Treatment of Algae-Induced Tastes and Odors

by Chlorine, Chlorine Dioxide and Permanganate

by

Lisa Webster Buffin

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APPROVED:

Robert C. Hoehn, Chairman

Andrea M. Dietrich

Bruce C. Parker

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(ABSTRACT)

Chlorine ($Cl_{2(aq)}$), chlorine dioxide (ClO_2) and potassium permanganate ($KMnO_4$) were evaluated as oxidants for the removal of grassy and cucumber odors associated with the pure compounds, cis-3-hexenol and trans-2, cis-6-nonadienal, respectively, and for the removal of fishy odors associated with a culture of an alga, Synura petersenii. The effects of the oxidants on the pure compounds were assessed both by Flavor Profile Analysis (FPA) and gas chromatography/mass spectrometry (GC/MS). The effects of the oxidants on the algae culture were evaluated by FPA only. In addition, an unoxidized sample of Synura petersenii was analyzed by gas chromatography coupled with mass spectrometry (GC/MS) for possible identification of fishy-smelling compounds.

Chlorine (1-6 mg/L) and $KMnO_4$ (0.25-4 mg/L) markedly reduced grassy and cucumber odors associated with the two compounds. Gas chromatography/mass spectrometry confirmed that these compounds were reduced to below method detection limits. Levels of $Cl_{2(aq)}$ required (up to 6 mg/L) to reduce the grassy odors associated with cis-3-hexenol were higher than those of $KMnO_4$. The high $Cl_{2(aq)}$ doses may have contributed to

the formation of chemical odors observed by panelists. Two isomers of chlorohexenol were confidently identified as by-products of cis-3-hexenol chlorination and may have contributed to the chemical odors that developed after $Cl_{2(aq)}$ treatment. Chlorine and $KMnO_4$ (both at 10 mg/L) either reduced or destroyed the fishy odor associated with the culture of Synura petersenii; however, oxidation caused either the development or unmasking of fruity, cucumber, melon and grassy odors.

Chlorine dioxide (3 mg/L) did not reduce the grassy and cucumber odors associated with cis-3-hexenol and trans-2, cis-6-nonadienal, respectively. Gas chromatography and mass spectrometry confirmed that concentrations of these compounds were not reduced to below method detection limits. Furthermore, at a concentration of 10 mg/L, ClO_2 did not effectively reduce either the fishy or other objectionable odors associated with Synura petersenii culture.

Hexanal, with an odor described as "green" or "like lettuce heart," and trans-2, cis-6-nonadienal (cucumber odor) were confirmed as algal products in a two-week-old culture of Synura petersenii. In addition, decatrienal was confidently identified as a product of Synura and may have contributed to the fishy odor associated with this alga.

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INTRODUCTION

Water utilities have always been plagued with taste-and-odor problems because consumers associate poor aesthetic quality with a nonpotable supply. Although most complaints do not originate from toxic concentrations of contaminating compounds (Bartels et al., 1986), the water-supply industry is concerned with the public's rejection of a safe water supply in favor of a nonpotable one. Although tastes and odors are regarded as secondary, or aesthetic, contaminants, they are the primary criteria by which consumers judge their drinking water supply (Manwaring et al., 1986), and can cause considerable, as well as expensive, problems for a water utility.

Algae are one of the principal sources of compounds that cause objectionable tastes and odors; however, few basic data exist regarding algae-produced tastes and odors other than those associated with geosmin and 2-methylisoborneol (MIB), which cause earthy/musty odors. Furthermore, research is lacking in the area of treatment methods for the odor compounds, particularly research focused on the effectiveness of oxidants in eliminating certain tastes and odors. This research focused on the treatment of algae-produced "grassy", "cucumber", and "fishy" odors, which frequently evoke

complaints from consumers.

The objective of this research was to evaluate the effects of three oxidants that are commonly used during drinking water treatment on solutions of two, odoriferous, organic compounds, one that produces grassy odors and another that produces cucumber odors, and on an algae culture that smelled fishy. The oxidants included aqueous chlorine ($Cl_{2(aq)}$), chlorine dioxide (ClO_2), and potassium permanganate ($KMnO_4$). The effects of the oxidants, which were added at dosages typically used during drinking water treatment, on the two odor-causing compounds were assessed both by sensory analyses, which were conducted by a trained panel, and by gas chromatography and mass spectrometry (GC/MS). The effects of the oxidants on the algae culture were evaluated only by sensory analysis. In addition, an untreated sample of the fishy-smelling algae culture was analyzed by GC/MS in an effort to identify the fishy-smelling compound(s), since no such compound has been identified. The specific analytical procedures are described later in this thesis. Of particular interest during the sensory analyses was the detection of any alterations in both the characteristics and intensities of the odors that might have occurred after the oxidants were added. The GC/MS analyses were intended to reveal both the extent of reduction in the odor-compound concentrations and the appearance of by-products.

LITERATURE REVIEW

Tastes and Odors in Drinking Water

Tastes and odors are the primary criteria by which consumers judge their drinking water supply according to a 1985 survey conducted by the American Water Works Association Research Foundation (Manwaring et al., 1986). This same survey showed that the majority of consumers who purchased bottled water did so because their tap water had objectionable tastes and odors. "In fact, the water customer appears to be more concerned about off-flavors, color, and turbidity than about the presence of health-threatening concentrations of nonodorous chemical compounds" (Bartels et al., 1986). The public takes a safe water supply for granted and may reject an aesthetically unpleasant potable supply for a nonpotable one. This is indeed a public health concern and is the reason why research in taste and odor treatment for drinking water supplies is so important.

Algae Generated Tastes and Odors and the Need for Research

One of the principal sources of objectionable tastes and odors in drinking water supplies is algae. Two of the most widely researched compounds in taste-and-odor research, geosmin and 2-methylisoborneol (MIB), are produced by algae.

Particularly associated with blue-green algae, or cyanobacteria, (Slater and Blok, 1983; Tabachek and Yurkowski, 1976), these compounds produce earthy and musty odors, respectively, and "create more problems in public water supplies than any other types of odor" (Mallevialle and Suffet, 1987). Because complaints about earthy/musty odors are perhaps the most frequent of all complaints received concerning tastes and odors in drinking water (Table 1), most of the analytical techniques and treatment methods described in this area of research are specifically aimed at solving this particular problem (Hwang et al., 1984; Lalezary et al., 1986; McGuire et al., 1981). Other odors, however, which are associated with other groups of algae as well as the blue-greens, frequently provoke complaints from consumers. Typical descriptors such as "grassy", "cucumber", and "fishy" are often used. Apart from geosmin and MIB, "work is just beginning....on determining the specific chemicals that cause odors or off-flavors commonly found in drinking water" (Mallevialle and Suffet, 1987). Some odor causing compounds occurring during algae blooms include alkenes, saturated and unsaturated alcohols, aldehydes, ketones, esters, thioesters and sulfides (Jüttner, 1983). Other than this, few basic data regarding algae-generated tastes and odors can be found in the literature.

Table 1. Odor classification by frequency (Mallevialle and Suffet, 1987)

CLASSIFICATION*	DESCRIPTOR
Group 1	Musty, earthy, moldy
Group 2	Chlorinous
Group 3	Grassy, hay-like, woody
Group 4	Marshy, swampy, septic, sewage
Group 5	Fragrant (vegetable or flowery)
Group 6	Fishy
Group 7	Medicinal, phenolic, antiseptic
Group 8	Chemical, hydrocarbon, miscellaneous

* Classification used by Philadelphia Suburban Water Company

Numerous problems associated with data collection exist, which may explain the lack of basic data. First, a direct link cannot always be made between the occurrence of a compound in a water supply and the presence of an algal species. Second, biological and chemical conversions of the algal metabolites can occur. For example, blue-green algae are capable of reducing a large number of carbonyl compounds to form the corresponding alcohols (Jüttner and Hans, 1986). Furthermore, conversion between isomers can take place (Jüttner, 1983), often resulting in a change in odor characteristics. Age and environmental factors--such as light, temperature, pH, mechanical damage and the presence of metals and/or sensitizers--can influence the formation of particular compounds. Changes can also occur during compound concentration and storage (Takeoka et al., 1986). Finally, even when a compound is known, an oxidant's effectiveness at the water treatment level is often unknown.

Several efforts have been made to minimize the problems that complicate taste-and-odor-research. For example, axenic cultures have been grown under controlled conditions and the volatile chemicals they produce have been analyzed (Jüttner, 1983). Also, reference compounds, with similar odor characteristics as the unknown compound have been used for standardizing odor descriptors (Mallevialle and Suffet, 1987) and evaluating treatment. Finally, the effectiveness of

various water-treatment procedures for removing tastes-and-odors from water has been evaluated at treatment plants by a sensory-based procedure such as Flavor Profile Analysis (FPA) (Bartels et al., 1989). The research reported in this thesis was conducted with these factors in mind.

Grassy, Cucumber and Fishy Odors Produced by Algae

Grassy Odors

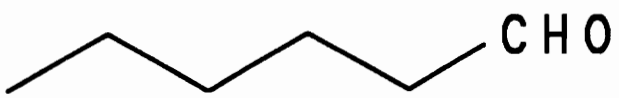
Grassy odors are associated with many groups of algae (Table 2) and are often resistent to conventional treatment processes (Mallevialle and Suffet, 1987). Usually associated with one or several six-carbon compounds (Figure 1); such as cis-3-hexenol, trans-2-hexenal, cis-3-hexenal and n-hexanal (Badings, 1970; Josephson and Lindsay, 1986; Karahadian and Lindsay, 1989); these odors may vary from coarse and/or oily green-plant-like notes, such as those characteristic of decaying vegetables, to fresher aromas, such as those associated with green leaves, fresh cut grass, and/or green apples.

Hexanal and cis-3-hexenal are liberated in higher plants during "trauma" or wound formation (Galliard, 1978), and it is feasible to expect the same for algae (Jüttner, 1981). Similiarly, the "leaf aldehydes" may be reduced to "leaf alcohols." Although hexanal has been detected during a bloom of Stephanodiscus hantzchii (a diatom) (Jüttner, 1984) and in

Table 2: Algae associated with the "grassy" descriptor

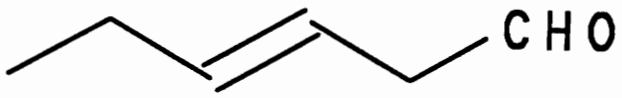
Blue-green algae (Cyanobacteria): Anabaena, Anabaenopsis, Anacystis, Aphanizomenon, Cylindrospermum, Gloeotrichia, Gomphosphaeria, Oscillatoria, Rivularia
Diatoms: Closterium, Cosmarium, Scenedesmus, Staurastrum, Synedra
Green Algae: Actinastrum, Closterium, Cosmarium, Dictyosphaerium, Nitella, Pediastrum, Scenedesmus, Spirogyra, Staurastrum

After Mallevialle and Suffet, 1987, and Palmer, 1962.



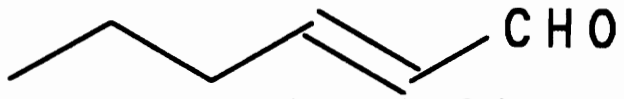
Hexanal

(lettuce heart/green)



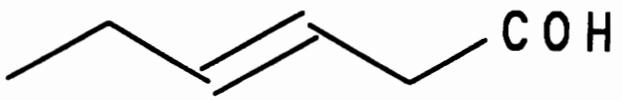
3-Hexenal

(3Z-isomer: green)



2-Hexenal

(2E-isomer: sweet/fruity/leafy/green)



3-Hexenol

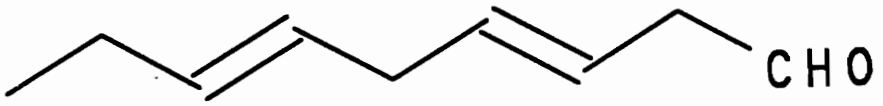
(3Z-isomer: grassy/green apples)

Figure 1. Compounds associated with grassy or "green" odors.

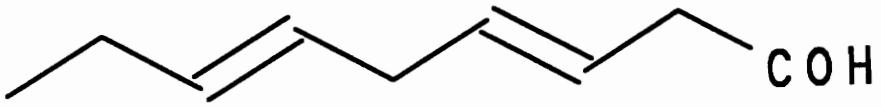
an axenic culture of Anabaena oscillarioides (a blue-green alga) (Möhren and Jüttner, 1983), no direct link has beenmade between this compound and the grassy descriptor associated with any type of algae. Cis-3-hexenol, which was found as a biogenic product that exhibited an intense odor in a eutrophic shallow lake (Jüttner, 1984), was chosen as the reference compound for bench testing and sensory analysis instead of hexanal or hexenal because its odor is readily described as "fresh cut grass" or "green apples." In contrast, descriptors arising from hexanal solutions include "green" (Badings, 1970), "lettuce heart" (Burlingame, 1992), "pumpkin" (Burlingame, 1992) and various other vegetable-like associations.

Cucumber Odors

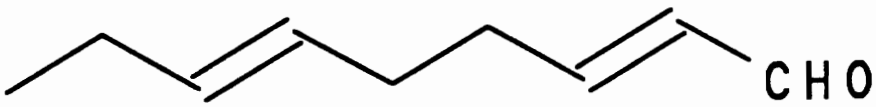
Cucumber odors are associated with three types of freshwater algae: Peridinium (a dinoflagellate), Synura (a chrysophyte), and Uroglenopsis (a chrysophyte) (Palmer, 1962). These algae, when abundant, may produce even more offensive fishy odors (Mallevialle and Suffet, 1987). The compound responsible for the cucumber odor in Synura petersenii has been identified as trans-2,cis-6-nonadienal (Hayes and Burch, 1989). It has been isolated also from Japanese kelps (Kajiwara et al., 1988), and is considered the major contributor to the aroma of cucumbers (Kemp et al., 1974).



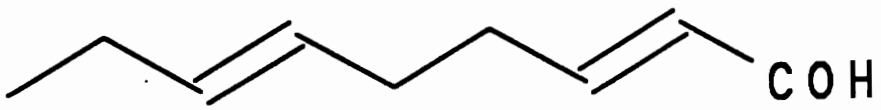
3,6-Nonadienal
(no information available)



3,6-Nonadienol
(3Z,6Z-isomer: muskmelon/watermelon)



2,6-Nonadienal
(2E,6Z-isomer: cucumber)



2,6-Nonadienol
(2E,6Z-isomer: violets)

Figure 2. Some C_9 compounds associated with cucumbers, muskmelons and watermelons.

Synura also produces a muskmelon odor (Palmer, 1962), which can possibly be attributed to trans-2,cis-6-nonadienal as well as to other related, nine-carbon compounds (Figure 2). Some higher plants, notably the major curcurbits (cucumber, Cucumis sativus L.; muskmelon, Cucumis melo; and watermelon, Citrullus vulgaris), seem to have evolved several structurally similar, odoriferous compounds (Kemp, 1975; Kemp et al., 1971; Kemp et al., 1974; Kemp et al., 1974). In cucumbers, trans-2, cis-6-nonadienal is related to the auto-oxidative or enzymatic peroxidation of linolenic acid, an omega-3 polyunsaturated fatty acid (Grosch, 1971) and is formed only when the fruit is cut or ruptured in the presence of oxygen (Fleming, 1968). Trans-2,cis-6-nonadienal also contributes desirable characteristic cucumber or melon aromas to certain species of freshly harvested fish and is again formed from the auto-oxidative or enzymatic oxidation of omega-3 polyunsaturated fatty acids, namely eicosapentaenoic and docosahexaenoic acids (Karahadian and Lindsay, 1989; Josephson and Lindsay, 1986). A similar mechanism is suspected in Synura (Jüttner, 1981; Hayes and Burch, 1989). Because trans-2,cis-6-nonadienal has been isolated and identified as an algal odor-causing compound, it was chosen for bench testing and sensory evaluation during this research project.

Fishy Odors

Fishy odors are associated with numerous types of algae (Table 3). The odors are produced often by the same algae that are responsible for the fragrant odors and are often difficult to remove from water supplies (Mallevialle and Suffet, 1987). Although compounds such as dimethylamine and trimethylamine are routinely chosen as "fishy" reference standards, these compounds do not always impart the same odor to water as do many groups of algae. Descriptions of "fishy" vary from "clamshells" (Peridinium) to "cod liver oil" (Uroglena and Synura, both chrysophytes) (Mallevialle and Suffet, 1987). Dimethylamine and trimethylamine are more often associated with "rotten fish" and "ammonia" type odors.

Two compounds that have been implicated as possible sources of fishy odors are trans-2,cis-4-decadienal and trans-2,cis-4-heptadienal. Jüttner (1981) stated that Synura's offensive cod liver oil-like odor was traceable to these two compounds during a heavy algae bloom in Germany's Wahnbach Reservoir in 1978; however, in a later article (Jüttner, 1983), the same bloom was described as having an "offensive, rancid odor" (Jüttner, 1983), not a "fishy" one. This corresponds better to the descriptors numerous students in the Virginia Tech Laboratory used to describe the two compounds. The presence of the these compounds was further associated with a bloom of a Dinobryon species (also a chrysophyte) in a

Table 3. Algae associated with the "fishy" descriptor

Chrysophytes: Chrysosphaerella, Dinobryon, Mallomonas, Synura, Uroglenopsis
Diatoms: Asterionella, Cyclotella, Pandorina, Stephanodiscus, Tabellaria
Dinoflagellates: Ceratium, Glenodinium, Peridinium
Euglenoids: Euglena
Green Algae: Dictyosphaerium, Eudorina, Gonium, Pandorina, Volvox
Yellow-green Algae: Tribonema

After Mallevialle and Suffet, 1987, and Palmer, 1977

eutrophic shallow lake, but no odor descriptor was given (Jüttner, 1984). Jüttner (1981) attributed the cod liver oil-like odor of Synura mainly to trans-2,cis-4-decadienal, which was present in amounts far above its odor threshold; however, he also isolated major amounts of this compound, as well as other compounds, from a culture of Poteriochromonas malhamensis, another chrysophyte, although "pineapple" and "rancid" were the descriptors given to the odor emanating from the algae sample (Jüttner, 1983).

