8	(1.5)	(0.27)	(0.82)
mid N	8	79,700	20	8	1.46	0.05	0.8
mid N	14	301,600	20	8	(0.6)	(<0.05)	(0.69)
mid N	18	437,900	20	8	0.34	<0.05	0.63
mid N	29	341,500	20	8	0.24	<0.05	0.52
mid N	39	245,700	20	8	0.17	<0.05	0.47
low N	0	3,600	20	8	0.83	0.36	0.96
low N	3	7,100	20	8	(0.7)	(0.21)	(1.0)
low N	8	21,900	20	8	0.49	0.05	1.05
low N	14	26,500	20	8	(0.25)	(<0.05)	(0.97)
low N	18	37,500	20	8	0.19	<0.05	0.91
low N	29	35,400	20	8	0.19	<0.05	0.83
low N	39	25,200	20	8	0.19	<0.05	0.76
DY III	0	6,600	20	8	4.19	1.37	1.16
DY III	3	15,000	20	8	(3.3)	(0.75)	(0.95)
DY III	7	137,500	20	8	2.42	0.16	0.74
DY III	14	320,600	20	8	(1.6)	(0.07)	(0.4)
DY III	19	365,000	20	8	0.97	<0.05	0.22
DY III	28	465,100	20	8	0.97	<0.05	0.14
DY III	40	705,600	20	8	0.68	<0.05	0.20

*Brackets ( ) indicate interpolated values

(continued)

Table F.8 (continued)

Compound production (ng/10,000 cells)			Compound concentration (ng/mL)	
Intracellular decatrienal	Extracellular decatrienal	Total decatrienal	Intracellular decatrienal	Total decatrienal
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
1.857	0.175	2.032	37.43	40.96
3.162	0.092	3.253	116.03	119.39
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
7.008	0	7.008	9.39	9.39
1.836	0	1.836	5.25	5.25
5.887	0.568	6.455	60.34	66.16
9.401	0	9.401	104.73	104.73
0	0	0	0	0
0	0	0	0	0
5.374	0	5.374	18.54	18.54
10.492	0.506	10.998	123.59	129.55
6.279	0.178	6.457	134.56	138.37
6.569	0	6.569	244.29	244.29
2.918	0	2.918	96.01	96.01
0	0	0	0	0
0	0	0	0	0
2.754	0	2.754	21.95	21.95
4.815	0	4.815	145.23	145.23
3.982	0	3.982	174.37	174.37
2.132	0.176	2.308	72.80	78.81
4.541	0	4.541	111.56	111.56
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
2.901	0	2.901	10.27	10.27
6.250	0	6.250	15.75	15.75
0	0	0	0	0
0	0	0	0	0
6.450	0.364	6.813	88.68	93.68
9.646	1.356	11.003	309.26	352.74
8.924	0.683	9.607	325.71	350.65
3.474	1.382	4.856	161.57	225.83
0.963	0.059	1.022	67.98	72.14

(continued)

Table F.8 (continued)

Compound production (ng/10,000 cells)			Compound concentration (ng/mL)	
Intracellular nonadienal	Extracellular nonadienal	Total nonadienal	Intracellular nonadienal	Total nonadienal
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.404	0	0.404	8.15	8.15
0.905	0	0.905	33.22	33.33
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
2.063	0	2.063	21.15	21.15
1.641	0	1.641	18.28	18.28
0	0	0	0	0
0	0	0	0	0
1.290	0	1.290	4.45	4.45
2.579	0.489	3.068	30.38	36.14
2.270	0	2.270	48.65	48.65
2.546	0	2.546	94.68	94.68
1.323	0	1.323	43.51	43.51
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0.707	0	0.707	21.32	21.32
3.912	0	3.912	171.29	171.29
0.376	0.110	0.485	12.83	16.57
0.664	0	0.664	16.32	16.32
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
2.641	0	2.641	36.31	36.31
4.715	0.197	4.912	151.16	157.47
3.040	0.167	3.207	110.97	117.07
0.793	0.219	1.012	36.86	47.06
0.067	0	0.067	4.70	4.70

Table F.9

Nonparametric results for *Synura petersenii*

Treatment	Rank sum for population cell density		Rank sum for total decatrienal production	
high light (LL)*		15.0		14.0
low temperature (LT)	10.0	8.0	21.5	15.0
low phosphorus (LP)	23.0	17.0	25.0	22.0
mid nitrogen (MN)	26.0	25.0	18.0	18.0
low nitrogen (LN)	13.0	13.0	15.5	11.0
regular DY III (Reg)	33.0	27.0	25.0	25.0
Friedman's S	20.457	15.057	4.086	7.857
P	< 0.001	0.010	0.395	0.164

	Multiple comparisons†	
	population cell density	total decatrienal production
Treatments: (for k = 5, n = 7, and α = 0.052)	LT LN LP MN Reg	LN MN LT LP Reg
Conclusion:	LT ≠ MN, Reg and LN ≠ Reg	No significant differences
Treatments: (for k = 6, n = 5, and α = 0.047)	LT HL LP LN MN Reg	LN HL LT MN LP Reg
Conclusion:	LT ≠ MN, Reg	No significant differences

*The high light experiment ended at 25 days, because of an abrupt increase in incubator temperature. Analyses that include high light (k = 6) involve only the data obtained from 0-20 days (e.g., five sample dates, n = 5); analyses without high light (k = 5) involve the entire 40 day incubation period (e.g., seven sample dates, n = 7).

† The lines beneath the treatments indicate that the attained values were not significantly different.

