THE EFFECT OF THE FORM AND CONCENTRATION OF NITROGEN ON GEOSMIN PRODUCTION BY AN AQUATIC ACTINOMYCETE (*STREPTOMYCES* ISOLATE WM1C1)

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ABSTRACT

Actinomycetes are organisms that produce geosmin which is one of the organic compounds responsible for the earthy (musty) odor in water. This study was designed to isolate an actinomycete, confirm it as a geosmin producer, and determine the effects of the form and concentration of nitrogen on the production of geosmin by the isolate.

Five actinomycetes were isolated from the muds of Lake Waco; four were identified as Streptomyces and one as a possible Nocardia sp. or Micromonospora sp., and two Streptomyces spp. were confirmed to be geosmin producers by GC-MS. The effects of nitrogen on one Streptomyces sp. were reported as affecting threshold odor number (R.O.N.), biomass production (mg dry wt), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}). The forms of nitrogen used were NO_3-N as KNO_3, NH_3-N as NH_4Cl, and organic nitrogen (ORG-N) as asparagine and the concentration range of nitrogen was 0.000 $mg \cdot L^{-1}$ to 0.600 $mg \cdot L^{-1}$.

T.O.N. values increased linearly as concentrations of NO_3-N and NH_3-N increased while ORG-N

(0.300 $mg \cdot L^{-1}$) inhibited geosmin production. Biomass production increased linearly as concentrations of NO_3-N and ORG-N increased (0.000 $mg \cdot L^{-1}$ to 0.600 $mg \cdot L^{-1}$) but low concentrations of all three nitrogen forms did not promote significantly larger biomass proudction than the nitrogen-free media. Specific T.O.N. increased as concentrations of NH_3-N and NO_3-N increased with NH_3-N producing significantly higher Specific T.O.N. per flask.

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LITERATURE REVIEW

Reports of Tastes and Odors in Water Supplies

The quality of water may be affected by the presence of either a disagreeable taste or odor, or both. These disagreeable tastes and odors were given attention as early as the eighteenth century as noted in 1798 by Joseph Brown, a New York physician:

> Water . . . that is clear and from a running source, that boils leguminous vegetables tender, in which soap readily dissolves, and (which) has no bad flavor may be pronounced good water (Allen 1960).

One of the first reports of a disagreeable odor was by Farlow (1883) who reported that the water supplies in several eastern cities in the United States had a "pigpen" odor. Disagreeable odors and tastes in impoundments (lakes and reservoirs) and in rivers have been reported from Iowa (Morris et al. 1963), Oklahoma (Silvey and Roach 1975), South Carolina (Rashke et al. 1975), southern California (McGuire et al. 1981), from Japan (Kikuchi et al. 1973a), and Finland (Persson 1980).

Besides affecting water quality, the materials responsible for tastes and odors can be absorbed into fish muscle and make the meat unpalatable. This has been reported in salmon from a river in the British Isles (Thaysen 1936), in rainbow trout from fish farms in Canada (Yurkowski and Tabachek 1974), and in bream from the Porvoo Sea area in Finland (Persson 1979).

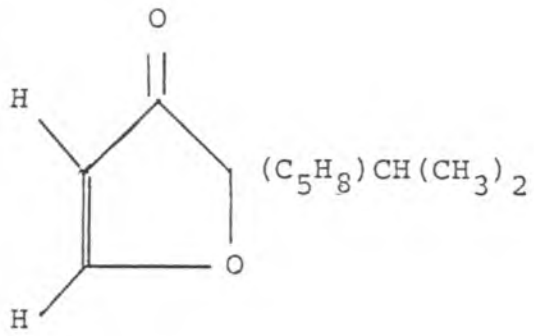
Biochemical Sources of Taste and Odor Metabolites