Both -dienals have been associated with undesirable odors in foods. Badings (1970) gave the descriptors "fatty, oily" and "frying odor," respectively, to the trans-2,trans-4 and trans-2,cis-4 isomers of heptadienal. Similarly, he gave the descriptors "deep-fried" and "frying odor," respectively, to the trans-2,trans-4 and trans-2,cis-4 isomers of decadienal, stating that "all 2,4-dienals contribute to fatty or fried flavors." "Fast-food-restaurant-like" was the descriptor given by students in the Virginia Tech Laboratory. Swoboda and Peers (1977) identified the class of compounds with a 2,4-dienal functional group as being the major odor contributors to the fishy taint in butterfat, adding that the fishy odor does not have the same impact when individual components are smelled at the odor port of the chromatograph.

Swoboda and Peers (1977) did find that a fishy description was associated with the emergence from the odor

port of trans-2,cis-4,cis-7-decatrienal, another compound with a 2,4-dienal functional group. Meijboom and Stroink (1972) identified trans-2,cis-4,cis-7-decatrienal as the "fishy off-flavor occurring in strongly auto-oxidized oils containing linolenic or omega-3, 6, 9 etc. fatty acids." This compound, as well as its isomer, are highly contributory to burnt/fishy or cod liver oil-like flavors in oxidized fish or fish oils (Karahadian and Lindsey, 1989), with the trans,trans,cis-isomer contributing more of a "green, fishy" (Karahadian and Lindsey, 1989) or a "sweet, greeny, cucumber and melon-like flavor" (Meijboom and Stroink, 1972).

Because evidence indicates that a complex mixture of 2,4-heptadienals, 2,4-decadienals and 2,4,7-decatrienals together elicit the cod liver oil-like odor in foods (Badings, 1973; Josephson and Lindsay, 1986; Ke et al., 1975; Swoboda and Peers, 1977;) and because trans-2,cis-4,cis-7-decatrienal could not be purchased, no reference compound was chosen. Instead, a fishy-smelling Synura petersenii culture was analyzed for possible identification of the odor-causing compounds since the role of the 2,4-dienals is still unclear and because the fishy-smelling trans-2,cis-4,cis-7-decatrienal has yet to be identified in algae. Identifying such a compound could lead to a suitable reference standard, aiding in the development of treatment methods.

Oxidation with Chlorine, Chlorine Dioxide and Potassium Permanganate

Oxidation: An Overview

This research focused on oxidation for removal of tastes and odors in drinking water because it is commonly used during drinking water treatment. Furthermore, the chemical structures of the organic compounds chosen for this project (aldehyde and alcohol), were considered in that full oxidation would theoretically result in nonvolatile carboxylic acids or carbon dioxide. However, oxidation reactions and their rates depend on numerous factors, including (Bartels et al., 1989) the relative oxidation potentials, the method in which the oxidant attacks, the functional group on the organic molecule, and environmental factors; such as pH, temperature, concentrations of reactants and products, chemical competition, the presence of catalysts and interference by other substances. Even if an odor compound can be oxidized, the kinetics of the reaction may make treatment impossible under typical water-treatment plant conditions. Furthermore, certain oxidants may contribute to the formation of unwanted and even toxic compounds through other reactions. As stated earlier, the oxidants chosen for evaluation during this research included $Cl_{2(aq)}$, ClO_2, and $KMnO_4$.

Chlorine

Chlorine is generally not effective in controlling odors associated with the two most widely researched algal taste-and-odor compounds, geosmin and MIB (Bartels et al., 1989; Lalezary et al., 1986). Results of another study (Bartels et al., 1989) showed likewise that its performance on other taste-and-odor compounds, not necessarily algal, was poor. Bartels et al., citing Weber (1972), stated that "under water treatment conditions, chlorine probably plays a minor role in organic oxidation, and substitution reactions....are favored." Such reactions can lead to the formation of unwanted chlorinated byproducts, such as trihalomethanes or other taste-and-odor compounds such as chloroanisoles, which smell earthy. Bruchet et al. (1992) showed that chlorination of natural nitrogenous organic compounds could produce odors linked to aliphatic aldehydes and other by-products. Sigworth (1957) showed that chlorination and superchlorination were generally unsuccessful in completely solving taste-and-odor problems. It is well-known that chlorine itself can contribute to taste-and-odor problems. "Chlorinous" is the second most frequently observed odor in drinking water (Mallevialle and Suffet, 1987). Nevertheless, there is some evidence that heavy doses of chlorine in water can control some algal tastes-and-odors (Harlock and Dowlin, 1958; Riddick, 1951).

Chlorine Dioxide

Chlorine dioxide, which is superior to chlorine in that it forms fewer chlorinated by-products, is also generally ineffective in controlling earthy/musty tastes and odors (Bartels et al., 1989; Lalezary et al., 1986). Of particular interest to this study were chlorine dioxide's reactions with aldehydes and alcohols. Aliphatic alcohols are resistant to oxidation by chlorine dioxide, even at elevated temperatures, and react only under extreme conditions (Masschelein, 1979; Rav-Acha, 1984; Somsen, 1960). Aldehydes react with ClO_2 at neutral pH and moderate temperatures (Somsen, 1960; Masschelein, 1979); however, the extent to which these reactions occur under water treatment plant conditions is still questionable (Rav-Acha, 1984). For example, Hoigné (1985) and Hoigné and Bader (1982) found that aldehydes cannot react significantly with ClO_2 at dosages commonly used during water treatment because the reaction rate constant is too low under these conditions. Furthermore, aldehydes were found in a sample of ClO_2-treated Ohio River water which suggests that they were not further oxidized to their respective carboxylic acids (Stevens, 1982). Chlorine dioxide treatment has been shown to be successful in controlling fishy odors in at least one case in Canada (Walker et al., 1986). The fishy odors were presumed to be algal metabolites. In contrast, Mallevialle and Suffet (1987), citing Anselme (1986), stated

that treatment with ClO_2 has also produced fishy odors in a few cases.

Potassium Permanganate

Potassium permanganate has been reported to be effective for the removal of some tastes and odors. Again, like chlorine and chlorine dioxide, it is generally ineffective in controlling earthy/musty tastes and odors (Bartels, et al., 1989; Lalezary et al., 1986). This oxidant was shown to be generally ineffective against most of the compounds tested by Bartels et al. (1989), but reacted the most quickly (1.2 h) with the aldehyde. No conclusion could be drawn from this observation. In general, not much lab-scale data associated with taste-and-odor removal by potassium permanganate exists, although the anecdotal evidence is abundant.

Reduction of potassium permanganate leads to the formation of several insoluble manganese oxide species, sometimes of a colloidal nature, which appear to remain stable over long periods of time (Morgan and Stumm, 1964). The precipitate that is generated can adsorb some organics (Colthurst and Singer, 1982), and this may have some implication for taste-and-odor removal.

Sensory Analyses

Threshold Odor Number

In the United States, the threshold odor number (TON) method is routinely used to monitor the organoleptic quality of drinking water (Standard Methods, 1989). The TON is the highest sample dilution with odor-free water which still yields a definitely perceptible odor. Even though the TON method was used to establish secondary maximum contaminant levels, it has many drawbacks: 1) An overall intensity rating is given to the sample rather than individual intensities for each odor component (Mallevialle and Suffet, 1987). 2) This method relies on dilution of a sample, but dilution itself may change the odor characteristics of the sample. The TON, furthermore "corresponds to the dilution required to diminish the concentration of the most readily perceived chemical to a point where it is barely detected" (Mallevialle and Suffet, 1987). This chemical may not be the one which produces the objectionable odor in the undiluted sample. 3) The normal variation in population sensitivity can result in large differences in TON assignment (Mallevialle and Suffet, 1987) because this method uses an open-ended scale.

Because of the drawbacks, a second sensory method, Flavor Profile Analysis (FPA), was developed.

Flavor Profile Analysis

First described by Cairncross and Sjöström (1950), the FPA method was initially developed in the food industry and later adapted for water treatment purposes by the Metropolitan Water District of Southern California (Krasner et al., 1985). Described as proposed Standard Method 2170 in the supplement to Standard Methods (1990), FPA is currently being used by a few large water utilities across the United States and in France (Meng and Suffet, 1992). Its advantages over the TON method are the following (Mallevialle and Suffet, 1987): 1) individual contributors to the overall odor of a sample are given individual intensity ratings, 2) the sample is not diluted 3) a trained panel of at least four members is required and 4) the intensity scale is close-ended and is divided into seven discrete intensities: 1 = threshold, 2 = very slight, 4 = slight, 6 = slight to moderate, 8 = moderate, 10 = moderate to strong, 12 = strong (Krasner et al., 1985). This scale structure results in more consistent data. The FPA method requires considerable time and training, and the list of reference standards is far from complete (Bartels et al., 1986). Furthermore, the method has still not been fully standardized, although a quality assurance/quality control program was described by Meng and Suffet (1992). Despite these problems, the FPA method was chosen for this research because the data obtained are usually more consistent than

those obtained with the TON method. Differences in individual odor sensitivities are moderated because a panel is involved and an average is obtained. Furthermore, the FPA data may be related to data obtained from chemical analyses performed.

Chemical Analyses

Isolation Techniques

Several compound-isolation techniques were reviewed for their applicability to this research and to the selected compounds. The mechanics of each procedure will not be presented here, only the advantages and disadvantages as related to this research.

Purge and trap. Purge and trap has been used successfully for the recovery of certain trihalomethanes (Pankow et al., 1982); however, its use is limited mainly to nonpolar, highly volatile compounds (Durell et al., 1987) of low molecular weight and low water solubility.

Liquid/liquid extraction. Liquid/liquid extraction has the advantage of being a straightforward technique. Furthermore, it recovers compounds over a broad range of molecular weights and polarities (Mallevialle and Suffet, 1987) though it is especially useful for nonpolar compounds. On the other hand, highly volatile compounds are often lost, and the technique requires considerable time and the use of a fume hood because large quantities of extraction solvent are

used. Information regarding its use for the recovery of taste-and-odor compounds of biological origin is scarce.

Steam distillation-extraction. This method has several advantages. It is not time consuming, does not require large amounts of solvent, can be performed under vacuum for the recovery of heat-sensitive compounds, and results in relatively high recoveries of volatile, water-immiscible organic compounds (Parliment, 1986). It is "one of the mainstays in the flavor field" (Parliment, 1986). Second, it has been found to be more effective than closed-loop stripping for compounds with higher molecular weights and higher polarities (Mallevialle, et al., 1984), especially esters, ketones, aldehydes, phenols and alcohols (Mallevialle and Suffet, 1987). The only disadvantages associated with this technique are the poor recovery of certain acids (Janda and Pehal, 1984) and the requirement for hood space.

Closed-loop stripping. Information regarding the use of closed-loop stripping analysis (CLSA) in the field of tastes-and-odors in drinking water is abundant (Grob and Zürcher, 1976; Hwang et al., 1984; McGuire et al., 1981). This technique is suitable for the analysis of compounds of medium-to-high volatility and low-to-medium polarity (Durell et al., 1987). It is not especially time-consuming and requires extremely small volumes of extraction solvent. Above all, it was available for this research.

The CLSA technique does have several disadvantages: It is better suited for nonpolar compounds (Mallevialle and Suffet, 1987), contamination can vary with stripping time (Durell et al., 1987), recoveries can vary with stripping temperature (Mallevialle and Suffet, 1987), addition of salt can improve recoveries but results in clogging of the filter, filter resistance can affect air flow and thus recoveries (Hwang et al., 1984), leakage in the system and adsorptive material in the pumping circuit can result in contamination and high background (Grob and Zürcher, 1976), differences in headspace can result in differences in stripping efficiencies (Mallevialle and Suffet, 1987) and algae samples require more headspace because foaming during stripping is significant, and the foam can contaminate the system.

Although steam distillation-extraction was the preferred isolation method for this research, CLSA was selected primarily because it was available. It was suitable nevertheless for recovering the compounds of interest and has been used widely in taste-and-odor research throughout the world.

Identification Techniques

Gas chromatography coupled with mass spectrometry (GC/MS) is routinely used in taste-and-odor research and is described throughout the literature. Although another method exists, gas chromatography coupled with Fourier Transform Infrared

Spectroscopy (GC/FTIR), a GC/MS was available for this study and was the preferred method.

MATERIALS AND METHODS

The specific methods and materials that were used during this research project are described in this chapter, including the preparation of odorous compounds and the oxidants that were used to treat them, the analyses of the test solutions and the untreated algae culture by gas chromatography and mass spectrometry (GC/MS), and the evaluations of the organoleptic properties of the solutions and treated algae culture after Flavor Profile Analysis (FPA).

Reagents

The following chemicals were used during this study: acetone (CAS# 67-64-1; Fisher Scientific, Fairlawn, NJ), pH 7 buffer solution concentrate (CAS# 7778-77-0, CAS# 1310-73-2, CAS# 7732-18-5; Fisher Scientific), carbon disulfide, 99.99 percent (CAS# 75150; Aldrich Chemical Company, Milwaukee, WI), 1-chlorodecane, 98 percent (CAS# 28519-06-4; ChemService, Westchester, PA), 1-chlorooctane, 99 percent (CAS# 111-85-3; ChemService), 1-chloropentane, 99 percent (CAS# 543-59-9; Aldrich), Chromerge® solution (Fisher Scientific), n-hexanal, 99 percent (CAS# 66-25-1; Aldrich), cis-3-hexenol, 98 percent (CAS# 928-96-1; Aldrich), hydrochloric acid, 36.5-38 percent (CAS# 7647-01-0; Fisher Scientific), methanol (CAS# 67-56-1;

Fisher Scientific), methylene chloride (CAS# 75-09-2; Fisher Scientific), nitric acid, 69-71 percent (CAS# 7697-37-2; Fisher Scientific), nitrogen gas (CAS# 7727-37-9; AIRCO, Murrey Hill, NJ), trans-2,cis-6-nonadienal, 95 percent (CAS# 557-48-2; Aldrich), phenylarsine oxide solution, 0.00564N (CAS# 637-03-6, CAS# 1310-73-2, CAS# 7732-18-5; Fisher Scientific), potassium iodide (CAS# 7681-11-0; Fisher Scientific), potassium permanganate (CAS# 7722-64-7; Fisher Scientific), sodium chlorite flakes (CAS# 7758-19-2; Eastman Kodak Company, Rochester, NY), sodium hypochlorite solution, 5.25 percent (Wonder Bleach, Fairless Hills, PA), anhydrous sodium sulfite (CAS# 7757-83-7; Fisher), and sulfuric acid, 95-98 percent (CAS# 7664-93-9, CAS# 7732-18-5; Fisher Scientific).

Oxidation Experiments--Preparation

Glassware and Teflon-coated materials

All glassware was washed with standard dishwashing detergent and tap water and then cleaned with Fisher Chromerge® solution, which was prepared and used according to the manufacturer's directions. The glassware was then rinsed thoroughly with tap water, Milli-Q (Millipore Corporation, Norwalk, CT) reagent water, and acetone, in that order. It was allowed to dry completely in a laboratory drying oven.

All Teflon-coated materials were washed with standard

dishwashing detergent, rinsed with tap water and then rinsed with Milli-Q water. They were then rinsed several times with acetone and placed in the oven until dry.

Oxidant Stock Solutions

Chlorine. The chlorine ($Cl_{2(aq)}$) stock solution was prepared by diluting a standard household 5.25 percent sodium hypochlorite solution with Milli-Q reagent water to a concentration of 1 milligram per milliliter (mg/mL) as Cl_2. The density of the bleach was assumed to be 1 gram per milliliter (g/mL). The $Cl_{2(aq)}$ stock solution was freshly prepared before each $Cl_{2(aq)}$ oxidation experiment and the titer measured with a Hellige Pocket Comparator and N, N-DIETHYL-p-phenyl-enediamine (DPD) tablets (ORBECO Analytical Systems, Inc., Farmingdale, NY). Titer measurements were based on two dilutions: 1 mL of stock solution in enough Milli-Q water for a total volume of 1000 mL and 4 mL of stock solution in enough Milli-Q water for a total volume of 1000 mL, resulting in 1 milligram per liter (mg/L) and 4 mg/L free chlorine, respectively. After titer measurements, the stock solution was used directly in oxidation experiments. All transfers were made with a bulb pipette with the tip well below the surface of the experimental solution. The pH of the $Cl_{2(aq)}$ stock solution always remained between 5 and 6. The solution pH was checked using 0-13 pH paper test strips (Micro Essential Laboratory, Brooklyn, NY).