Table F.10

Synura petersenii population density regression results

Dependent variable cell density (log cells/mL)	R-squared = 0.756	Adjusted R-squared = 0.714	Standard error of estimate = 0.425
Model variables	Coefficient	T	P (2 tail)
constant	2.33	6.385	< 0.001
age	0.093	6.831	< 0.001
light intensity	-0.011	-1.796	0.081
temperature	0.061	3.330	0.002
age * age	-0.001	-4.248	< 0.001
initial NO_3-N * initial AHP*	0.541	2.036	0.049
initial NH_3-N * initial AHP	-1.25	-1.499	0.143

Analysis of variance

Source	Sum-of-squares	Degrees of freedom	P
regression	19.6	6	< 0.001
residual	6.33	35	

*Acid hydrolyzable phosphorus

Table F.11

Synura petersenii intracellular 2t,4c,7c-decatrienal regression results

Dependent variable intracellular 2t,4c,7c-decatrienal (ng/10,000 cells)	R-squared = 0.456	Adjusted R-squared = 0.363	Standard error of estimate = 2.584
Model variables	Coefficient	T	P (2 tail)
constant	-0.326	-0.147	0.884
age	0.305	3.698	< 0.001
light intensity	-0.096	-2.612	0.013
temperature	1.31×10^{-4}	0.001	0.999
age * age	-0.005	-2.785	0.009
initial NO_3-N * initial AHP*	3.59	2.223	0.033
initial NH_3-N * initial AHP	-9.60	-1.892	0.067

Analysis of variance

Source	Sum-of-squares	Degrees of freedom	P
regression	196	6	0.001
residual	234	35	

*Acid hydrolyzable phosphorus

Table F.12

Synura petersenii extracellular 2t,4c,7c-decatrienal regression results

Dependent variable extracellular 2t,4c,7c-decatrienal (ng/10,000 cells)	R-squared = 0.453	Adjusted R-squared = 0.359	Standard error of estimate = 0.259
Model variables	Coefficient	T	P (2 tail)
constant	-0.383	-1.726	0.093
age	0.021	2.551	0.015
light intensity	-0.003	-0.871	0.389
temperature	0.005	0.461	0.648
age * age	-4.12×10^{-4}	-2.370	0.023
initial NO_3-N * initial AHP*	0.041	0.251	0.803
initial NH_3-N * initial AHP	0.321	0.631	0.532

Analysis of variance

Source	Sum-of-squares	Degrees of freedom	P
regression	1.95	6	0.001
residual	2.36	35	

*Acid hydrolyzable phosphorus

Table F.13

Synura petersenii total 2t,4c,7c-decatrienal production regression results

Dependent variable total 2t,4c,7c-decatrienal (ng/10,000 cells)	R-squared = 0.468	Adjusted R-squared = 0.377	Standard error of estimate = 2.692
Model variables	Coefficient	T	P (2 tail)
constant	-0.710	-0.308	0.760
age	0.326	3.796	< 0.001
light intensity	-0.099	-2.592	0.014
temperature	0.005	0.046	0.964
age * age	-0.005	-2.902	0.006
initial NO_3-N * initial AHP*	3.63	2.158	0.038
initial NH_3-N * initial AHP	-9.28	-1.756	0.088

Analysis of variance

Source	Sum-of-squares	Degrees of freedom	P
regression	223	6	< 0.001
residual	254	35	

*Acid hydrolyzable phosphorus

Table F.14

Synura petersenii intracellular 2t,6c-nonadienal regression results

Dependent variable intracellular 2t,6c-nonadienal (ng/10,000 cells)	R-squared = 0.382	Adjusted R-squared = 0.276	Standard error of estimate = 1.032
Model variables	Coefficient	T	P (2 tail)
constant	-1.36	-1.535	0.134
age	0.092	2.797	0.008
light intensity	-0.035	-2.404	0.022
temperature	0.053	1.204	0.237
age * age	-0.002	-2.419	0.021
initial NO_3-N * initial AHP*	1.54	2.384	0.023
initial NH_3-N * initial AHP	-3.89	-1.920	0.063

Analysis of variance

Source	Sum-of-squares	Degrees of freedom	P
regression	23.1	6	0.007
residual	37.3	35	

*Acid hydrolyzable phosphorus

Table F.15

Synura petersenii total 2t,6c-nonadienal production regression results

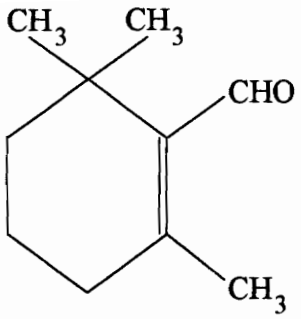
Dependent variable total 2t,6c-nonadienal (ng/10,000 cells)	R-squared = 0.395	Adjusted R-squared = 0.291	Standard error of estimate = 1.057
Model variables	Coefficient	T	P (2 tail)
constant	-1.470	1.623	0.114
age	0.097	2.860	0.007
light intensity	-0.037	-2.472	0.018
temperature	0.058	1.277	0.210
age * age	-0.002	-2.487	0.018
initial NO_3-N * initial AHP*	1.613	2.441	0.020
initial NH_3-N * initial AHP	-4.069	-1.960	0.058

Analysis of variance

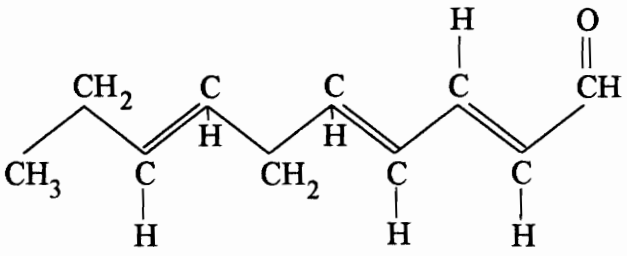
Source	Sum-of-squares	Degrees of freedom	P
regression	25.6	6	0.005
residual	39.1	35	