Chlorine dioxide. Chlorine dioxide (ClO_2) was generated by a modification of the procedure described in Section 4500B of Standard Methods for the Examination of Water and Wastewater (1989). The modifications, described by White (1986), included pre-cooling the receiving solution and increasing the concentrations of the reactants. The stock solution was adjusted to pH 6 with sodium bicarbonate ($NaHCO_3$) and the solution stored in the refrigerator in a dark-brown, glass bottle with a ground glass stopper. The titer was measured before each experiment according to Section 4500E of Standard Methods (1989) by titration with a 0.00564N phenylarsine oxide solution as the titrant and a Fischer-Porter Model 17T1010 Amperometric Titrator (Warminister, PA). Chlorine, ClO_2, and chlorite ion (ClO_2^-) concentration were measured. The ClO_2 stock solution was generated as necessary to provide a high concentration of ClO_2 and little, if any, excess Cl_2. Inevitably, all of the ClO_2 solutions that were prepared contained excess ClO_2^-. Transfers were made with a bulb pipette, the tip being submerged well below the surface of the experimental solution.

Potassium permanganate. The potassium permanganate ($KMnO_4$) stock solution was prepared by dissolving 1 g of $KMnO_4$ crystals in 1000 mL of Milli-Q reagent water. The stock solution was stored in a capped, dark-brown bottle in the refrigerator. The titer was checked periodically by the

method described in Section 3500E of Standard Methods (1989). All transfers were made by a bulb pipette with the tip submerged well below the surface of the experimental solution.

Organic Compound Stock Solutions

Trans-2,cis-6-nonadienal. The stock solutions were prepared as follows: One mL of methanol was added to a 5.0 mL volumetric flask. Six hundred microliters (uL) of trans-2,cis-6-nonadienal (mol. wt. 138; density = 0.866) were transferred to the flask with a glass syringe and the solvent flush technique. Methanol was then added to adjust the total volume to 5.0 mL. This solution was labeled "trans-2,cis-6-nonadienal stock solution #1." One milliliter of methanol was then added to a separate 5.0 mL volumetric flask. Next, five hundred microliters of nonadienal stock solution #1 were transferred to this flask with a glass syringe and the solvent flush technique. The volume was then adjusted to 5.0 mL with methanol. This solution was labeled "trans-2,cis-6-nonadienal stock solution #2" and was the standard working solution for all oxidation experiments. The resulting concentration was 9.9 micrograms per microliter (ug/uL). Initially, acetone was used as the stock solution solvent but was later found to contribute to the formation of an odorous, chlorinated by-product that interfered with the organoleptic evaluations of the treated solutions.

Cis-3-hexenol. The cis-3-hexenol (mol. wt. 100; density

= 0.846) stock solution was made in exactly the same manner as nonadienal stock solution #1. This solution was labeled "cis-3-hexenol stock solution" and was the standard working solution for all oxidation experiments. The resulting concentration was 100 ug/uL.

Other Materials

Other materials included Teflon-coated magnetic stirring bars, one-liter CLSA glass bottles with tapered, ground-glass stoppers, Teflon tape, magnetic-stirrer plates, aluminum foil, and Milli-Q reagent water.

Oxidation Experiments--General Procedure

Trans-2,cis-6-nonadienal Experiments

Milli-Q water was buffered with the pH 7 concentrate according to directions to make 1.0-L of solution. For each experiment, 1.0-L of buffered solution was placed in an aluminum foil-covered CLSA bottle containing a Teflon-coated stirring bar. Two uL of trans-2,cis-6-nonadienal stock solution #2 were added to the buffered solution with a glass syringe by flushing the compound volume with an additional 0.2 uL of methanol; the tip of the needle was submerged below the surface of the solution during transfer. The appropriate amount of oxidant was added with a bulb pipette. The tip of the pipette was maintained below the surface of the solution during transfer. The bottle was quickly stoppered, sealed with

Teflon tape, placed on a magnetic-stirrer plate and allowed to stir at a moderate setting for one hour. Chlorine and ClO_2 residuals were determined and recorded, and the treated solutions were then dosed with a predetermined amount of Na_2SO_3 to quench the oxidant residuals. Color changes, if any, were noted during experiments with $KMnO_4$ solutions, and the permanganate residuals were quenched with Na_2SO_3 to produce manganese dioxide (MnO_2) which is yellow-brown in color. After reduction of the residual oxidant, each sample was either stripped by the CLSA technique and analyzed by GC/MS or used for FPA.

Cis-3-hexenol Experiments

These experiments were conducted in exactly the same manner as the trans-2,cis-6-nonadienal experiments with only a modification in cis-3-hexenol dosage. Each cis-3-hexenol experiment required 5 uL of stock solution.

Residual Measurements

Chlorine residuals were measured with a Hellige Pocket Comparator and DPD tablets. Chlorine dioxide residuals were measured by amperometric titration in the manner previously described. Potassium permanganate residuals were not determined, but color changes were noted. All residuals were reduced with a predetermined amount of Na_2SO_3.

Oxidation Experiments--Special Procedures

All Experiments

As mentioned previously, the CLSA bottles were covered with aluminum foil to prevent any interference from ultraviolet light. Furthermore, the buffered Milli-Q water was analyzed beforehand to determine if it had any considerable oxidant demand.

Chlorine Dioxide Experiments

The ClO_2 experiments were conducted in duplicate.

Oxidation Experiments--Quality Assurance/Control

For every experimental sample, a blank containing a proportional amount of methanol was oxidized under the same conditions as the odor compound and either stripped by the CLSA technique or used as a blank for FPA evaluation.

Algae Experiments

The algae experiments consisted of two parts: 1) stripping 90 mL and 900 mL, respectively, of a two-week-old, fishy-smelling Synura petersenii culture (including cells) with a CLSA and analyzing the extract by gas chromatography/mass spectrometry, and 2) evaluating oxidized ($Cl_{2(aq)}$, ClO_2, and $KMnO_4$) algae samples and unused medium by FPA. The alga was obtained from the Virginia Tech Biology Department. The medium (DYIII) (Lehman, 1976) also was

Table 4. Medium ingredients for Synura culture

Ingredient	Concentration in mg/L
$CaCl_2 + 2 H_2O$	75
$NaSiO_3 + 9 H_2O$	15
$MgSO_4 + 7 H_2O$	50
NH_4NO_3	5
$NaNO_3$	20
KCl	3
$Na_2EDTA + 2 H_2O$	8
$FeCl_3 + 6 H_2O$	2.4
$MnCl_2 + 4 H_2O$	0.72
$ZnSO_4 + 7 H_2O$	0.18
$NaMoO_4 + 2 H_2O$	0.05
$CoCl_2 + 6 H_2O$	0.03
H_3BO_3	4.56
Biotin	0.0005
Thiamine	0.2
Vitamin B_{12}	0.0005
Sodium glycerophosphate	10
2-[N-Morpholino]ethane Sulfonic Acid	200

Adapted from Lehman, 1976.

stripped by the CLSA technique to obtain control data. The medium formulation is listed in Table 4.

Dosages of the oxidants during these experiments were necessarily greater than those applied during previous experiments, because the oxidant demand of the medium was quite large. An oxidant concentration of 10 mg/L was used for each sample and was based on the $KMnO_4$ dose necessary to maintain a pink color after one hour. No residual measurements were made.

Closed Loop Stripping

Preparation

Glassware. All glassware, excluding pieces with metal attachments, were cleaned as stated previously. Parts with metal attachments were rinsed thoroughly several times with acetone and allowed to dry in a laboratory oven.

Filters. The 1.5 mg activated carbon filter (Tekmar, Cincinnati, OH) was cleaned before stripping by passing several filter-holder volumes of acetone, methylene chloride and carbon disulfide (CS_2) through it, in that order. The time required to empty the last CS_2 rinse was noted. Filters with flow rates lower than 0.75 milliliter per minute (mL/min) were not used. Filters were cleaned after extraction by passing several filter-holder volumes of CS_2 through them. They were dried at low heat in a laboratory drying oven

several minutes, then allowed to cool. Filters not used immediately were wrapped in aluminum foil and stored for future use. Filters used for pre-stripping, or auxiliary filters, were cleaned once a week. Occasionally, each filter was cleaned with 1N nitric acid (HNO_3), followed by several filter-holder volumes of Milli-Q reagent water and solvent (acetone, methylene chloride and carbon disulfide, in that order) by the procedure stated previously.

Teflon Materials. All Teflon connector sleeves, O-ring covers, and vial caps were rinsed thoroughly with acetone and placed in the laboratory oven until dry.

External Standards. The use of external and internal standards for quantification purposes was initiated but later abandoned; however, chloroalkane standards were routinely added to extracts to maintain extract solution composition throughout all of the experiments. The external standard for trans-2,cis-6-nonadienal consisted of 350 ng/uL each of 1-chlorooctane and 1-chlorodecane in acetone. The external standard for cis-3-hexenol consisted of 350 ng/uL each of 1-chloropentane and 1-chlorooctane in acetone. For the trans-2, cis-6-nonadienal standard, 4.0 uL chlorooctane and 4.1 uL chlorodecane were injected into approximately one mL of acetone in a 10 mL volumetric flask. The total volume was adjusted to 10 mL. The standard for cis-3-hexenol was prepared in the same manner by dilution of 4.0 uL 1-

chloropentane and 4.0 uL 1-chlorooctane in acetone.

General Procedure

A Brechbühler AG CLSA system (Schlieren, Switzerland) equipped with a Model MB-21 bellows pump (Metal Bellows Corp., Sharon, Mass.) was used for all stripping procedures. An auxiliary filter was placed in the proper location in the CLSA. The sparger was placed into the CLSA bottle containing either the water sample from the oxidation experiment or the algae samples, and the bottle was then submerged into the preheated (30°C) water bath. All joints were connected, after which the trap heater was turned on and allowed to reach its set temperature of 45°C. The sample was stripped for 10 seconds (s), the auxiliary filter was removed, and the actual filter put into place. After all joints were again connected, the trap heater was allowed to reach 45°C, and the sample was stripped for 1.5 hours (hr). After this time, the filter was removed and allowed to cool for 30 s. During this time, approximately 1 uL of CS_2 was placed in the 50-uL glass sample vial with a glass syringe, and the filter was then connected to the sample vial with the Teflon sleeve, without dead space between the glass filter holder and the vial. The filter was extracted by placing 5 uL of CS_2 above the filter and alternately separating and reconnecting the filter and vial ten times while it was still within the Teflon sleeve. The

vial was then cooled with ice while it remained tightly connected to the filter holder within the Teflon sleeve. By doing so, the CS_2 was drawn below the carbon filter. The filter/vial assembly was shaken lightly to completely transfer the CS_2 extract to the sample vial. The whole procedure was repeated two more times, resulting in a final extract of approximately 10 uL. For the trans-2,cis-6-nonadienal and cis-3-hexenol experiments, 2 uL of external standard were added, resulting in a total volume of 12 uL; however, calculations based on these standards were later abandoned. The total volume of the extracts from the algae experiments was 10 uL because no standards were used. All extracts were analyzed immediately to reduce the likelihood of change in volatile composition due to storage.

Quality Assurance/Quality Control

As stated previously, a blank containing a proportional amount of methanol was oxidized under the same conditions as the odor compound for every experiment. These blanks were stripped in the same manner as the oxidized, odor-compound solutions. All removable CLSA glass parts were cleaned after each sample run. All glass/metal connections were cleaned once a week.

Gas Chromatography/Mass Spectrometry

Preparation and Materials

All extracts were analyzed with a Hewlett Packard 5890 Series II Gas Chromatograph (Avondale, PA) equipped with a Hewlett Packard 5970 Series Mass Selective Detector (Avondale, PA). A J & W Scientific (Folsom, CA) DB-5 phase, fused silica capillary column was used in all experiments. Column dimensions were 30m length x 0.25mm inside diameter, with a film thickness of 0.25 um.

General Procedure

The operational conditions during the GC/MS analyses were as follows:

Carrier gas: Helium

Linear velocity on the column: 29 centimeters/second (cm/s)

Sample size: 2.0 uL, splitless, vent opened at 1.00 min

Injector temperature: 220° C

Detector Temperature: 280° C

Source Pressure: 4 x 10^{-5} torr or less

Electron Energy: 70 eV

Mass range scanned: 40 to 450 atomic mass units (amu)

Scan Time: 1.05 scans/s

The temperature programming differed according to the compound or substance being analyzed. During analyses of the trans-2,cis-6-nonadienal solutions, the following parameters were used:

Initial temperature: 30°C Hold 2 min.

Ramp 1: 30°C - 110°C at 5°C/min

Ramp 2: 110° - 115°C at 2°C/min

Ramp 3: 115° - 250°C at 5°C/min

During analyses of the cis-3-hexenol solutions, the parameters were as follows:

Initial temperature: 30°C Hold 2 min.

Ramp 1: 30°C - 250°C at 5°C/min Hold 4 min.

The temperature conditions during analyses of the algae samples were as follows:

Initial temperature: 25°C Hold 2 min.

Ramp 1: 25°C - 280°C at 5°/min Hold 2 min.

Compound identification. Relative retention times and mass spectra were used to identify compounds. An identification was confirmed if both the relative retention time and the mass spectrum of an unknown compound matched the relative retention time and mass spectrum of the purchased known compound using the same temperature program. Confident identifications were based on comparison with known fragmentation patterns and/or mass spectral data from the NBS/Wiley Mass Spectral Library (1989) or literature sources.

Method Detection Limits

Trans-2,cis-6-nonadienal. Method detection limits were determined by dosing one liter of buffered water under the same conditions as in the oxidation experiments with

consecutively lower amounts of trans-2,cis-6-nonadienal, stripping the solution, and analyzing the extracts. Duplicates at each concentration were analyzed. If characteristic mass/charge peaks 69 and 70 were detected in one or both of the duplicates, a lower dosage was used. If these peaks were not detected in one of the duplicates, a third replicate was examined and a lower dosage was used upon detection of both of the peaks. The method detection limit, 250 ng/L, was the lowest dosage at which mass/charge peaks 69 and 70 were detected. The next lowest dosage examined was 200 ng/L.

Cis-3-hexenol. Method detection limits were determined in the same manner as those for trans-2,cis-6-nonadienal. The simultaneous presence of characteristic mass/charge peaks 67 and 69 was used to confirm the detection of cis-3-hexenol, and the method detection limit was 12 ug/L. The next lowest dosage examined was 10 ug/L.

Flavor Profile Analysis (FPA)--Advanced Preparation

Screening of Potential Panel Members

Fifteen students at Virginia Tech were initially screened as candidates for the Flavor Profile Analysis panel. The screening consisted of three parts: (1) odor recognition, (2) intensity rating, and (3) two triangle tests. Each of these

elements of the screening procedure is described in the following paragraphs.

Preparation. All candidates were notified of the screening one week in advance and were asked to refrain from using any perfume, hair-spray or other odorous materials, to refrain from eating, drinking or smoking for at least 30 minutes prior to test time, to report any illnesses or allergies, and to wash their hands immediately before test time with an odorless soap which was provided. Each panelist was given a test sheet in the screening room and asked to include his/her name, age, sex, the time, and whether or not he or she had refrained from eating, drinking, smoking and the use of odorous materials. Odor-free water was available between samples and the panelists were asked to wait several minutes between the evaluation of each sample to avoid fatigue of the sense of smell.

Odor recognition. The odor recognition test consisted of ten samples: two of two different reference compounds prepared at readily perceived strengths, five of common household goods, duplicate tap water samples and one Milli-Q water sample. The reference compounds were trans-2-nonenal ("cucumber, oily") and n-heptanal ("rancid walnut oil"). The common household goods used were soap, cloves, orange extract, peppermint extract and cod liver oil. All samples were in aluminum foil-covered Erlenmeyer flasks to prevent visual

recognition. The odor-panel candidates were asked to identify each sample to the best of his or her knowledge without consulting other candidates.

Intensity rating. Six samples were evaluated during this part of the screening. Two reference compounds, n-hexanal and n-heptanal, were prepared at three different concentrations each, all at above threshold levels. The candidates were asked to smell each sample and to rate them from one to three in order of intensity and from zero to two if no odor was perceptible in one of the samples.

Triangle tests. The two triangle tests were conducted with three samples each, two of which contained the same material. The first test included two clove samples and one 2,4,6-trichloroanisole sample. The second test consisted of two cis-3-hexenol samples and one trans-2,cis-6-nonadienal sample. The candidates were asked to choose which of the samples did not belong in each of the two groups.

Final panelists. Panelists were selected on the basis of their performance, degree of motivation, and availablity. In rating panelist performance, the order of sampling was noted in order to account for any change in concentration over time during the screening. Care was taken to avoid the inclusion of individuals with either dominant or passive personalities.

Compound dosage. A major objective of this research was to determine the effects of $Cl_{2(aq)}$, ClO_2, and $KMnO_4$ on selected

odor-causing compounds. Oxidant dosages were to be representative of those applied during water treatment, while the concentrations of odor-causing compounds were arbitrarily selected to be those that evoked a response of 6 on the odor-intensity scale when the test solutions were evaluated by the FPA panel. While the concentration of trans-2,cis-6-nonadienal required to produce the intensity level was only 20 ug/L the required cis-3-hexenol concentration ranged from 2 to 5 mg/L, which was much greater than could be reasonably added to test solutions if the oxidant concentrations were to be restricted to reasonable levels. Thus, the concentration was reduced to 500 ug/L, but that solution elicited only a 4 (slight odor) response from the FPA panelists, which was not unexpected because sensory responses have been shown to be related by the Weber-Fechner Law (Wright, 1982) to the logarithm of odorant concentrations of those substances. Reducing the FPA intensity of the test solution was deemed more acceptable an option than increasing the oxidant dosages to extremely high levels. Lower concentrations of cis-3-hexenol were not used because descriptor identification (grassy/green apples) at these levels proved to be difficult. These data are presented later in Chapter 4. The concentrations were not necessarily representative of actual concentrations in raw water during odor episodes because, in most cases, these are not known.