*Acid hydrolyzable phosphorus

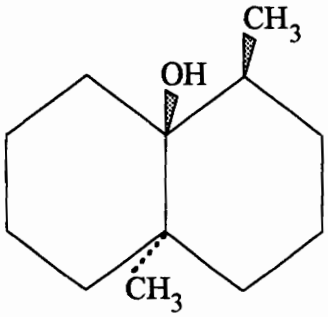
APPENDIX G
STRUCTURES OF SELECTED ODOROUS ALGAL METABOLITES



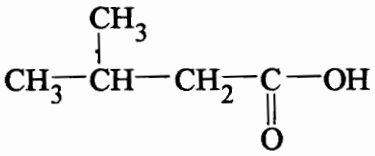
A. *B*-cyclocitral



B. 2t,4c,7c-decatrienal

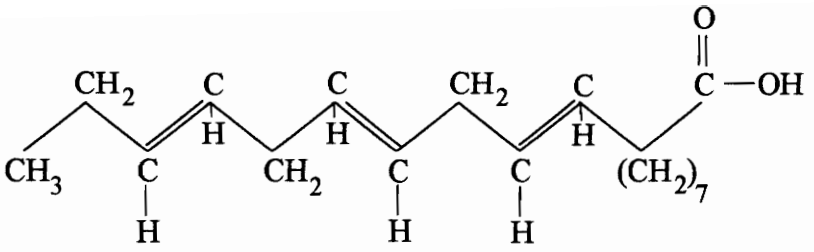


C. geosmin

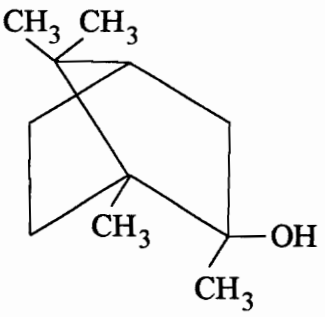


D. isovaleric acid

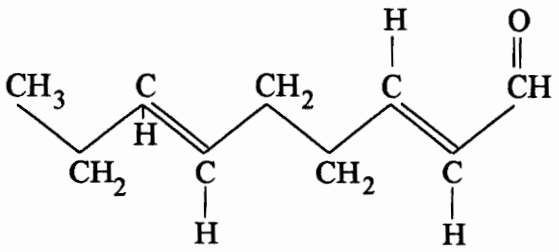
Figure G.1 Structures of selected odorous algal metabolites: (A) *B*-cyclocitral, (B) 2t,4c,7c-decatrienal, (C) geosmin, and (D) isovaleric acid.



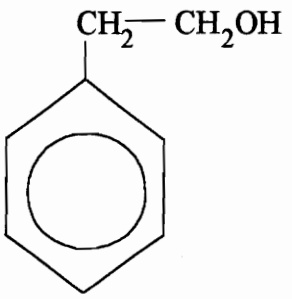
A. linolenic acid



B. MIB



C. 2t,6c-nonadienal



D. phenethyl alcohol

Figure G.2 Structures of selected odorous algal metabolites: (A) linolenic acid, (B) MIB, (C) 2t,6c-nonadienal, and (D) phenethyl alcohol.

REFERENCES

Aaronson, S., T. Berner, and Z. Dubinsky. 1980. Microalgae as a source of chemicals and natural products. In *Algae biomass: production and use*. Edited by G. Shelef and C. J. Soeder. New York: Elsevier-North Holland Biomedical Press.

Aldrich Chemical Co. 1994. *Flavors & fragrances*. Milwaukee, Wis.: Aldrich Chemical Co.

Amoore, J. E. 1986. The chemistry and physiology of odor sensitivity. *J. AWWA*. 78(3):70-76.

Anholt, R. R. H. 1992. Molecular aspects of olfaction. In *Science of olfaction*. Edited by M. J. Serby and K. L. Chobor. New York: Springer-Verlag.

Anselme, C., I. H. Suffet, and J. Mallevialle. 1988. Effects of ozonation of tastes and odors. Journal AWWA. 80(10):45-51.

APHA, AWWA, and WPCF. 1992. *Standard Methods for the Examination of Water and Wastewater*. 18th ed. Washington, D.C.:APHA.

Arctander, S. 1969. *Perfume and flavor chemicals, Vol. 1 and 2*. Montclair, NY: Arctander.

AWWA. 1993. *Flavor profile analysis: screening and training of panelists*. Denver, Co.:AWWA.

Barclay, W., C. Wyman, R. A. Lewin, and L. Cheng. 1987. Development of microalgal systems for the production of liquid fuels. In *Algal Biotechnology*. Edited by T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen. New York: Elsevier Applied Science.

Bauld, J, and T. D. Brock. 1974. Algal excretion and bacterial assimilation in hot spring algal mats. *J. Phycol*. 10:101-106.

Becker, E. W. 1986. Nutritional properties of microalgae: potentials and constraints. In *CRC Handbook of microalgal mass culture*. Edited by A. Richmond. Boca Raton, Fla.: CRC Press.

Bell, W. 1980. Bacterial utilization of algal extracellular products. 1. The kinetic approach. *Limnol. Oceanogr*. 25(6):1007-1020.

Bell, W., and R. Mitchell. 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bulletin*. 143(2):265-277.

Bell, W., and E. Sakshaug. 1980. Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. *Limnol. Oceanogr*. 25(6):1021-1033.

Belly, R. T., M. R. Tansey, and T. D. Brock. 1973. Algal excretion of ^{14}C-labeled compounds and microbial interactions in *Cyanidium caldarium* mats. *J. Phycol.* 9:123-127.

Berg, K., W. W. Carmichael, O. M. Skulberg, C. Denestad, and B. Underdal. 1987. Investigation of a toxic water-bloom of *Microcystis aeruginosa* (*Cyanophyceae*) in Lake Akersvatn, Norway. *Hydrobiologia*. 144:97-103.

Borowitzka, M. A. 1988a. Vitamins and fine chemicals from micro-algae. In *Micro-algal biotechnology*. Edited by M. A. Borowitzka and L. J. Borowitzka. New York: Cambridge University Press.

Borowitzka, M. A. 1988b. Fats, oils and hydrocarbons. In *Micro-algal biotechnology*. Edited by M. A. Borowitzka and L. J. Borowitzka. New York: Cambridge University Press.

Brouers, M., H. de Jong, D. J. Shi, and D. O. Hall. 1987. Immobilized cyanobacteria for sustained ammonia production. In *Algal Biotechnology*. Edited by T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen. New York: Elsevier Applied Science.

Budavari, S. et. al., Eds. 1989. *The Merck Index*. 11th ed. Rahway, NJ:Merck

Burlingame, G., R. M. Dann, and G. L. Brock 1986. A case study of geosmin in Philadelphia's water. *Journal AWWA*. 78(3):56-61.

Cairncross, S. E., and L. B. Sjöström. 1950. Flavor profiles: a new approach to flavor problems. *Food Technology*. 4:308-311.

Carlucci, A. F., and P. M. Bowes. 1970a. Production of vitamin B12, thiamin, and biotin by phytoplankton. *J. Phycol.* 6:351-357.

Carlucci, A. F., and P. M. Bowes. 1970b. Vitamin production and utilization by phytoplankton in mixed culture. *J. Phycol.* 6:393-400.

Carmichael, W. W., and P. R. Gorham. 1977. Factors influencing the toxicity and animal susceptibility of *Anabaena flos-aquae* (*Cyanophyta*) blooms. *Journal of Phycology*. 13:97-101.

Carmichael, W. W., and P. R. Gorham. 1980. Freshwater cyanophyte toxins: types and their effects on the use of micro algae biomass. In *Algae biomass: production and use*. Edited by G. Shelef and C. J. Soeder. New York: Elsevier-North Holland Biomedical Press.

Carmichael, W. W., and N. A. Mahmood. 1984. Toxins from freshwater cyanobacteria. In *Seafood toxins*, American Chemical Society Series. 262:377-389.

Chan, H. W.-S. 1977. Photo-sensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways. *JAOCS* 54:100-104.

Cohen, Z. 1986. Products from microalgae. In *CRC Handbook of microalgal mass culture*. Edited by A. Richmond. Boca Raton, Fla.: CRC Press.

Collins, M. 1978. Algal toxins. *Microbiological Review*. 42(4):725-746.

Cranwell, P. A., M. E. Creighton, and G. H. M. Jaworski. 1988. Lipids of four species of freshwater chrysophytes. *Phytochemistry*. 27(4):1053-1059.

de la Noüe, J., and D. Proilx. 1987. Tertiary treatment of urban wastewaters by chitosan-immobilized *Phormidium* sp. In *Algal Biotechnology*. Edited by T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen. New York: Elsevier Applied Science.

Dillon, P. J., and F. H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. *Limnology and Oceanography*. 19(5):767-773.

Dravnieks, A., F. C. Bock, and F. H. Jarke. 1981. Sensory structure of odor mixtures. In *Odor quality and chemical structure*. Edited by H. R. Moskowitz and C. B. Warren. ACS Symposium Series #148. Washington, D. C.: American Chemical Society.

Dravnieks, A. 1985. *Atlas of odor character profiles*. ASTM Data Series Publication. Philadelphia, PA: ASTM

Eichelberger, J. W. L. E. Harris, and W. L. Budde. 1975. Reference compound to calibrate ion abundance measurements in gas chromatography-mass spectrometry. *Analytical Chemistry*. 47(7):995-1000.

Engen, T. 1982. *The perception of odors*. New York: Academic Press.

Fogg, G. E., and B. Thake. 1987. *Algal cultures and phytoplankton ecology*. 3rd ed. Madison, Wisconsin: University of Wisconsin Press.

Forss, D. A. 1973. Odor and flavor compounds from lipids. *Prog. Chem. Fats & Other Lipids*. 13:177-258.

Frankel, E. N. 1980. Lipid oxidation. *Prog. Lipid Res*. 19:1-22.

Frankel, E. N. 1982. Volatile lipid oxidation products. *Prog. Lipid Res*. 22:1-33.

Frankel, E. N., W. E. Neff, and T. R. Bessler. 1979. Analysis of autoxidized fats by gas chromatography-mass spectrometry: V. Photosensitized oxidation. *Lipids*. 14(12):961-967.

Frankel, E. N., W. E. Neff, and E. Selke. 1981. Analysis of autoxidized fats by gas chromatography- mass spectrometry: VII. Volatile thermal decomposition

products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate, and linolenate. *Lipids*. 16(5):279-285.

Frankel, E. N., W. E. Neff, and E. Selke. 1983. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VIII. Volatile thermal decomposition products of hydroperoxy cyclic peroxides. *Lipids*. 18(5):353-357.

Fulco, A. J. 1977. Fatty acid desaturation in microorganisms. In *Polyunsaturated fatty acids*. Edited by W. H. Kunau and R. T. Holman. Champaign, Ill.: American Oil Chemist's Society.

Galliard, T., and H. W.-S. Chan. 1980. Lipoxygenases. In *The biochemistry of plants: a comprehensive treatise. Vol. 4 Lipids: structure and function*. Edited by P. K. Stumpf and E. E. Conn. New York: Academic Press.

Galliard, T., and D. R. Phillips. 1976. The enzymic cleavage of linoleic acid to C_9 carbonyl fragments in extracts of cucumber (*Cucumis sativus*) fruit and the possible role of lipoxygenase. *Biochimica et Biophysica Acta*. 431:278-287.

Gerber, N. N., and H. A. Lechavalier. 1965. Geosmin, an earthy-smelling substance isolated from actinomycetes. *Applied Microbiology*. 13:935-938.