Training of Panelists

At least half of the panel members attended a training workshop conducted at Virgina Tech by Gary Burlingame of the Philadelphia Water Department, Philadelphia, PA. This workshop was based on a training manual being developed for the American Water Works Association (AWWA) but not yet in print. All panelists underwent additional training regarding the numeric scale of intensity, which is based on evaluations of sugar solutions at six different concentrations and the relationship between the taste response to these standards and the odor responses to solutions containing odoriferous substances.

FPA--General Procedure

The FPA was conducted according to proposed method 2170 in Standard Methods (1991). The temperature of the samples was 45°C. Published threshold odor concentrations for trans-2, cis-6-nonadienal and cis-3-hexenol in water are 13 ng/L (Devos, et al., 1990) and 70 ug/L (Stahl, 1973), respectively, which are the concentrations at which the odors are barely perceptible. No samples were analyzed for their taste intensity. A few modifications of the method were made:
1) All glassware was cleaned by the procedure previously described glassware used during the oxidation experiments. After each flask was oven-dried, 200 mL of Evian (Evian,

France) bottled water was heated to boiling as described in Standard Methods. This rendered the glassware odor-free.
2) Bottled water was used for odor-free water as described in the AWWA Training Manual; however, all samples of the odoriferous materials were prepared in Milli-Q reagent water. Milli-Q water was included in the FPA evaluations in order to account for any odor contribution it might make to the sample.

FPA--Quality Assurance/Control

Odor-free samples and duplicates were used occasionally as according to the proposed Standard Method 2170. As stated previously, blanks (containing an amount of methanol proportional to the amount added to samples when the odor compound dissolved in methanol was added to the samples) were oxidized under the same conditions as the solutions of odor compounds. These controls were evaluated by the FPA panel.

FPA--Data Analyses

FPA data were calculated according to the procedure described in proposed Standard Method 2170. Averages of the odor intensity were calculated if at least 50 percent of the panelists agreed on a given descriptor. An intensity rating of zero was assigned if a panelist did not agree on a given description. Descriptors for which there was less than 50 percent panelist agreement were listed as "other notes" and

were listed separately and not included in group results. In this case, an average intensity was calculated by dividing the sum of all intensity ratings by the number of ratings. The quotient was recorded with the descriptor, "no consensus."

RESULTS

This chapter presents results divided into two categories: 1) the GC/MS and FPA results obtained from the oxidation of the pure compounds, trans-2,cis-6-nonadienal and cis-3-hexenol and the FPA results obtained from the oxidation of the Synura petersenii culture, and 2) the GC/MS results from the CLSA of the untreated Synura petersenii culture.

Oxidation Experiments

Figure 3 displays the graph that was the basis for selection of the odor compound concentration. As stated previously, initial concentrations were selected on the basis of an average response value of 6 from the FPA panel. However, the cis-3-hexenol concentration was reduced to evoke a 4 response. Note from Figure 3 the differences in odor intensities at the various compound concentrations. Note also the typical plant-level molar concentrations of the oxidants in relationship to the concentrations of the two odor compounds.

Typical mass spectra of the two organic compounds used in the study--trans-2,cis-6-nonadienal and cis-3-hexenol--are shown in Figures 4 and 5, respectively. These spectra were obtained from the CLSA and GC/MS analyses of the unoxidized

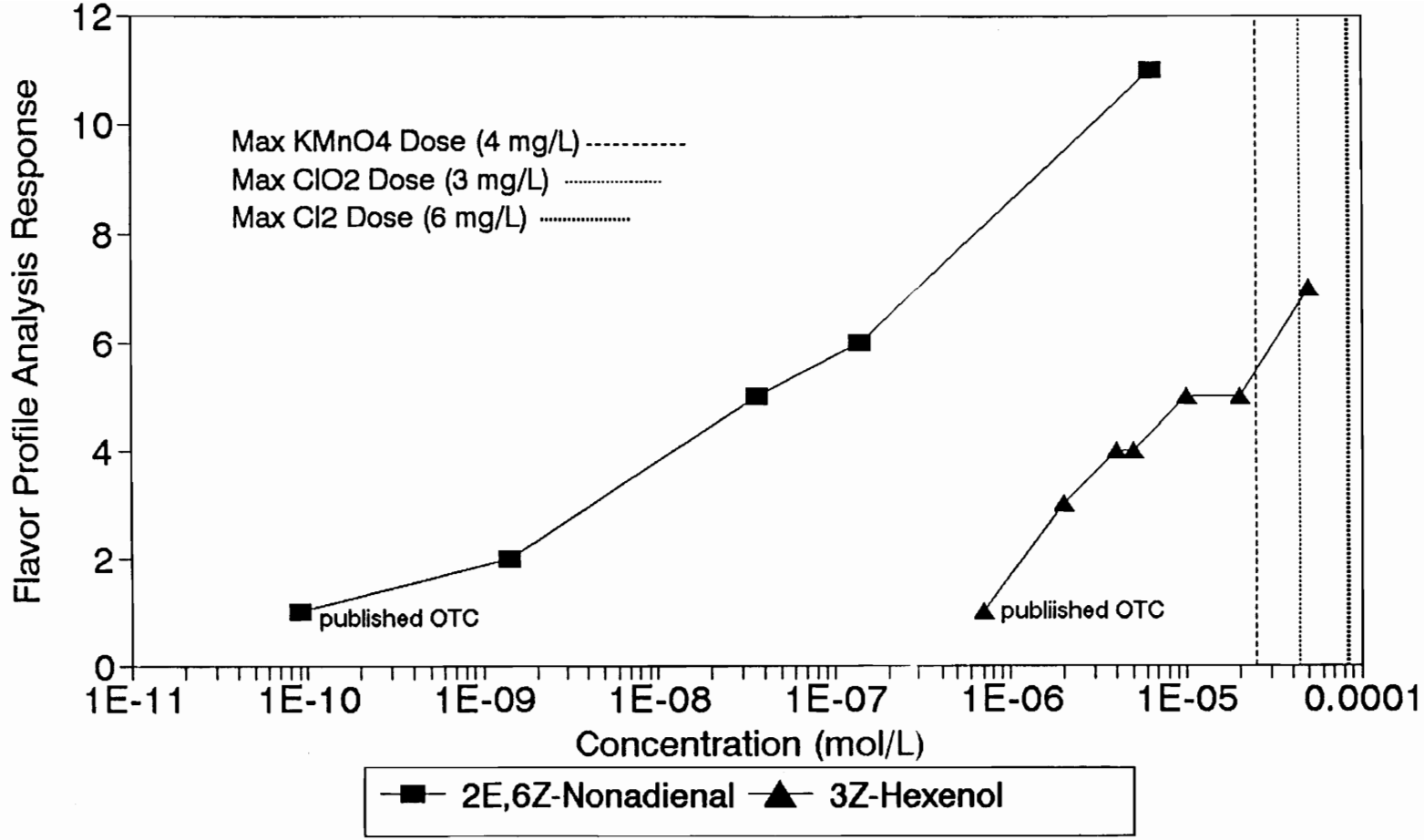


Figure 3. Weber-Fechner relationship between FPA response and concentrations of trans-2,cis-6-nonadienal and cis-3-hexenol, respectively. Oxidant dosages are shown in comparison.
(a. OTC = Odor Threshold Concentration)

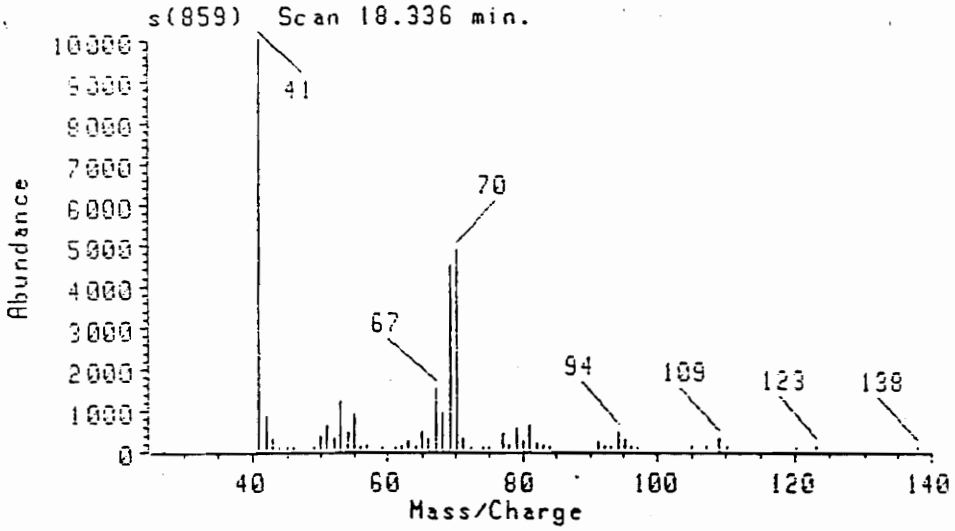


Figure 4. Typical mass spectrum of trans-2, cis-6-nonadienal

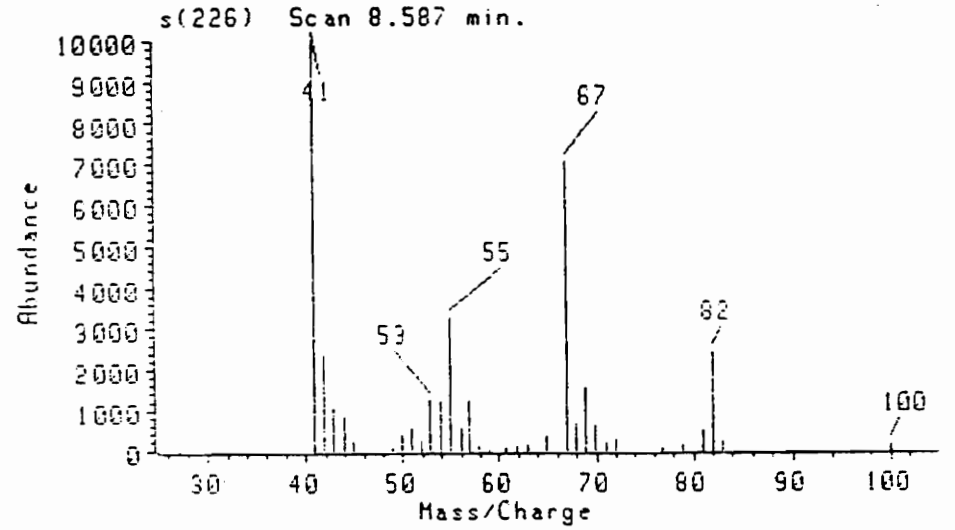


Figure 5. Typical mass spectrum of cis-3-hexenol

test solutions. The original concentrations in the solutions were 20 ug/L trans-2, cis-6-nonadienal and 500 ug/L cis-3-hexenol.

Oxidation of trans-2, cis-6-nonadienal

Table 5 summarizes the effects of $Cl_{2(aq)}$, ClO_2 and $KMnO_4$ on trans-2, cis-6-nonadienal. Figure 6 shows the effects of $Cl_{2(aq)}$ on the odor characteristics of trans-2,cis-6-nonadienal (20 ug/L, pH 7). Note the marked decrease in odor intensity after oxidation with 2 mg/L free chlorine. Also note that the cucumber odor was not detected at this level and that odor-free was the descriptor used by the FPA panel. Table 5 shows that the panelists were able to detect the cucumber odor at concentrations below 250 ng/L, which was the method detection limit (MDL) for trans-2, cis-6-nonadienal during this research.

Chlorine dioxide, even at a relatively high concentration of 3 mg/L, did not decrease the odor intensity of trans-2,cis-6-nonadienal (Figure 7), but the FPA panel descriptor changed slightly from cucumber in the control to cucumber/fruit rind in the treated sample (Figure 7). The slightly greater intensity after oxidation is an artifact of the average of the individual responses. The compound was detected by GC/MS at levels higher than 250 ng/L (Table 5), which is far above the threshold odor concentration.

Potassium permanganate markedly reduced the average

Table 5: Effects of oxidants on the odor characteristics of trans-2,cis-6-nonadienal[a]

Oxidant	Oxidant Dose (mg/L)	Oxidant Residual (mg/L)[b]	Detected by GC/MS	FPA Descriptor and Average Intensity[c]
none	----	----	yes	cucumber 5.0
$Cl_{2(aq)}$	1	0.8	no	cucumber/melon 2.3
$Cl_{2(aq)}$	2	2	no	odor-free
ClO_2	3	0.8-1.0	yes	cucumber/fruit rind 6
$KMnO_4$	0.25	----[d]	no	cucumber 1.2
$KMnO_4$	0.5	----[d]	no	odor-free
$KMnO_4$	1	----[d]	no	odor-free sweet 0.75

[a] The trans-2, cis-6-nonadienal dose was 20 ug per liter of pH 7 buffered Milli-Q reagent water. The method detection limit for this compound was 250 ng/L. The published threshold odor concentration is 13 ng/L (Devos et. al, 1990).

[b] Residuals were measured after a retention time of one hour and subsequently quenched with Na_2SO_3.

[c] Data were calculated according to proposed Standard Method 2170 (Standard Methods, 1990). For raw data including "other notes" see Appendix Table A-1.

[d] All $KMnO_4$ solutions remained pink after one hour. No residual measurements were made.

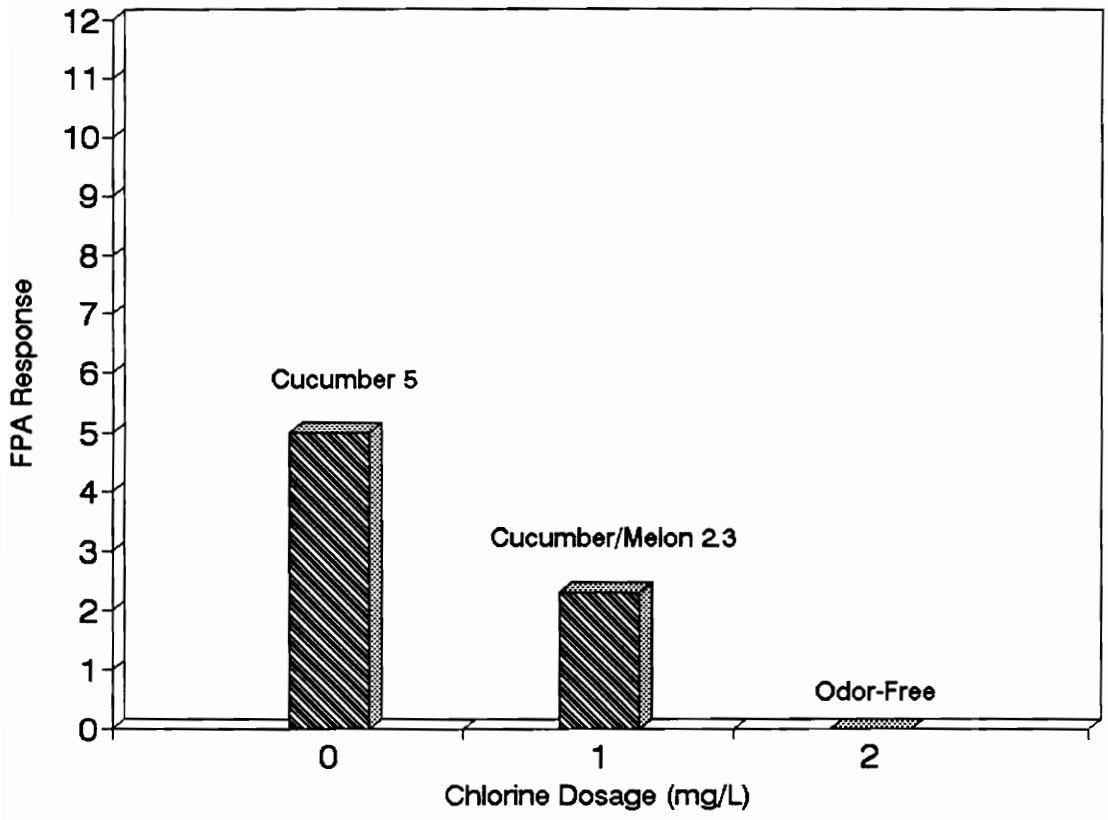


Figure 6. The effect of chlorine on the odor characteristics of trans-2, cis-6-nonadienal (20 ug/L) in water at pH 7.