Gerber, N. N. 1969. A volatile metabolite of actinomycetes, 2-methylisoborneol. *J. Antibiot*. 22(10):508-509.

Gorham, P. R., J. McLachlan, U. T. Hammer, and W. K. Kim. 1964. Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb. de Breb.). *International Association of Theoretical and Applied Limnology*. 15:796-804.

Grob, K., and F. Zurcher. 1976. Stripping of trace organic substances from water - equipment and procedure. *J. Chromatogr*. 117:285-294.

Grosch, W. 1987. Reactions of hydroperoxides - products of low molecular weight. In *Autoxidation of unsaturated lipids*. Edited by H. W.-S. Chan. New York: Academic Press.

Gurr, M. I., and A. T. James. 1980. *Lipid biochemistry: an introduction*. London: Chapman and Hall.

Hamilton, R. J. 1989. The chemistry of rancidity in foods. In *Rancidity in foods*. 2nd ed. Edited by J. C. Allen and R. J. Hamilton. New York: Elsevier Applied Science

Hanson, M. J., and H. G. Stefan. 1984. Side effects of 58 years of copper sulfate treatment of the Fairmont Lakes, Minnesota. *Water Resources Bulletin*. 20(6):889-900.

Harper, R., E. C. Bate Smith, and D. G. Land. 1968. *Odour description and odour classification: a multidisciplinary examination*. New York: American Elsevier Publishing.

Harris, G. P. 1986. *Phytoplankton ecology: structure, function, and fluctuation*. Cambridge: Cambridge University Press.

Hatanaka, A., T. Kajiwara, and T. Harada. 1975. Biosynthetic pathway of cucumber alcohol: trans-2,cis-6-nonadienol via cis-3,cis-6-nonadienal. *Phytochemistry*. 14:2589-2592.

Hayes, K. P., and Burch, M. D. 1989. Odorous compounds associated with algal blooms in south Australian waters. *Wat. Res.* 23:115-121.

Hellebust, J. A. 1974. Extracellular products. In *Algal Physiology and Biochemistry*. Edited by W. D. Stewart. London: Blackwell Scientific Publications.

Henatsch, J. J., and F. Jüttner. 1983. Volatile odorous excretion products of different strains of *Synechococcus* (cyanobacteria). *Wat. Sci. Tech.* 15:259-266.

Henley, D. E. 1970. Odorous metabolite and other selected studies of Cyanophyta. Doctoral dissertation, North Texas State Univ., Denton, Texas.

Herbst, V., and J. Overbeck. 1978. Metabolic coupling between the alga *Oscillatoria redekei* and accompanying bacteria. *Naturwissenschaften*. 65:598-599.

Hering, J. G., and F. M. M. Morel. 1988. Humic acid complexation of calcium and copper. *Environmental Science & Technology*. 22(10):1234-1237.

Hess, J. L., and N. E. Tolbert. 1967. Glycolate pathway in algae. *Plant Physiol.* 42:371-379.

Hitchcock, C., and B. W. Nichols. 1971. *Plant lipid biochemistry*. New York: Academic Press.

Hoffmann, G. 1962. Vegetable oils. In *Symposium on foods: lipids and their oxidation*. Edited by H. W. Schultz, E. A. Day, and R. O Sinnhuber. Westport, Conn.: AVI Publishing

Hollander, M., and D. A. Wolfe. 1973. *Nonparametric statistical methods*. New York: Wiley & Sons.

Hoson, T. 1992. Growth characteristics of the musty odor producing alga, *Oscillatoria tenuis*. *Wat. Sci. Tech.* 25(2):177-184.

Hrudey, S. E., D. Rector, and N. Motkosky. 1992. Characterization of drinking water odour arising from spring thaw for an ice-covered upland river source. *Wat. Sci. Tech.* 25(2):65-72.

Hsieh, R. J., and J. E. Kinsella. 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. In *Advances in food and nutrition research, Vol 33*. Edited by J. E. Kinsella. New York: Academic Press.

Hsieh, T. C. Y., S. S. Williams, W. Vejaphan, and S. P. Meyers. 1989. Characterization of volatile components of Mahaden fish (*Brevoortia tyrannus*) oil. *JAOCS* 66(1):114-117.

Hwang, C. J., S. W. Krasner, M. J. McGuire, M. S. Moylan, and M. S. Dale. 1984. Determination of subnanogram per liter levels of earthy-musty odorants in water by the salted closed-loop stripping method. *Envir. Sci. Tech.* 18(7):535-539.

Izaguirre, G. 1992. A copper-tolerant Phormidium species from Lake Mathews, California, that produces 2-methylisoborneol and geosmin. *Wat. Sci. Tech.* 25(2):217-223.

Izaguirre, G., C. J. Hwang, S. W. Krasner, and M. J. McGuire. 1982. Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. *Appl. Envir. Micro.* 43(3):708-714.

Jones, R. A. and G. F. Lee. 1982. Recent advances in assessing impact of phosphorus loads on eutrophication-related water quality. *Water Research.* 16(5):503-515.

Josephson, D. B. 1991. Seafood. In *Volatile compounds in foods and beverages.* Edited by H. Maarse. New York: Marcel Dekker.

Josephson, D. B., R. C. Lindsay, and D. A. Stuiber. 1985. Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. *J. Food Sci.* 50:5-9.

Jurs, P. C., C. L. Ham, and W. E. Brügger. 1981. Computer-assisted studies of chemical structure and olfactory quality using pattern recognition techniques. In *Odor quality and chemical structure*. Edited by H. R. Moskowitz and C. B. Warren. ACS Symposium Series #148. Washington, D. C.: American Chemical Society.

Jüttner, F. 1976. β-Cyclocitral and alkanes in *Microcystis* (Cyanophyceae). *Z. Naturforschung.* 31(9/10):491-495.

Jüttner, F. 1979a. Nor-carotenoids as the major volatile excretion products of *Cyanidium*. *Z. Naturforschung.* 34c:186-191.

Jüttner, F. 1979b. The algal excretion product, geranylacetone: a potent inhibitor of carotene biosynthesis in *Synechococcus*. *Z. Naturforschung.* 34c:957-960.

Jüttner, F. 1981. Detection of lipid degradation products in the water of a reservoir during a bloom of *Synura uvella*. *Appl. Envir. Micro.* 41:100-106.

Jüttner, F. 1983. Volatile odorous excretion products of algae and their occurrence in the natural aquatic environment. *Wat. Sci. Tech.* 15:247-257.

Jüttner, F. 1984a. Characterization of *Microcystis* strains by alkyl sulfides and β-cyclocitral. *Z. Naturforschung.* 39c:867-871.

Jüttner, F. 1984b. Dynamics of the volatile organic substances associated with cyanobacteria and algae in a eutrophic shallow lake. *Appl. Envir. Micro.* 47(4):814-820.

Jüttner, F. 1987. Volatile organic substances. In *The Cyanobacteria.* New York: Elsevier Science Publishers

Jüttner, F., and O. Bogenschütz. 1983. Geranyl derivatives as inhibitors of the carotenogenesis in *Synechococcus* PCC 6911 (cyanobacteria). *Z. Naturforschung.* 38c:387-392.

Karahadian, C., and R. C. Lindsay. 1989. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *JAOCS.* 66(7):953-960.

Kay, R. A. 1991. Microalgae as food and supplement. *Critical reviews in food, science, and nutrition.* 30(6):555-573.

Ke, P. J., R. G. Ackman, and B. A. Linke. 1975. Autoxidation of polyunsaturated fatty compounds in mackeral oil: formation of 2,4,7-decatrienals. *JAOCS* 52:349-353.

Kenyon, C. N., R. Rippka, and R. Y. Stanier. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. *Arch. Microbiol.* 83:216-236.

Kenefick, S. L., S. E. Hrudey, E. E. Prepas, N. Motkosky, and H. G. Peterson. 1992. Odorous substances and cyanobacterial toxins in prairie drinking water sources. *Wat. Sci. Tech.* 25(2):147-154.

Khiari, D., L. Brenner, G. A. Burlingame, and I. H. Suffet. 1992. Sensory gas chromatography for evaluation of taste and odor events in drinking water. *Wat. Sci. Tech.* 25(2):97-104.

Krasner, S. W., S. E. Barrett, M. S. Dale, and C. J. Hwang. 1989. Free chlorine versus monochloramine for controlling off-tastes and off-odors. *Journal AWWA.* 81(2):86-93.

Krasner, S. W., C. J. Hwang, and M. J. McGuire. 1983. A standard method for quantification of earthy-musty odorants in water, sediments, and algal cultures. *Wat. Sci. Tech.* 15(6/7):127-138.

Lange, W. 1974. Chelating agents and blue-green algae. *Can. J. Microbiol.* 20:1311-1321.

Lee, R. E. 1989. *Phycology*. 2nd ed. New York: Cambridge University Press.

Lee, G. F., W. Rast, and R. A. Jones. 1978. Eutrophication of water bodies: insights for an age-old problem. *Environmental Science & Technology*. 12(8):900-908.

Lehman, J. T. 1976. Ecological and nutritional studies on *Dinobryon* Ehr.: seasonal periodicity and the phosphate toxicity problem. *Limnol. Oceanogr.* 21:646-658.

Ley, S. H. 1987. Cultivation and application of microalgae in people's Republic of China. In *Algal Biotechnology*. Edited by T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen. New York: Elsevier Applied Science.

Lovell, R. T. 1983. Off-flavors in pond cultured channel catfish. *Wat. Sci. Tech.* 15(6/7):67-73.

Mallevialle, J., and I. H. Suffet, eds. 1987. *Identification and treatment of tastes and odors in drinking water*. Denver, Co.: AWWA and Lyonnaise des Eaux.

Martin, J. F. 1992. The use of sodium carbonate peroxyhydrate to treat off-flavor in commercial catfish ponds. *Wat. Sci. Tech.* 25(2):315-321.

Martin, J. F., and I. H. Suffet. 1992. Chemical etiologies of off-flavor in channel catfish aquaculture. *Wat. Sci. Tech.* 25(2):73-79.

Materassi, R., C. Paoletti W. Balloni, and G. Florenzano. 1980. Some considerations on the production of lipid substances by microalgae and cyanobacteria. In *Algae biomass: production and use*. Edited by G. Shelef and C. J. Soeder. New York: Elsevier-North Holland Biomedical Press.

McC. Gamble, E. A. 1898. Applicability of Weber's Law to smell. *Am. J. Psychol.* 10(1):82-142.

McFeters, G. A., S. A. Stuart, and S. B. Olson. 1978. Growth of heterotrophic bacteria and algal extracellular products in oligotrophic waters. *Appl. Envir. Micro.* 35:383-391.

McGuire, M. J., R. M. Jones, E. G. Means, G. Izaguirre, and A. E. Preston. 1984. Controlling attached blue-green algae with copper sulfate. *Journal AWWA.* 76(5):60-65.

McIntosh, R. 1984. Intensive microalgae culture for production of lipids for fuel. *In Energy Applications of Biomass*. Edited by M. Z. Lowenstein. New York: Elsevier Applied Science.

McKnight, D. M., and F. M. M. Morel. 1979. Release of weak and strong copper-complexing agents by algae. *Limnology and Oceanography*. 24(5):823-837.

Means, E. G., and M. J. McGuire. 1986. An early warning system for taste and odor control. *Journal AWWA*. 78(3):77-83.

Medsker, L. L., D. Jenkins, and J. F. Thomas. 1968. Odorous compounds in natural waters: Ab earthy-smelling compound associated with blue-green algae and actinomycetes. *Environmental Science & Technology*. 2:461-464.