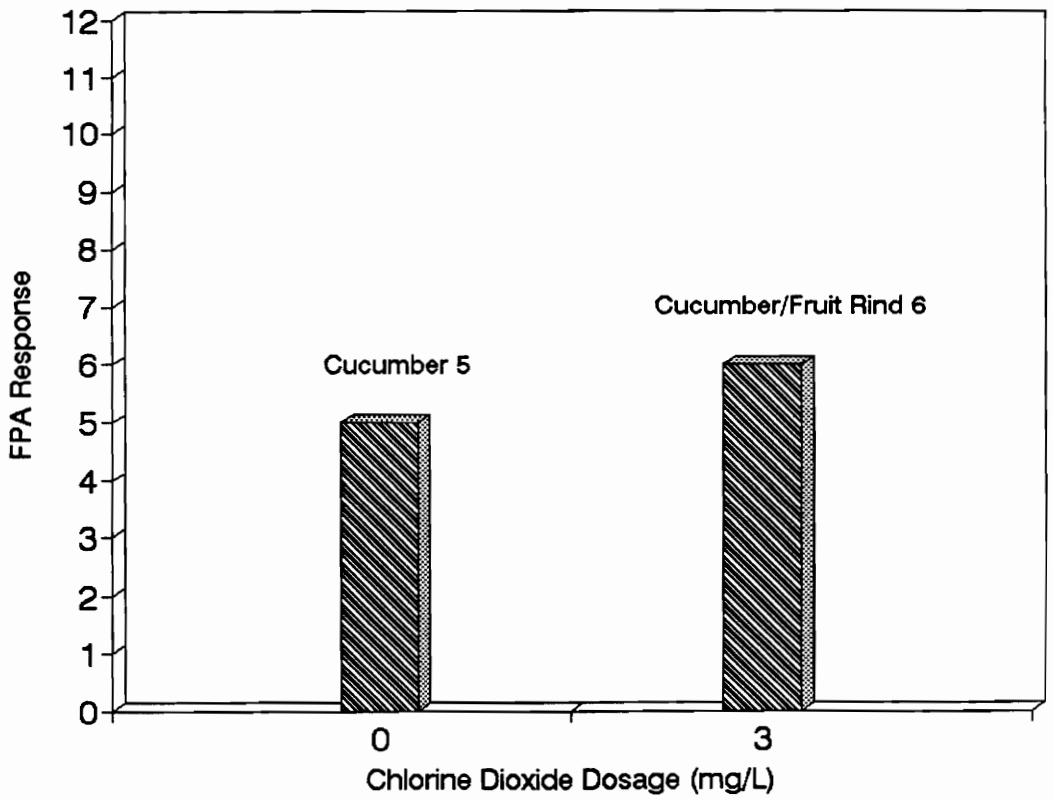


Figure 7. The effect of chlorine dioxide on the odor characteristics of trans-2, cis-6-nonadienal (20 ug/L) in water at pH 7.

cucumber-odor intensity from 5 in the control to 1.2 with only 0.25 mg/L $KMnO_4$ (Figure 8). The FPA panelists were able to detect the compound at concentrations below the MDL (Table 5). Although 2 mg/L free chlorine was required to render the trans-2,cis-6-nonadienal sample odor-free according to the FPA panel, the same effect was observed when only 0.5 mg/L $KMnO_4$ was added. When the samples treated with 1 mg/L $KMnO_4$ were evaluated by the panel, the descriptors were equally divided between odor-free and sweet.

Table 6 shows the sensory results obtained from the evaluation of the oxidized control samples. Each control contained the same amount of methanol as was present in the odor compound dose subjected to oxidation. Note that the FPA panel detected few, if any, odors in the controls, and those that were detected were described either as unidentified or stale. Note the extremely low intensity ratings assigned to each control.

Oxidation of cis-3-hexenol

The effects of chlorine, chlorine dioxide, and potassium permanganate on the FPA profile of cis-3-hexenol are shown in Table 7. Note that the concentration of cis-3-hexenol required to produce an FPA intensity ratio of only 3.6 was 500 ug/L, whereas the concentration of trans-2,cis-6-nonadienal required to produce a rating of 5 was only 20 ug/L (Table 5).

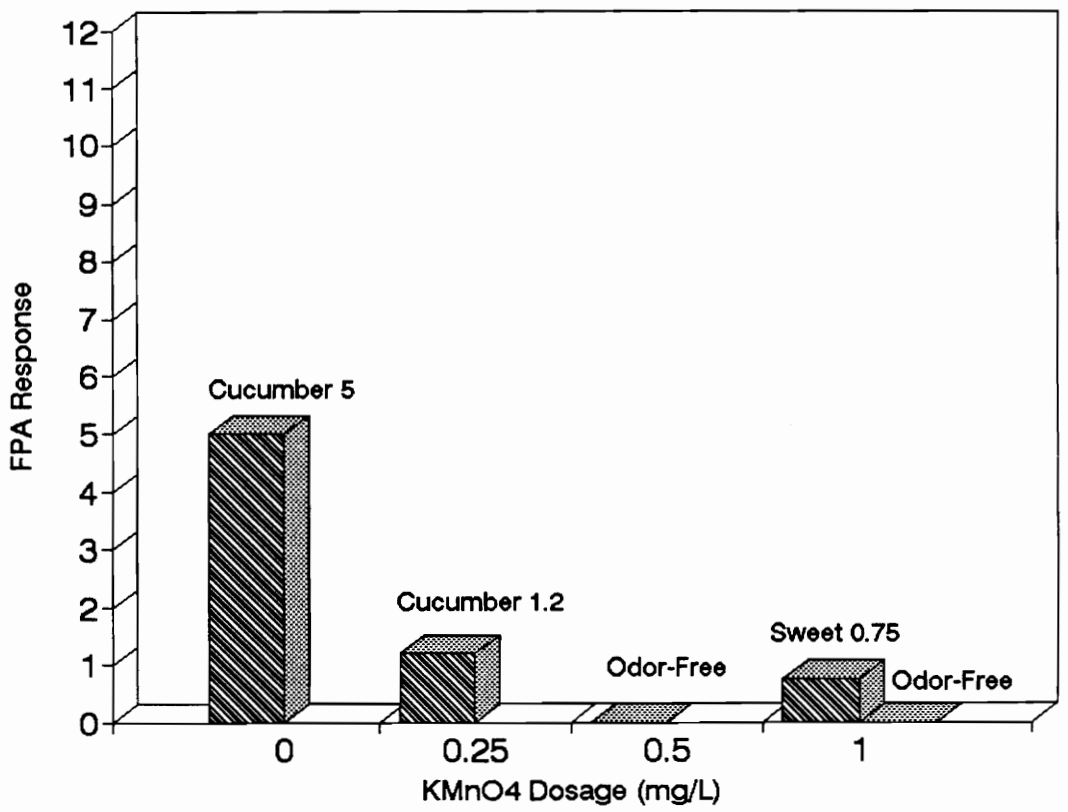


Figure 8. The effect of potassium permanganate on the odor characteristics of trans-2, cis-6-nonadienal (20 ug/L) in water at pH 7.

Table 6: Effects of oxidants on the odor characteristics of the methanol control[a] used during trans-2,cis-6-nonadienal experiments

Oxidant	Oxidant Dose (mg/L)	Oxidant Residual (mg/L)[b]	FPA Descriptor and Average Intensity[c]
none	----	----	unidentified 0.75
$Cl_{2(aq)}$	1	0.8	no consensus
$Cl_{2(aq)}$	2	2	unidentified 1
ClO_2	3	0.9-1	odor-free
$KMnO_4$	0.25	----[d]	odor-free
$KMnO_4$	0.5	----[d]	odor-free
$KMnO_4$	1	----[d]	odor-free stale 1

[a] The control consisted of 2.2 uL methanol per liter of pH 7 buffered Milli-Q reagent water.

[b] Residuals were measured after a retention time of one hour and subsequently quenched with Na_2SO_3.

[c] Data were calculated according to proposed Standard Method 2170 (Standard Methods, 1990). For raw data including "other notes" see Appendix Table A-2.

[d] All $KMnO_4$ solutions remained pink after one hour. No residual measurements were made.

Table 7: Effects of oxidants on the odor characteristics of cis-3-hexenol[a]

Oxidant	Oxidant Dose (mg/L)	Oxidant Residual (mg/L)[b]	Detected by GC/MS	FPA Descriptor and Average Intensity[c]
none	----	----	yes	grassy/green apples 3.6
Cl_2	3	0	yes	no consensus
Cl_2	4	0	yes	no consensus
Cl_2	6	2	no	chemical 1.7
ClO_2	3	2	yes	fruity/grassy 3
$KMnO_4$	2	----[d]	yes	sweet 0.5
$KMnO_4$	3	----[d]	no	no consensus
$KMnO_4$	4	----[d]	no	no consensus

[a] The cis-3-hexenol dose was 500 ug per liter of pH 7 buffered Milli-Q reagent water. The method detection limit for this compound was 12 ug/L. The published threshold odor concentration is 70 ug/L (Stahl, 1973).

[b] Residuals were measured after a retention time of one hour and subsequently quenched with Na_2SO_3.

[c] Data were calculated according to proposed Standard Method 2170 (Standard Methods, 1990). For raw data including "other notes" see Appendix Table A-3.

[d] All $KMnO_4$ solutions remained pink after one hour. No residual measurements were made.

Table 7 shows also that the panel could not agree on an odor descriptor for several samples. Figure 9 shows the effects of $Cl_{2(aq)}$ on the odor characteristics and intensity of cis-3-hexenol. A high chlorine dose was required to reduce 500 ug/L of cis-3-hexenol to below the MDL (12 ug/L). Notice from Table 7 that no $Cl_{2(aq)}$ residual was present after one hour when the dosages were 3 and 4 mg/L, and the FPA panel reached no consensus regarding the odor descriptors. The FPA descriptor changed from "grassy/green apples" in the control to "chemical" when 6 mg/L chlorine was added (Table 7). Even though the $Cl_{2(aq)}$ residual was 2 mg/L, the descriptor "chlorinous" was not used by the panel.

Table 7 and Figure 10 show that ClO_2 at a dosage of 3 mg/L was ineffective against cis-3-hexenol at pH 7. The descriptor, however, changed from grassy/green apples to fruity/grassy.

In contrast to ClO_2, $KMnO_4$ was quite effective against the compound (Figure 11). The average intensity rating was reduced from 3.6 in the control to 0.5 in the sample treated with 2 mg/L $KMnO_4$, although the compound was still detected by GC/MS. The FPA panel was unable to reach a consensus regarding the odor of samples treated with greater concentrations of the oxidant.

Table 8 shows the panel evaluations of the methanol

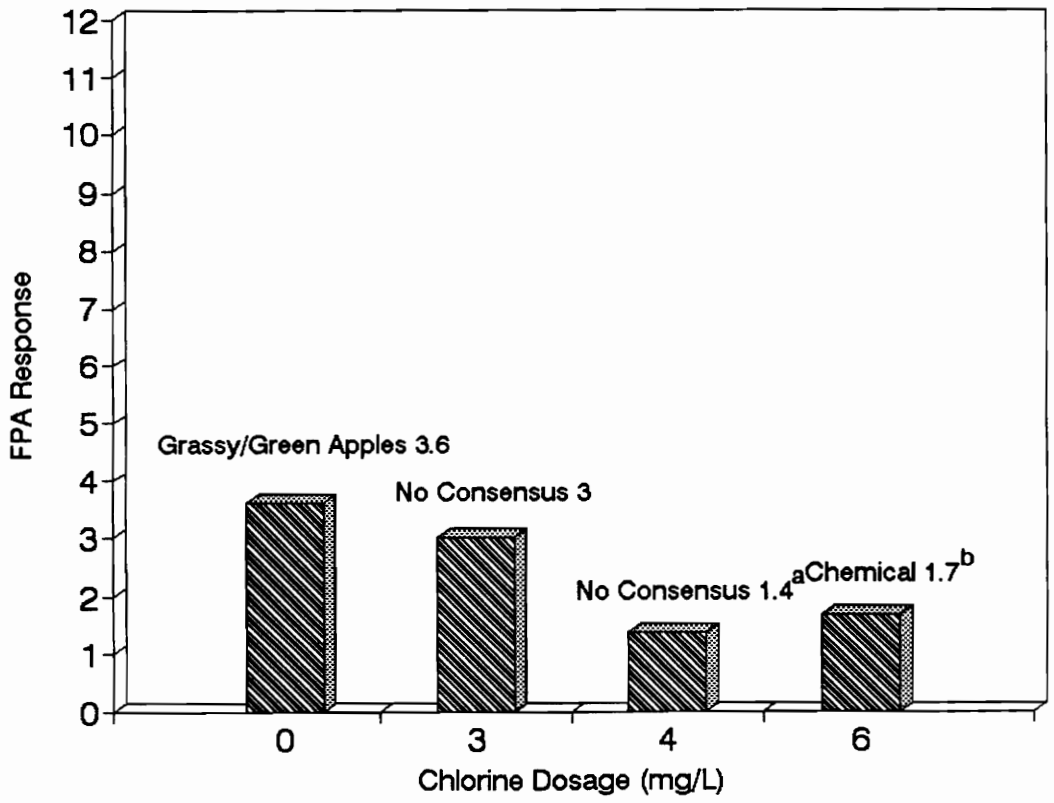


Figure 9. The effect of chlorine on the odor characteristics of cis-3-hexenol (500 ug/L) in water at pH 7. (a. The intensity rating, 3, is the average of the individual responses: odor-free 0, chlorinous 4, chlorinous 2, chemical 4, chemical 4, fruity/sweet 4, fruity/sweet 4, old hay 2. b. The intensity rating, 1.4, is the average of the individual responses: odor-free 0, odor-free 0, pungent 2, hay 1, earthy 1, chemical 1, sweet 4, chalky 2.)

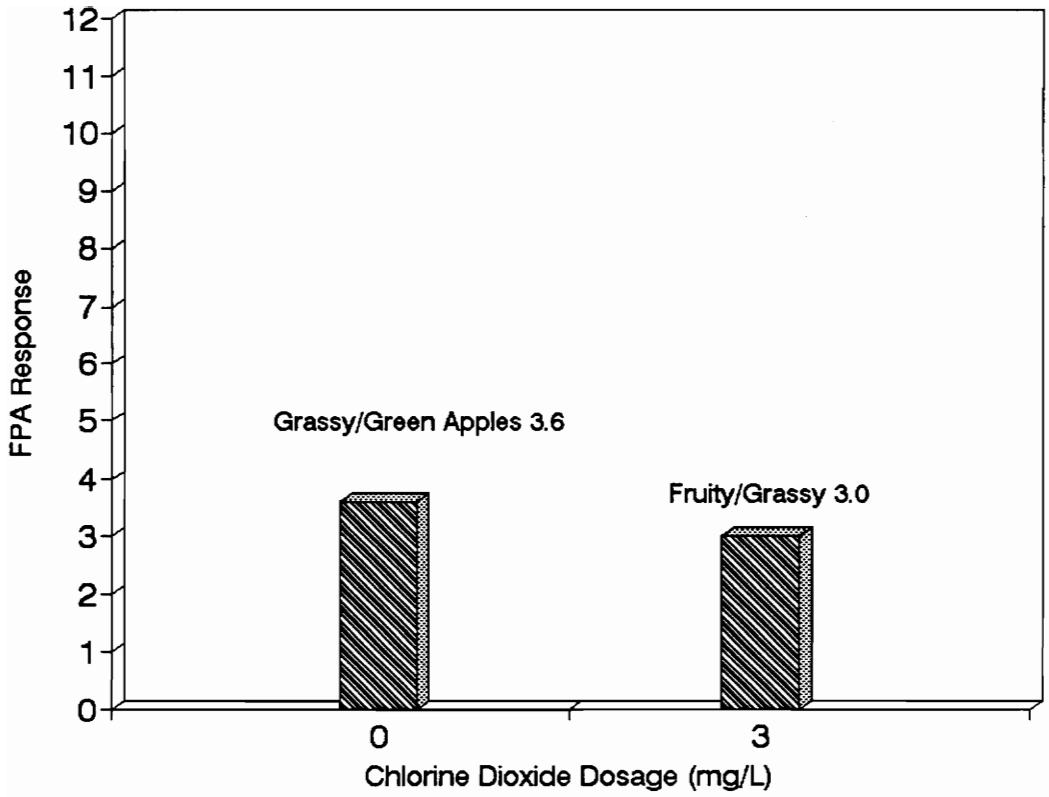


Figure 10. The effect of chlorine dioxide on the odor characteristics of cis-3-hexenol (500 ug/L) in water at pH 7.

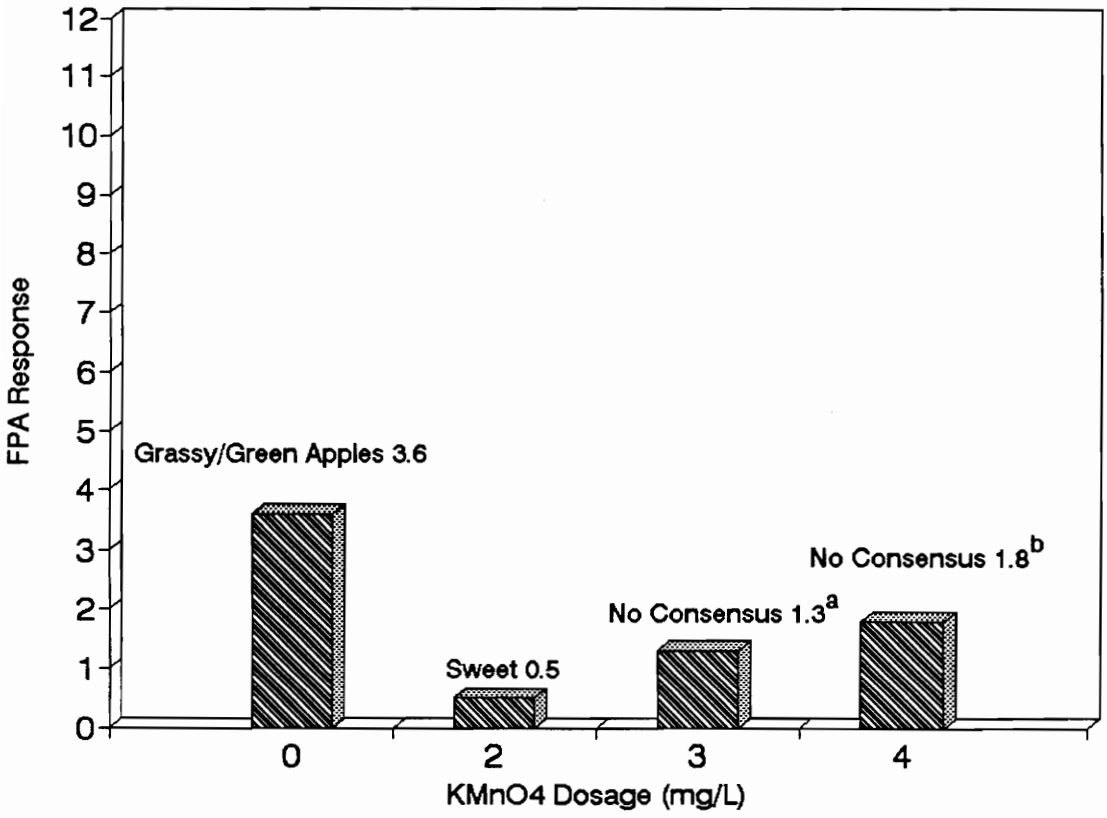


Figure 11. The effect of potassium permanganate on the odor characteristics of cis-3-hexenol (500 ug/L) in water at pH 7. (a. The intensity rating, 1.3, is the average of the individual responses: spicy 1, phenol 2, chlorinous 2, sweet 2, odor-free 0, sweet 1. b. The intensity rating, 1.8, is the average of the individual responses: sweet 2, alcohol 1, swimming pool 2, fishy 2, sweet 2, unidentified 2.)