Meng, A. K., and I. H. Suffet. 1992. Assessing the quality of flavor profile analysis data. *Journal AWWA*. 84:89-96.

Miwa, M., and K. Morizane. 1988. Effect of chelating agents on the growth of blue-green algae and the release of geosmin. *Wat. Sci. Tech*. 20(8/9):197-203.

Monie, W. D. 1956. Algae control with copper sulfate. *Water & Sewage Works*. 103(9):392-397.

Moskowitz, H. R. 1981. Psychophysical scaling and optimization of odor mixtures. In *Odor quality and chemical structure*. Edited by H. R. Moskowitz and C. B. Warren. ACS Symposium Series #148. Washington, D. C.: American Chemical Society.

Naes, H., H. Aarnes, H. C. Utkilen, S. Nilsen, and O. M. Skulberg. 1985. Effect of photon fluence rate and specific growth rate on geosmin production of the cyanobacterium *Oscillatoria brevis* (Kutz.) Gom. *Appl. Envir. Micro*. 49(6):1538-1540.

Naes, H., and A. F. Post. 1988. Transient states of geosmin, pigments, carbohydrates, and proteins in continuous cultures of Oscillatoria brevis induced by changes in nitrogen supply. *Arch. Microbiol*. 150:333-337.

Naes, H., H. C. Utkilen, and A. F. Post. 1988. Factors influencing geosmin production by the cyanobacterium *Oscillatoria brevis*. *Wat. Sci. Tech*. 20(8/9):125.131.

Nalewajko, C. 1977. Extracellular release in freshwater algae and bacteria: extracellular products of algae as a source of carbon for heterotrophs. In *Aquatic Microbial Communities*. Edited by J. Cairns. New York: Garland Publishing

Nalewajko, C., T. G. Dunstall, and H. Shear. 1976. Kinetics of extracellular release in axenic algae and in mixed algal-bacterial cultures: significance in estimation of total (gross) phytoplankton excretion rates. *J. Phycol.* 12:1-5.

Nalewajko, C., and D. R. S. Lean. 1972. Growth and excretion in planktonic algae and bacteria. *J. Phycol.* 8:361-366.

Nalewajko, C., and L. Marin. 1969. Extracellular production in relation to growth of four planktonic algae and of phytoplankton populations from Lake Ontario. *Can. J. Botany.* 47:405-413.

Novotny, V., and G. Chesters. 1981. *Handbook of nonpoint pollution: sources and management.* New York: Van Nostrand Reinhold

Orcutt, D. M., and G. W. Patterson. 1974. Effect of light intensity upon lipid composition of *Nitzschia closterium* (*Cylindrothexa fusiformis*). *Lipids.* 9(12):1000-1003.

Orcutt, D. M., and G. W. Patterson. 1975. Sterol, fatty acid, and elemental composition of diatoms grown in chemically defined media. *Comp. Biochem. Physiol.* 50B:579-583.

Palenik, B., and F. M. M. Morel. 1990. Amino acid utilization by marine phytoplankton: a novel mechanism. *Limnol. Oceanogr.* 35(2):260-269.

Palmer, C. M. 1980. *Algae and water pollution: the identification, significance, and control of algae in water supplies and in polluted water.* Castle House Publications.

Patrick, R. 1977. Ecology of freshwater diatoms and diatom communities. In *The biology of diatoms*, Botanical Monographs, Vol. 13. Edited by D. Werner. Berkeley: Univ. of California Press (Blackwell Scientific Publications).

Persson, P. E. 1988. Odorous algal cultures in culture collections. *Wat. Sci. Tech.* 20(8/9):211-213.

Poppe, W. L., J. S. Crossman, and S. W. Hixson. 1981. Phytoplankton effects on reservoir use. In *Phytoplankton-environmental interactions in reservoirs*; Vol. 1: Papers at Workshop, 10-12 April 1979, Monterey, California. Technical report E-81-13. Edited by M. C. Lorenzen. Vicksburg, Miss.: U. S. Army Engineer Waterways Experiment Station.

Preti, G., T. S. Gittelman, P. B Staudte, and P. Luitweiler. 1993. Letting the nose lead the way: malodorous components in drinking water. *Analytical Chemistry.* 65(15):699a-702a.

Punter, P. H., and B. Ph. M. Menco. 1981. The efficacy of n-aliphatic alcohols and n-aliphatic fatty acids on various membrane systems with special reference to olfaction and taste. In *Odor quality and chemical structure*. Edited by H. R. Moskowitz and C. B. Warren. ACS Symposium Series #148. Washington, D. C.: American Chemical Society.

Raman, R. K. 1985. Controlling algae in water supply impoundments. *Journal AWWA*. 77(8):41-43.

Raman, R. K., and B. C. Cook. 1988. *Guidelines for applying copper sulfate as an algicide: Lake Loami field study*. ILENR/RE-WR-88/19 Illinois Department of Energy and Natural Resources.

Rashash, D. M. C. 1991. An analysis of phytoplankton variations in the Occoquan Reservoir, 1970-1988. Masters thesis, VPI&SU, Blacksburg, Virginia.

Reichardt, W. 1981. Influence of methylheptenone and related phytoplankton norcarotenoids on heterotrophic aquatic bacteria. *Can. J. Microbiol.* 27:144-147.

Reynolds, C. S. 1984. *The ecology of freshwater phytoplankton*. Cambridge: Cambridge University Press.

Rezanka, O., J. Vokoun, J. Slavícek, and M. Podojil. 1983. Determination of fatty acids in algae by capillary gas chromatography-mass spectrometry. *J. Chromatography*. 268:71-78.

Rijn, J. van, and M. Shilo. 1985. Carbohydrate fluctuations, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fishponds. *Limnol. Oceanogr.* 30(6):1219-1228.

Rosen, B. H., B. W. Macleod, and M. R. Simpson. 1992. Accumulation and release of geosmin during the growth phases of *Anabaena circinalis* (kutz.) Rabenhorst. *Wat. Sci. Tech.* 25(2):185-190.

Ryther, J. H. 1984. Technology for the commercial production of macroalgae. In *Energy Applications of Biomass*. Edited by M. Z. Lowenstein. New York: Elsevier Applied Science.

Safferman, R. S., A. A. Rosen, C.I. Mashni, and M. E. Morris. 1967. Earthy-smelling substance from a blue-green alga. *Environmental Science & Technology*. 1:429-430.

Sävenhed, G., H. Borén, A. Grimvall, and A. Tjeder. 1983. Stripping techniques for the analysis of odorous compounds in drinking water. *Wat. Sci. Tech.* 15(6/7)139-148.

Sawyer, C. N. 1968. The need for nutrient control. *Journal WPCF*. 40(3):363-370.

Schindler, D. W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnology and Oceanography*. 23(3):478-486.

Seals, R. G., and E. G. Hammond. 1970. Some carbonyl flavor compounds of oxidized soybean and linseed oils. *JAOCS* 47:278-280.

Seifert, R. M., and R. G. Buttery. 1980. A convenient synthesis of (E,E,Z)-2,4,7-decatrienal. *J. Agric. Food Chem*. 28(1):68-70.

Selke, E., H. A. Moser, and W. K. Rohwedder. 1970. Tandem gas chromatography-mass spectrometry analysis of volatiles from soybean oil. *JAOCS* 47:393-397.

Sharaf, M. A., D. L. Illman, and B. R. Kowalski. 1986. Chemometrics. Vol 82 In: *Chemical Analysis: a series of monographs an analytical chemistry and its applications*. Edited by P. J. Elving, J. D. Winefordner, and I. M. Kolthoff. New York: Wiley-Interscience.

Shifrin, N. S., and S. W. Chisholm. 1980. Phytoplankton lipids: Environmental influences on production and possible commercial applications. In *Algae biomass: production and use*. Edited by G. Shelef and C. J. Soeder. New York: Elsevier-North Holland Biomedical Press.

Simpson, F. B., and J. B. Neilands. 1976. Siderochromes in cyanophyceae: isolation and characterization of schizokinen from *Anabaena* sp. *J. Phycol*. 12:44-48.

Slater, G. P., and B. C. Blok. 1983a. Volatile compounds of the Cyanophyceae- a review. *Wat. Sci. Tech*. 15:181-190.

Slater, G. P., and B. C. Blok. 1983b. Isolation and identification of odorous compounds from a lake subject to Cyanobacterial blooms. *Wat. Sci. Tech*. 15:229-240.

Smith, D. V., and H. J. Duncan. 1992. Primary olfactory disorders: anosmia, hyposmia, and dysosmia. In *Science of olfaction*. Edited by M. J. Serby and K. L. Chobor. New York: Springer-Verlag.

Stevens, S. S. 1970. Neural events and the psychophysical law. *Science*. 170:1043-1050.

Stumpf, P. K. 1980. Biosynthesis of saturated and unsaturated fatty acids. In *The biochemistry of plants: a comprehensive treatise. Vol. 4 Lipids: structure and function*. Edited by P. K. Stumpf and E. E. Conn. New York: Academic Press.

Tabachek, J. L., and M. Yurkowski. 1976. Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba. *J. Fisheries Res. Board of Canada*. 33(1):25-35.

Taylor, J. R. 1982. *An introduction to error analysis: the study of uncertainties in physical measurements*. Mill Valley, California: University Science Books.

Terao, J., and S. Matsushita. 1977. Products formed by photosensitized oxidation of unsaturated fatty acid esters. *JAOCS*. 54:234-238.

Thepenier, C., D. Chaumont, and C. Gudin. 1987. Mass culture of *Porphyridium cruentum*: a multiproduct strategy for the biomass valorisation. In *Algal Biotechnology*. Edited by T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen. New York: Elsevier Applied Science.

Tressl, R., D. Bahri, and K. H. Engel. 1981. Lipid oxidation in fruits and vegetables. In: *Quality of selected fruits and vegetables of North America*. Edited by R. Teranishi and H. Barrera-Benitez. ACS Symposium Series #170. Washington: American Chemical Society.

Tuwiner, S. B. 1976. Copper sulfate helps control microorganisms in reservoirs. *Water & Sewage Works*. 123(12):68-70.

Utkilen, H. C., and M. Frøshaug. 1992. Geosmin production and excretion in a planktonic and benthic *Oscillatoria*. *Wat. Sci. Tech*. 25(2):199-206.

Whitfield, F. B., and J. H. Last. 1991. Vegetables. In *Volatile compounds in foods and beverages*. Edited by H. Maarse. New York: Marcel Dekker.

Williams, L. R. V. W. Lambou, S. C. Hern, and R. W. Thomas. 1978. *Relationship of productivity and problem conditions to ambient nutrients: National Eutrophication Survey findings for 418 eastern lakes*. EPA 600/3-78-002. Las Vegas, Nev.:USEPA.

Wnorowski, A. U., and W. E. Scott. 1992. Incidence of off-flavors in South African surface waters. *Wat. Sci. Tech*. 25(2):225-232.

Wood, B. J. B. 1974. Fatty acids and saponifiable lipids. In *Algal physiology and biochemistry*. Edited by W. D. P. Stewart. London: Blackwell Scientific.

Wright, R. H. 1982. *The sense of smell*. Boca Raton, Florida:CRC Press.

Wu, J. T., and F. Jüttner. 1988. Differential partitioning of geosmin and 2-methylisoborneol between cellular constituents in *Oscillatoria tenuis*. *Arch. Microbiol*. 150:580-583.

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