Table 8: Effects of oxidants on the odor characteristics of the methanol control[a] used during cis-3-hexenol experiments

Oxidant	Oxidant Dose (mg/L)	Residual in ppm[b]	FPA Descriptor and Average Intensity[c]
none	----	----	unidentified 0.75
$Cl_{2(aq)}$	3	0	no consensus
$Cl_{2(aq)}$	4	0	odor-free
Cl_2	6	2	no consensus
ClO_2	3	0.9-1	odor-free
$KMnO_4$	2	----[d]	odor-free
$KMnO_4$	3	----[d]	odor-free
$KMnO_4$	4	----[d]	odor free

[a] The control consisted of 4.6 uL methanol per liter of pH 7 buffered Milli-Q reagent water.

[b] Residuals were measured after a retention time of one hour and subsequently quenched with Na_2SO_3.

[c] Data were calculated according to proposed Standard Method 2170 (Standard Method, 1990). For raw data including "other notes" see Appendix Table A-4.

[d] Solutions remained pink after one hour. No residual measurements were made.

controls before and after oxidation. Note that the odor of most of the blanks was described as odor-free. The odor of the other samples either could not be agreed upon or could not be identified.

Oxidation of algae in media

Figure 12 shows the effects of Cl_2, ClO_2 and $KMnO_4$ on the odor characteristics of DYIII medium both with and without Synura petersenii (including algal cells), respectively. Note the high background odor contributed by the medium after oxidation (Figure 12). Also note the lower intensity ratings given by FPA panelists to the samples containing Synura petersenii treated with $Cl_{2(aq)}$ and $KMnO_4$ samples in comparison to the ratings given to the ClO_2-treated samples containing the alga (Figure 12). Chlorine and $KMnO_4$ either effectively reduced or destroyed the fishy odor associated with Synura petersenii. The FPA panel could not agree on a descriptor for the ClO_2-treated sample, though one panelist did describe the odor as fishy. Note from Figure 12 the changes in FPA descriptors after the culture was treated with the oxidants.

Mass Spectra of Oxidized Compounds

Figures 13 and 14 display the mass spectra of the detected by-products formed after chlorination of cis-3-hexenol. These compounds were tentatively identified as isomers of chlorohexenol and have identical mass spectra but different GC retention times. These compounds were not

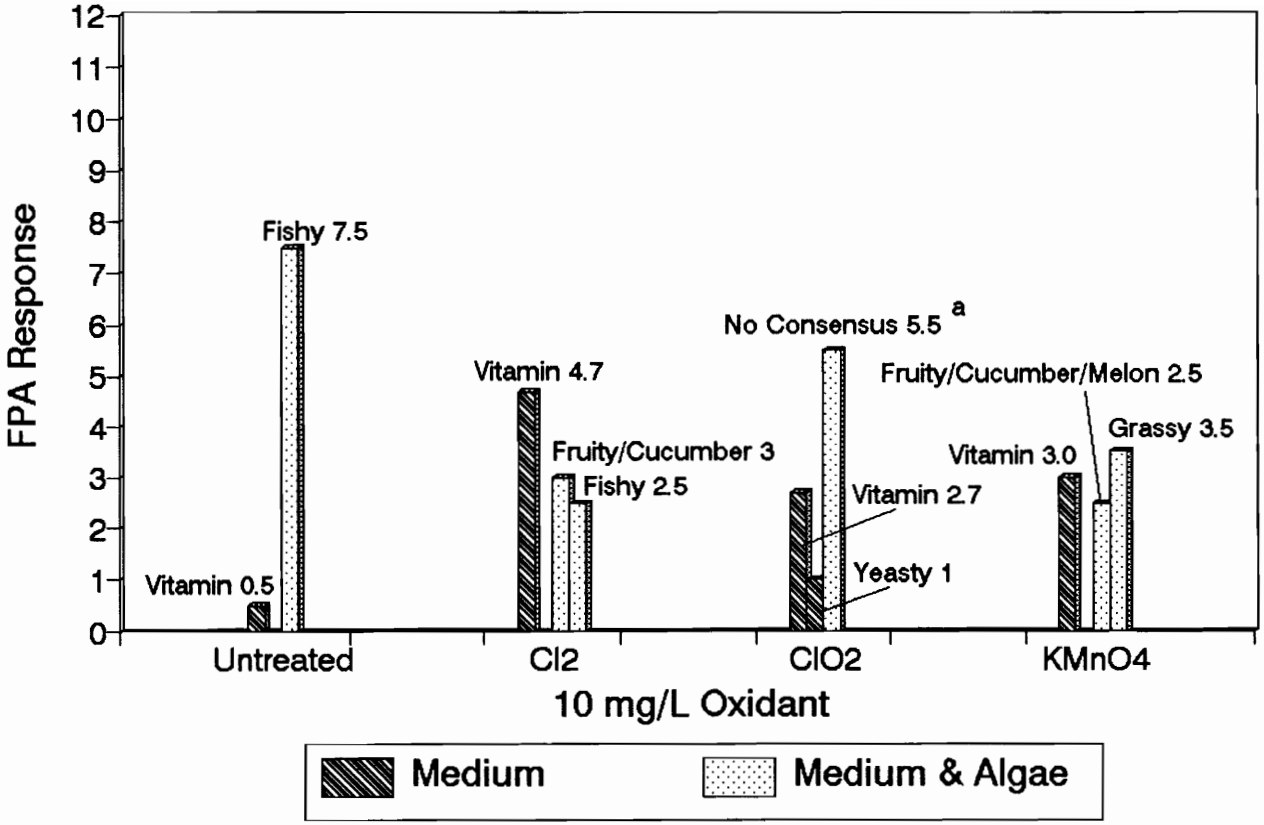


Figure 12. The effects of chlorine, chlorine dioxide and potassium permanganate on the odor characteristics of 14-day-old Synura petersenii and DYIII medium. (a. The intensity, 5.5, is an average of the individual responses: fishy 6, cucumber 6, vitamin 4, and urine 6.)

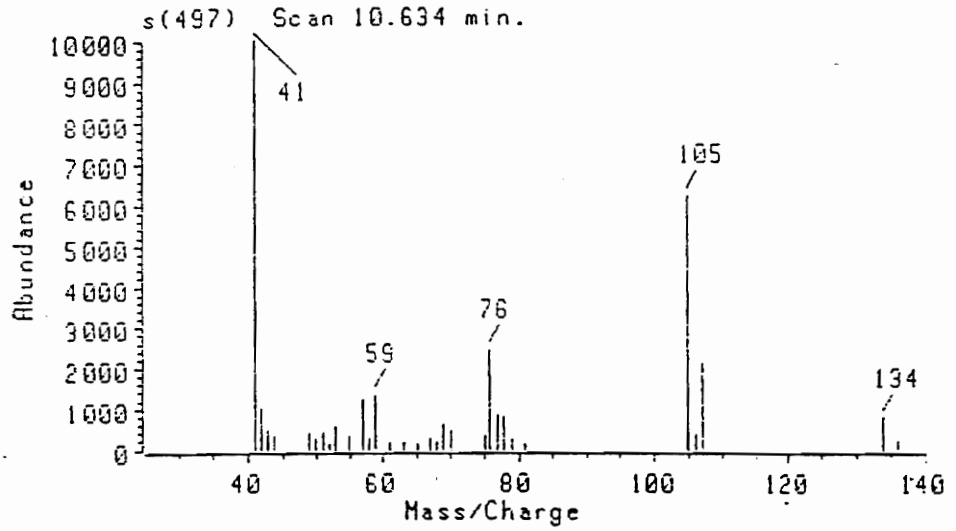


Figure 13. Mass spectrum of by-product formed after the chlorination of cis-3-hexenol, confidently identified as chlorohexenol, isomer 1.

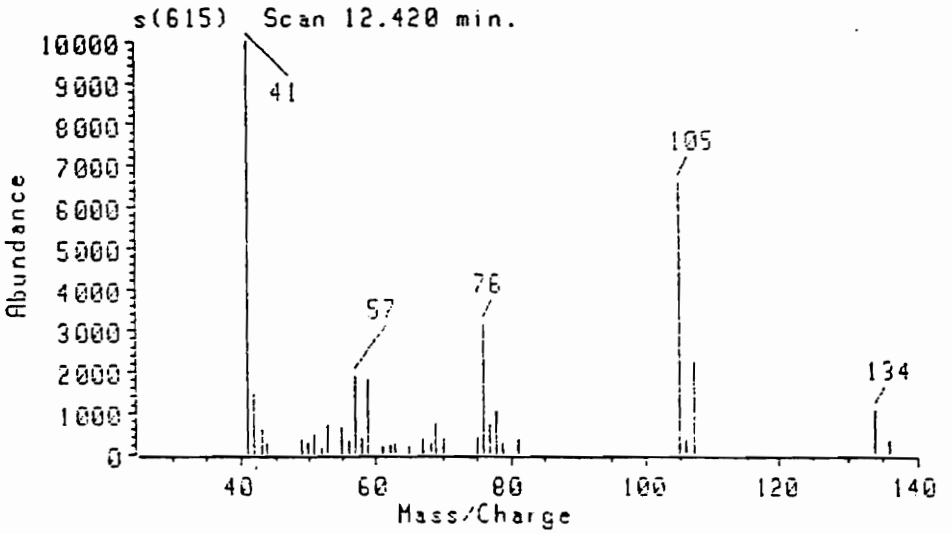


Figure 14. Mass spectrum of by-product formed after the chlorination of cis-3-hexenol, confidently identified as chlorohexenol, isomer 2

detected in either of the duplicate samples treated with ClO_2. No oxidation or substitution by-products were detected in any of the oxidized samples of trans-2, cis-6-nonadienal.

Figures 15 and 16 are the mass spectra of two artifacts formed during the oxidation experiments. Figure 15 shows the mass spectrum of a compound confidently (i.e. without confirmation by comparison of its mass spectrum to the spectrum of the purchased compound) identified as 1, 1, 1-trichloro-2-propanone. It appeared in chlorinated samples in which acetone (propanone) was used as the organic stock solution solvent. These samples had a characteristic odor that interfered with initial FPA panel evaluations of the test samples. Methanol was later used as the organic stock solution solvent. Figure 16 shows the mass spectrum of a unidentified compound detected after the closed-loop stripping and extraction of all trans-2,cis-6-nonadienal unoxidized controls and one of the ClO_2-treated samples. The compound was not detected in the trans-2, cis-6-nonadienal stock solution itself, nor was it detected in the stripped algae samples in which trans-2, cis-6-nonadienal was present.

Algae Compounds

These figures display the mass spectra of compounds at least confidently identified after closed-loop stripping and

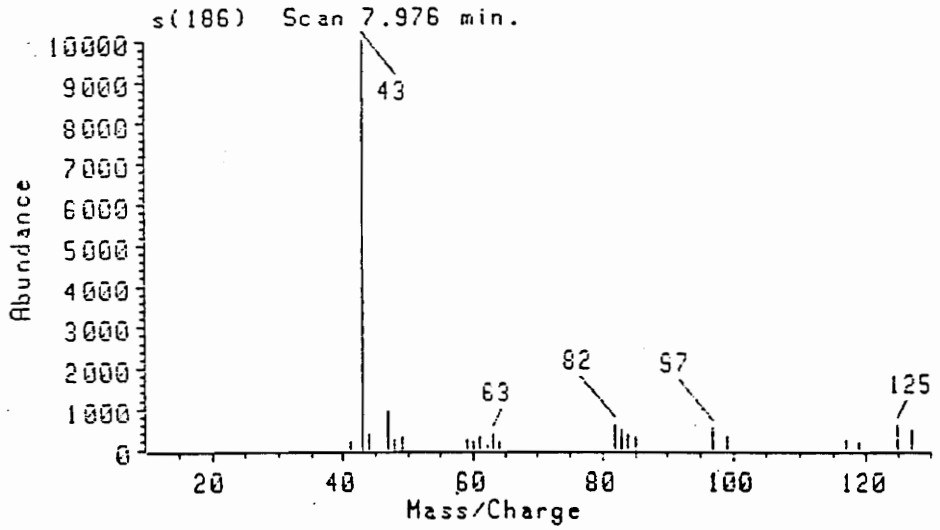


Figure 15. Mass spectrum of artifact confidently identified as 1, 1, 1-trichloro-2-propanone

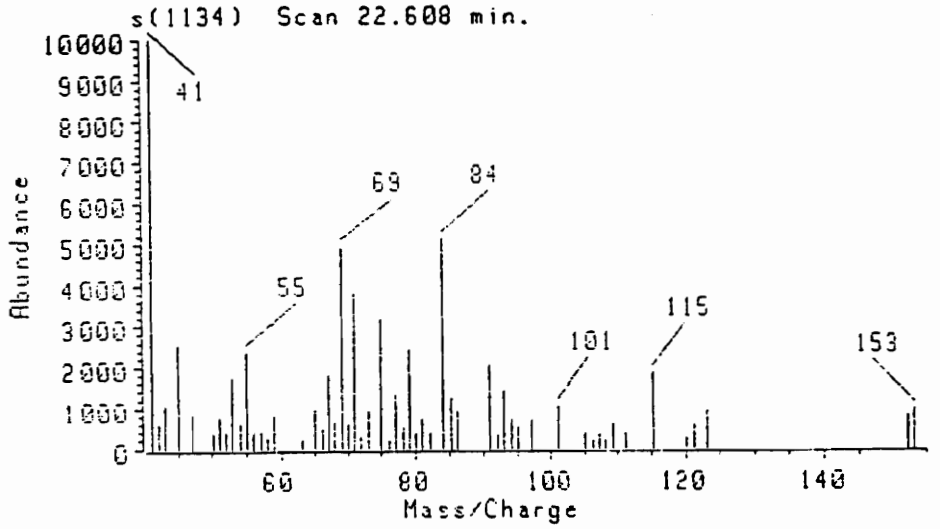


Figure 16. Mass spectrum of unidentified artifact formed during closed-loop stripping of unoxidized control samples containing trans-2, cis-6-nonadienal in methanol

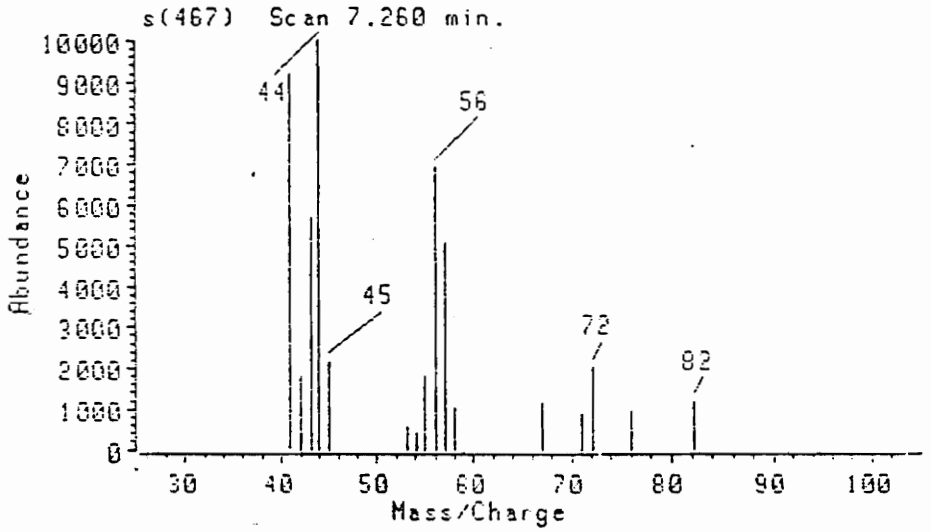


Figure 17. Mass spectrum of Synura petersenii product confirmed as n-hexanal

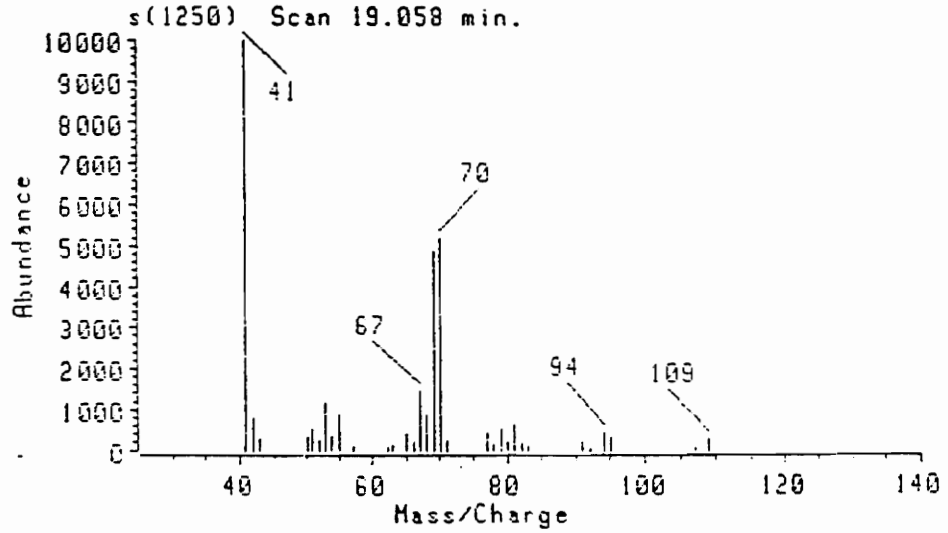


Figure 18. Mass spectrum of Synura petersenii product confirmed as trans-2, cis-6-nonadienal

extraction of both 90 mL and 900 mL samples of the Synura petersenii culture. Figure 17 is the mass spectrum of n-hexanal, which was confirmed as an algal product in both samples of Synura petersenii. Figure 18 is the mass spectrum of trans-2,cis-6-nonadienal, which was confirmed as an algal product in both volumes of Synura culture. Finally, Figure 19 is the mass spectrum of a compound detected in both samples of the Synura petersenii culture. This compound was confidently identified as an isomer of decatrienal, based on a comparison of the compound's mass spectrum with a published spectrum (Seifert and Buttery, 1980) of trans-2,trans-4,cis-7-decatrienal (Seifert and Buttery, 1980) (Figure 20). Figure 20 displays the mass spectrum of the detected compound and the published spectrum of trans-2,trans-4,cis-7-decatrienal. Because the published spectrum was listed as the two most intense ions every 14 mass units above mass/charge (m/e) 34, the spectrum of the detected compound was listed in this manner. For comparison purposes, m/e 39, which was detected by Seifert and Buttery (1980), was omitted in Figure 20 because the mass range scanned during the GC/MS analyses was 40-450 atomic mass units (amu). Note the similarity between the two spectra.

Although each oxidized algae sample was analyzed by GC/MS, logistic problems made it necessary to conduct the analysis 24 hours after the oxidation experiment. Trans-

2,cis-6-nonadienal and the suspected decatrienal were not detected by GC/MS in the oxidized samples, although the odors they produce were detected by the FPA panelists the previous day. Hexanal was detected by GC/MS.

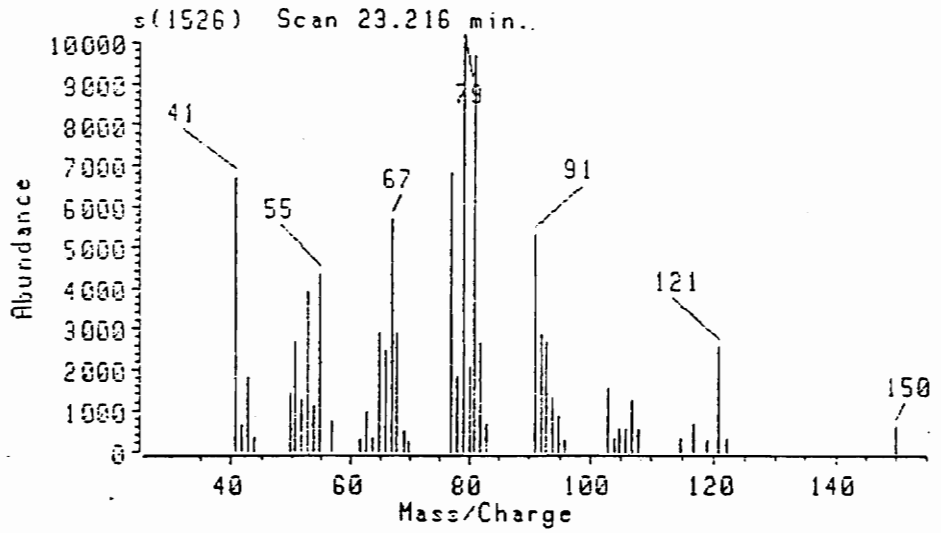


Figure 19. Mass spectrum of Synura petersenii product confidently identified as an isomer of decatrienal

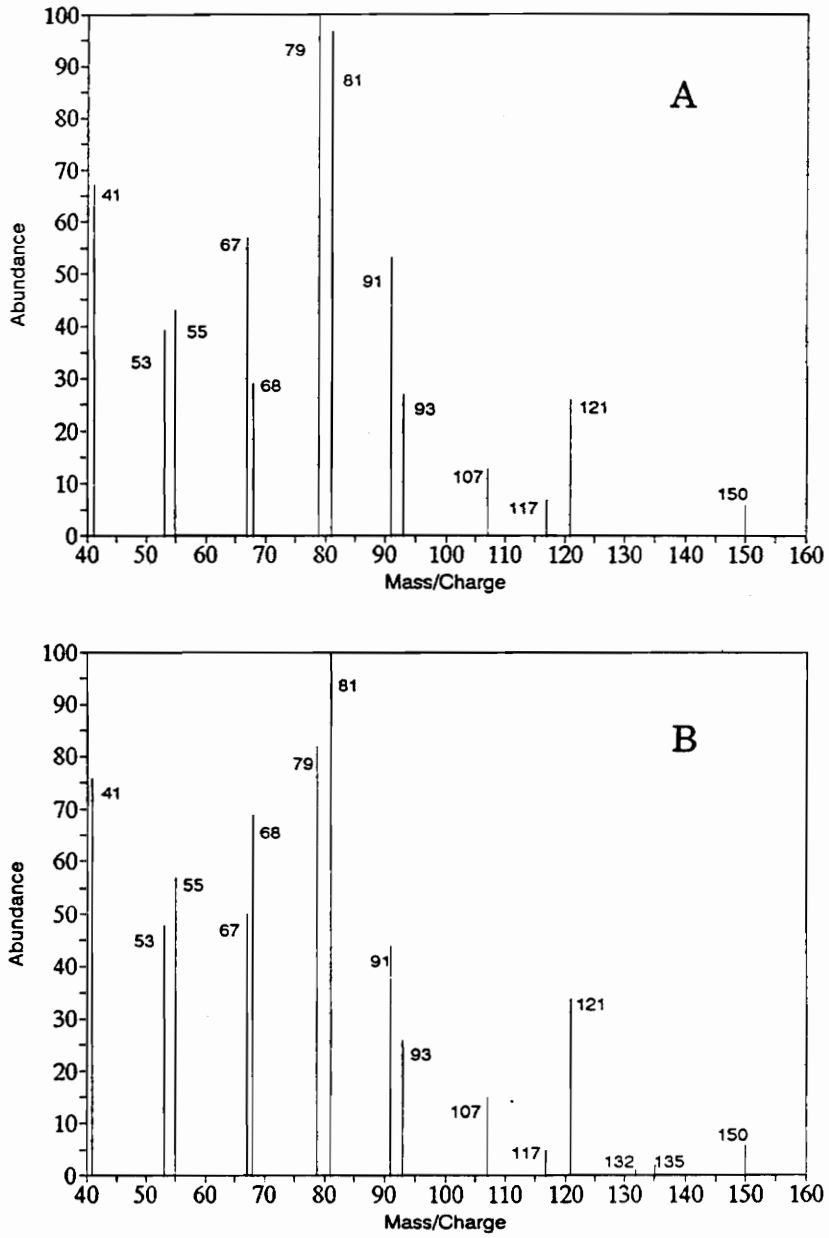


Figure 20. Comparison of mass spectrum of confidently identified isomer of decatrienal (A) with the published mass spectrum (Seifert and Buttery, 1980) of trans-2, trans-4, cis-7-decatrienal (B).

DISCUSSION

Oxidation Experiments

Cis-3-hexenol vs. Trans-2, cis-6-nonadienal

Before oxidation experiments and FPA could be conducted, it was necessary to select appropriate concentrations of trans-2,cis-6-nonadienal and cis-3-hexenol. Initially, concentrations were chosen to evoke a 6 response (slight to moderate) from the FPA panel. However, the odor intensity of cis-3-hexenol was much less than that of trans-2, cis-6-nonadienal, and a higher concentration of the compound was required. In fact, in order to obtain the same response, concentrations within the range of 1-5 mg/L (1-5 x 10^{-5} M) were necessary (Figure 3). These levels of cis-3-hexenol equal or exceed the concentrations of oxidants typically used during water treatment. If cis-3-hexenol is indeed one of the grassy odor-causing agents in drinking water (its presence has never been documented), this relationship may explain why such odors are "resistant to conventional treatment processes" (Mallevialle and Suffet, 1987); however, the presence of this compound at the high concentrations necessary to evoke an odor response in water supplies is questionable. Other C_6 compounds, with a lower odor threshold concentration (OTC), may be responsible for the grassy odors found in drinking

water. Trans-2, cis-6-nonadienal, with an OTC of 13 ng/L, elicited a 6 response at molar concentrations approximately 100 times less than the concentrations of cis-3-hexenol.

Effect of Oxidants on Trans-2,cis-6-nonadienal

Chlorine. Chlorine, at a concentration of 2 mg/L under neutral conditions, destroyed the cucumber odor associated with trans-2,cis-6-nonadienal (Table 5; Figure 6). This observation contrasts with most statements in the literature that $Cl_{2(aq)}$ does not effectively remove tastes and odors, but supports evidence by Burlingame et al. (1992). Burlingame et al. (1992) found that 2.4 mg/L of free Cl_2 at the intake of the Samuel Baxter Treatment Plant in Philadelphia effectively removed the cucumber flavor; however, a lower dosage of free Cl_2 (0.6 mg/L) did not eliminate the flavor. Because no by-products were detected by GC/MS, the oxidation by-products and/or chlorinated organics that were formed remain unknown. Lower chlorine-to-organic molar ratios, and perhaps a different compound-isolation technique, would be required for the detection of these by-products. In any case, trans-2,cis-6-nonadienal was reduced to below the threshold odor detection limit.

Chlorine dioxide. Chlorine dioxide, at a concentration of 3 mg/L under neutral conditions, did not destroy the cucumber odor associated with the aldehyde, trans-2,cis-6-nonadienal, even when a sizable ClO_2 residual remained (Table

5; Figure 7). This observation contrasts with published studies of ClO_2 and oxidation of model aldehydes by Masschelein (1979) and Somsen (1960), but is consistent with the results of experiments performed by Hoigné and Hoigné and Bader (Hoigné, 1985; Hoigné and Bader, 1982). According to Hoigné and Bader, the kinetic rate constants for reactions occurring between ClO_2 and aldehydes are too low under typical water treatment plant conditions. They reported that more than 100 days would be required to reduce the concentration of butanal and benzaldehyde by half if the ClO_2 dose was 1 mg/L in a plug-flow reactor (Hoigné, 1985; Hoigné and Bader, 1982). Hoigné also stated that "rate constants and product formation arising in nonaquatic systems or in cellulose or food bleaching are noncomparable" (Hoigné, 1985). These are the typical sources of information regarding reactions between ClO_2 and organic compounds, but the reactions described were carried out under conditions far different than those typical of conditions in water treatment plants. Furthermore, Burlingame et al. (1992) showed that ClO_2 (estimated at 0.5 mg/L) used by the Samuel Baxter Treatment Plant did not destroy the cucumber flavor in Philadelphia's drinking water.

Another factor that should be considered regarding reports of the effectiveness of ClO_2 for eliminating certain certain odor compounds is the amount of chlorine and chlorite present in the original stock solution. The results may may

be misinterpreted if either $Cl_{2(aq)}$ or ClO_2^- is present. During the oxidation experiments conducted during this project, little, if any, $Cl_{2(aq)}$ was present, which was especially important because $Cl_{2(aq)}$ destroyed the odor associated with trans-2,cis-6-nonadienal. Chlorite, in contrast, was present in all cases, and while trans-2,cis-6-nonadienal could theoretically react with chlorite at a low pH values (Masschelein, 1979), the reactions would be extremely slow in pH 7 buffered water. During these experiments, ClO_2^- did not seem to have any effect.

Potassium permanganate. Potassium permanganate destroyed the cucumber odor associated with trans-2,cis-6-nonadienal at the low concentration of 0.5 mg/L (Table 5; Figure 8). Theoretically, trans-2, cis-6-nonadienal should have been oxidized to its nonvolatile carboxylic acid. This product would not have been detected by CLSA, however. It is also unknown whether manganese dioxide ($MnO_{2(s)}$), which forms as a reduction product when permanganate is used as an oxidant, adsorbed the organic compounds and played a part in odor reduction.

Effect of Oxidants on Cis-3-Hexenol

Chlorine. Although $Cl_{2(aq)}$ effectively reduced the grassy and green apple odor associated with cis-3-hexenol, it did not completely eliminate offensive odors from the samples (Table 7; Figure 9). In fact, $Cl_{2(aq)}$ contributed to the formation of

two chlorinated by-products, confidently identified as isomers of chlorohexenol. These by-products may have been responsible for the chemical descriptor given to the most highly chlorinated sample. In this sample only the two isomers of chlorohexenol were detected; no cis-3-hexenol was found. The chlorine-to-organic molar ratio in these samples was low (from approximately 8:1 to 17:1) as compared to that in trans-2, cis-6-nonadienal samples (100:1 to 200:1). In fact, the concentration of cis-3-hexenol must be reduced to 14 ug/L or less in order to achieve the same molar ratios, a level far below the published odor threshold concentration (OTC) of 70 ug/L (Stahl, 1973).

Chlorine dioxide. Chlorine dioxide did not destroy the odor associated with cis-3-hexenol under the given experimental conditions (Table 7; Figure 10). This is supported by evidence from several sources (Masschelein, 1979; Rav-Acha, 1984; Somsen, 1960) that show that aliphatic alcohols are resistant to ClO_2 at neutral pH and room temperature. Temperatures greater than 70-80°C are necessary for reactions to take place (Somsen, 1960).

Potassium permanganate. Potassium permanganate, at a concentration of 2 mg/L, effectively reduced the average FPA odor intensity of cis-3-hexenol from 3.6 to 0.5 under the given experimental condition (Table 7; Figure 11). Cis-3-hexenol was detected in this sample by GC/MS after treatment,

but the MDL (12 ug/L) was far lower than the published odor threshold concentration (70 ug/L) (Stahl, 1973). Panelists could not agree on a descriptor for samples treated with more than 2 mg/L $KMnO_4$, probably because responses to samples containing low odorant conentrations can be influenced by other factors such as fatigue, expectation and interference, especially when much stronger samples present in the sample set are being evaluated. Panelists often evaluated strong-smelling algae samples (as part of another project) at the same time the oxidized samples were being evaluated. These could have affected panelist response and consensus regarding samples with much weaker odors.

Effect of Oxidants on Fishy-Smelling Algae

As stated previously, trans-2,cis-6-nonadienal and the suspected decatrienal were not detected by GC/MS in the oxidized algae samples. These compounds may have volatilized during storage, particularly if present at low concentrations. Only FPA results will be discussed.

Chlorine. Chlorine, at a concentration of 10 mg/L, effectively reduced the fishy odor associated with Synura petersenii, but fruity and cucumber odors, which were not detected by panelists in the unoxidized sample containing the alga, were detected in the oxidized sample (Figure 12). Trans-2, cis-6-nonadienal was confirmed as an algal product in the unoxidized sample (Figure 18), and it likely contributed

to the fruity/cucumber odor detected by the panel in the oxidized sample. One odor can commonly mask another, particularly if it is offensive (Burlingame, 1992). When the offensive odor is reduced to below its threshold level, the more subtle odor can be detected. The OTC of the fishy-smelling compound (or compounds) in this sample may have been higher than that of trans-2,cis-6-nonadienal, and, thus, its odor could have been reduced to below its threshold level more easily than trans-2,cis-6-nonadienal if both had been present at similar concentrations. Furthermore, low concentrations of the 2,4,7-decatrienal isomers contribute mild green-plant-like notes to oils, whereas high levels contribute distinct fishy notes (Karahadian and Lindsay, 1989). This fact may account for published observations that cucumber odors are noted when Synura is present in small numbers but fishy odors become dominant as Synura populations increase to high levels (Palmer, 1962). When fish oil oxidizes, the concentrations of both decatrienal isomers increase and cause fishy odors, which replace the melony/green aromas of unoxidized oil (Karahadian and Lindsay, 1989). Trans-2,trans-4,cis-7-decatrienal itself contributes a "sweet, greeny, cucumber and melon-like flavor" and is less "fishy" than the trans-2,cis-4,cis-7 isomer (Meijboom and Stroink, 1972). These facts could have important implications if the compound that was confidently identified by GC/MS (Figures 19 and 20) is indeed a

decatrienal or a combination of decatrienal isomers.

Chlorine treatment also enhanced the intensity of the vitamin odor of the uninoculated medium, but the vitamin odor was not detected in the oxidized algae samples (Figure 12). Masking is the logical explanation.

Chlorine dioxide. Chlorine dioxide did not effectively reduce offensive odors associated with Synura petersenii (Figure 12). Although the panel could not agree on a descriptor for the sample containing Synura petersenii treated with ClO_2, the odor intensity remained high after treatment. Four descriptors were given to the oxidized algae sample, one by each panelist. Fishy, cucumber and urine odors were among the odors detected. Because ClO_2 was shown to be ineffective against a model alcohol (cis-3-hexenol) and a model aldehyde (trans-2,cis-6-nonadienal) during this study (Tables 5 and 7; Figures 7 and 10), the fishy odor compound may be an aldehyde or alcohol since it was not destroyed.

The vitamin odor of the medium was detected in the oxidized sample containing the algae and appeared to be intensified by the treatment (Figure 12).

Potassium permanganate. As was shown earlier (Figure 12) $KMnO_2$ destroyed the fishy odor in the Synura petersenii culture, but, as was true of $Cl_{2(aq)}$ treatment, treatment with $KMnO_4$ produced fruity, cucumber and melon odors. In contrast, grassy odors were detected in the $KMnO_4$-treated sample but not

in the one treated with $Cl_{2(aq)}$. The fishy odor in the sample treated with $Cl_{2(aq)}$ may have masked the grassy odor. As stated previously, the compound causing the fishy odor was probably reduced to below its OTC more easily than trans-2, cis-6-nonadienal.

Although the intensity of the vitamin odor of the medium was enhanced after oxidation in the uninoculated medium, the vitamin odor was not detected in the oxidized algae sample.

Artifacts

Two artifacts were formed during the oxidation experiments, and these may be of interest to future researchers. One of the artifacts (Figure 15) was a compound confidently identified as 1,1,1-trichloro-2-propanone (trichloroacetone), which was produced after chlorination of samples dosed with organic compounds dissolved in acetone. These samples had a characteristic sweet odor, which would have interfered during the FPA sessions. The problem was solved by substituting methanol for the acetone.

The second artifact (Figure 16) was an unidentified compound with a tentative molecular weight of either 152 or 153. It was formed during the CLSA of all unoxidized control samples containing trans-2,cis-6-nonadienal in methanol and also in one of the ClO_2-treated nonadienal samples. Its mass spectrum was shown previously in Figure 16. The compound was detected in neither the nonadienal/methanol stock solution

itself, nor in the algae samples that contained trans-2,cis-6-nonadienal but no methanol. It may have been reaction product of trans-2, cis-6-nonadienal and methanol, which perhaps was induced by the heat in the CLSA and/or by reactions catalyzed by the activated carbon in the filter.

Algae Compounds

Three compounds were identified in both aliquots (90 mL and 900 mL samples) of the Synura petersenii culture. These were n-hexanal (Figure 17) and trans-2,cis-6-nonadienal (Figure 18), both of which were confirmed, and another compound that was confidently identified as an isomer of decatrienal (Figure 19). Trans-2,cis-6-nonadienal has been previously identified as the compound responsible for the cucumber odor associated with Synura petersenii (Hayes and Burch, 1989), but reports of n-hexanal production by Synura petersenii could not be found in the literature surveyed for this study. The compound has been reported to contribute to the characteristic odor of green plants and to "coarse, green plant-like aldehydic" notes in fish (Josephson and Lindsay, 1986). It was presumed to be a product of Synura uvella (Jüttner, 1983) and was identified as a product of Anabaena oscillarioides (Mohren and Juttner, 1983) and Phaeodactylum triconutum (Schobert and Elster, 1980).

No reports of decatrienal production by Synura petersenii

were found in the literature, although isomers have previously been identified in fish (Josephson and Lindsay, 1986; Karahadian and Lindsay, 1989), fish oils (Ke et al., 1975) and in other oils and fats containing auto-oxidized linolenic and other omega-3,6,9 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Badings, 1970; Badings, 1973; Meijboom and Stroink, 1972; Swoboda and Peers, 1977). The 2,4,7-decatrienal isomers contribute heavily to the fishy odor in all of these products; cod liver oil itself contains high quanities of EPA and DHA. The lipids of some algal species, including Synura petersenii, often are rich in omega-3 and/or other polyunsaturated fatty acids (Borowitzka and Borowitzka, 1988; Cranwell et al., 1988; Parker, 1992; Orcutt and Patterson, 1975), a fact that may help explain the odors associated with some algal blooms (Jüttner, 1981; Hayes and Burch, 1989).

Heptadienal, another typical by-product of omega-3 polyunsaturated fatty acid degradation, and decadienal, a typical by-product of omega-6 fatty acid degradation (Swoboda and Peers, 1977), were detected by Jüttner during a bloom of Synura uvella. The presence of these two compounds cannot fully explain the cod-liver-oil odor associated with the bloom, because both compounds cause "fatty" or "frying odors" (Badings, 1970), but they probably contribute to the overall rancidity of the odor. In most literature, the decatrienals

are considered to be the primary contributors to the burnt/fishy or cod-liver-oil odors (Karahadian and Lindsay, 1989; Karahadian and Lindsay, 1989; Karahadian and Lindsay, 1990) in fish and fish oils. The confidently identified decatrienal thus may contribute to the cod-liver-oil-like odor associated with Synura petersenii.

Trans-2,cis-6-nonadienal also originates from the degradation of omega-3 polyunsaturated fatty acid hydroperoxides, including those formed from EPA in fish (Figure 21). Eicosapentaenoic acid, furthermore, is the probable precursor of hexanal (Schobert and Elstner, 1980). Polyunsaturated fatty acids are extremely susceptible to oxidative degradation catalyzed by light in the presence of oxygen, metals such as copper, photosensitizers such as chlorophyll and polycyclic aromatic hydrocarbons, free radicals, and enzymes (Badings, 1970; Frankel, 1985; Galliard, 1978; Karahadian and Lindsay, 1989; Love, 1985). Normally, these fatty acids are stable components of healthy membrane lipids but become susceptible to peroxidation and thus degradation into volatile components when there is cellular damage or loss of cellular control such as that occurring during senescence or the aging process (Galliard, 1978). Volatile formation by light-induced, nonenzymatic peroxidation of polyunsaturated fatty acids in Phaeodactylum triconutum (a diatom) was suggested by Schobert and Elstner (1980).

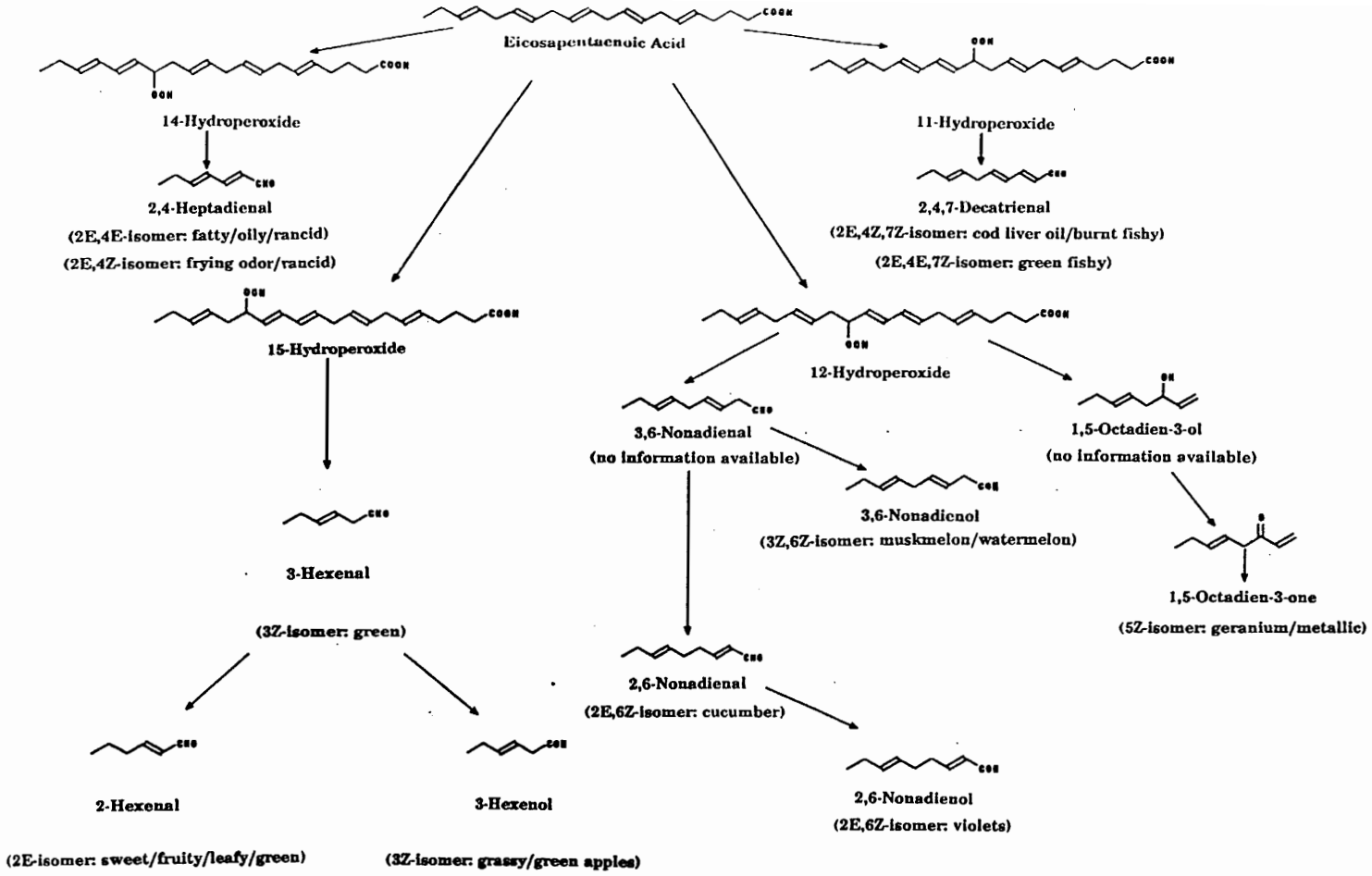


Figure 21. Odor compounds associated with the peroxidation of eicosapentaenoic acid. Modified from Josephson and Lindsay (1986) and Karahadian and Lindsay (1989).

If polyunsaturated fatty acids are precursors to some of the odors produced by algae, the process of lipid oxidation and its contributing factors could have very important implications for the treatment of algae-induced tastes-and-odors and for methods of algal control; however, further research should be conducted so that the relationships between these compounds and algae-induced taste-and-odor problems can be further elucidated.

Application of Research to Drinking Water Treatment

The results of this research have several very important applications to drinking water treatment. The structure, threshold odor concentration, masking ability, and origin of an odor-causing substance are all important factors one should consider before an oxidant is selected for use in treating water during an odor episode. These factors also should be considered when reference compounds for FPA analysis are selected.

Structure

The results of this study showed that ClO_2 reacts with neither alcohols nor aldehydes under water treatment conditions (Tables 5 and 7; Figures 7 and 10). This finding is substantiated by the work of Hoigné and Bader (1982). If an odor outbreak is associated with one compound in

particular, such as trans-2,cis-6-nonadienal (cucumber odor), the structure of the compound can be of utmost importance. If this compound is responsible for the odor, ClO_2 will not be an effective oxidant because ClO_2 does not effectively oxidize aldehydes. This fact was demonstrated by Burlingame et al. (1992) in Philadelphia. Chlorine and $KMnO_4$, in contrast, effectively destroy trans-2,cis-6-nonadienal, and, most likely, other aldehydes.

Threshold Concentration

Alcohols, in general, have significantly higher odor thresholds than the corresponding aldehydes (Eriksson, et al., 1976). This fact can be of significnce in the search for reference compounds. For example, the OTC of cis-3-hexenol is 70 ug/L (Stahl, et al., 1973). During this study, cis-3-hexenol, at a concentration of 500 ug/L, evoked only a 4 (slight) response from the FPA panelists. Its presence in drinking water has never been documented, which is unusual if it is indeed the compound responsible for the grassy odor. Concentrations in the range of 500 ug/L would have been easily detected. Perhaps the compound causing the odor is a C_6 aldehyde or a combination of C_6 compounds, as are found in grass and green plants.

According to Frankel (1982), "unsaturated aldehydes with an omega-3 double bond have particularly low (odor) threshold values," such as 3,6-nonadienal; 2,6-nonadienal; 2,4-

heptadienal; 3-hexenal and 2,4,7-decatrienal. During water treatment, a compound with an extremely low OTC may persist even after treatment of the water with an oxidant. For example, if cis-3-hexenol and trans-2,cis-6-nonadienal were present in water at equal concentrations of 80 ug/L, and enough oxidant were added to reduce both concentrations to 60 ug/L, the trans-2,cis-6-nonadienal would evoke an 8 (moderate) response and cis-3-hexenol would evoke a 0 (odor-free) response (Figure 3). This fact again suggests that aldehydes may be the most important contributors to algae-related taste-and-odor events and should be used as reference compounds.

Masking

Odors often change after oxidation during water treatment. For example, a source water may have a particular odor before oxidation and then develop other odors after treatment. The results of this project showed that the fishy odor of the alga, Synura petersenii, was replaced by fruity, grassy, cucumber and melon odors after oxidation with Cl_2 and/or $KMnO_4$ (Figure 12). These odors may have been present but masked by the offensive fishy odor in the unoxidized sample. Water treatment personnel should be aware that oxidants can remove masking compounds from water, thus revealing other odors, so that they will not confuse the elimination of a masking agent with the production of new, odorous compounds. By-products can indeed be produced, but

unmasking probably contributes to some of the odors detected by consumers. Compounds with higher threshold concentrations but more offensive odors may mask other compounds with lower threshold concentrations and less offensive odors (Burlingame, 1992).

Odor Compound Origin

Odor-compound origin may have important implications in the field of taste-and-odor removal of algae-produced compounds in drinking water. Most odor-related research in the field of water treatment focuses on the lower-molecular-weight compounds and not on their precursors. This study suggests that the omega-3- and other polyunsaturated fatty acids may be the precursors for many compounds associated with numerous odors that might be detected during some algae blooms (Figure 21). Although further research is necessary to determine if this is indeed true, the effect of oxidants on the precursors should be evaluated. Furthermore, copper (II) is known to catalyze fatty acid peroxidation, which may be an important consideration in systems where copper sulfate ($CuSO_4$) is used to control algal growth. The possiblility exists that the treatment itself may actually produce compounds that cause odors that did not exist prior to the application of $CuSO_4$. This effect on odor characteristics of water is distinctly different than that which results when algal cells rupture and release stored compounds into the

water.

Finally, the appearance of some odor-causing compounds in water may be caused by phenomena little-known to water-treatment specialists, namely that nonodorous fatty acids produced by certain algae may accumulate near the surface of a body of water and be converted into odorous compounds by either photosensitized oxidation or ultraviolet (UV) radiation.

SUMMARY AND CONCLUSIONS

Chlorine and $KMnO_4$ markedly reduced grassy and cucumber odors associated with cis-3-hexenol and trans-2, cis-6-nonadienal, respectively. Gas chromatography and mass spectrometry (GC/MS) confirmed that these compounds were reduced to below method detection limits. Higher levels of $Cl_{2(aq)}$ than $KMnO_4$ were necessary to reduce grassy odors associated with cis-3-hexenol and may have contributed to the formation of chemical odors. Two isomers of chlorohexenol were confidently identified as by-products of cis-3-hexenol chlorination and may have contributed to the chemical odor formed after treatment with $Cl_{2(aq)}$. Chlorine and $KMnO_4$ reduced or destroyed the fishy odor associated with Synura petersenii; however, oxidation led to the development or detection of fruity, cucumber, melon and grassy odors.

Treatment-plant levels of ClO_2 did not effectively reduce grassy and cucumber odors associated with cis-3-hexenol and trans-2,cis-6-nonadienal, respectively. Gas chromatography and mass spectrometry confirmed that these compounds were not reduced to below method detection limits. Chlorine dioxide did not destroy fishy and/or other objectionable odors associated with Synura petersenii.

Hexanal and trans-2,cis-6-nonadienal were confirmed as

algal products in a two-week-old culture of Synura petersenii. Decatrienal was confidently identified (but not confirmed) as a product of Synura and may have contributed to the fishy odor associated with this algae.

The conclusions derived from this study are as follows:

1. Chlorine and $KMnO_4$ can be used to destroy grassy and cucumber odors if these odors are caused by cis-3-hexenol and trans-2,cis-6-nonadienal, respectively. These oxidants can also be used to reduce or destroy the fishy odor associated with Synura petersenii.

2. Chlorine dioxide cannot be used to destroy grassy and cucumber odors associated with cis-3-hexenol and trans-2, cis-6-nonadienal, respectively, nor can it be used to destroy objectionable odors associated with Synura petersenii.

3. Treatment of fishy odors associated with Synura petersenii by $Cl_{2(aq)}$, $KMnO_4$, and ClO_2 can lead either to the development or the unmasking of fruity, cucumber, melon and grassy odors.

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VITA

Lisa Webster Buffin was born on October 13, 1966, one of four children of Harold and Virginia Webster of Manakin-Sabot, Virginia. She attended Goochland County High School and graduated valedictorian in 1985. A member of both Phi Beta Kappa and Phi Kappa Phi, she graduated magna cum laude from Virginia Tech in 1989 with a Bachelor of Science degree in Biology and a minor in Chemistry. After working one year in the Chemistry Department at Virginia Tech, Lisa began her graduate studies in Environmental Science and Engineering. She married Robert Buffin of Thizy, France, on April 27, 1992, and will join him in France after her graduation.