The objectionable tastes and/or odors in water are a combination of several known or suspected metabolites (Gerber 1979, Kikuchi et al. 1973b). Eight that have been produced *in vitro* and identified as possible contributors to taste and odor problems are: *trans*-1,10-dimethyl-*trans*-9-decalol (geosmin) (Gerber and Lechevalier 1965), 1,2,7,7-tetramethyl-2-norbornanol (2-methylisoborneol or MIB) (Gerber 1969, Medsker et al. 1969), 5-methyl-3-heptanone (Henley et al. 1969), isopropyl methoxylpyrazine (Gerber 1979), furfural (Kikuchi et al. 1973b), cadin-4-ene-1-ol (Gerber 1979), selina-4(14),7(11)-diene-9-ol (Gerber 1979), and butyric acid (Weete et al. 1979).

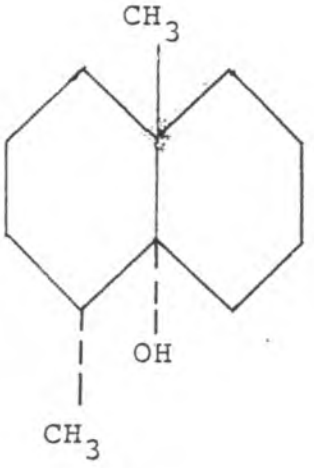
Geosmin and MIB appear to be the metabolites that cause most tastes and/or odors in water because of their high threshold odor numbers at low concentrations.

The threshold odor number (T.O.N.) is the dilution factor required before an odor in a water sample can be no longer detected by a group of judges (American Public Health Association 1981). In water, geosmin and MIB are detectable at concentrations of 0.2ppb and 0.1ppb respectively (Gerber 1979, Medsker et al. 1969).

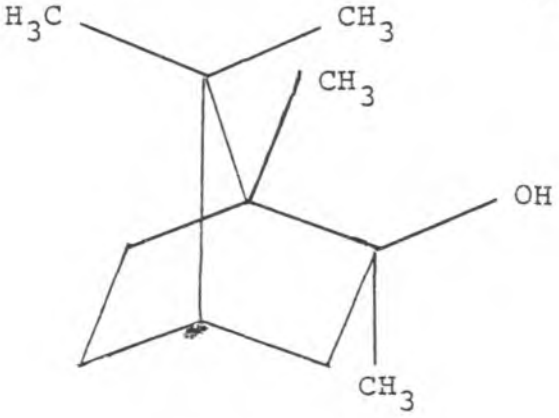
Geosmin ($C_{12}H_{22}O$) is derived from a sesquiterpene, C_{15}, precursor (Figure 1) (Bentley and Meganathan 1981, Gerber 1968). This metabolite, originally extracted from six different Streptomyces species (Gerber and Lechevalier 1965), was thought to be mucidone, a $C_{12}H_{18}O_2$ compound (Figure 1) that is also produced by Streptomyces (Doughtery and Morris 1967, Morris et al. 1963). After a comparison of geosmin with mucidone by T.O.N., gas chromatography, and odor designation, the metabolites were confirmed to be different (Gerber 1968), and the odor designation of mucidone was changed from "musty" to "estery" or "fruity" (Sipma et al. 1972). MIB ($C_{11}H_{20}O$) was first identified as a derivative of a sesquiterpene (Figure 1) (Gerber 1969), but has now been shown to be a derivative from a monoterpene, C_{10}, precursor (Bentley and Meganathan 1981).



MUCIDONE



GEOSMIN



METHYLISOBORNEOL

Figure 1. Structures of Mucidone, Geosmin, and Methylisoborneol (Adapted from Dougherty and Morris 1967 and Gerber 1979).

Biotic Sources of Taste and Odor Metabolites

The organisms responsible for producing the earthy, or musty, taste and odor in water are actinomycetes, blue-green algae, and a fungus. Gerber (1979) stated that actinomycetes were first suspected to cause taste and odor episodes in 1891 by Berthelot and Andre, and this was later confirmed in 1929 by Adams and in 1936 by Thaysen. However, actinomycetes are not the only organisms that are capable of producing earthy and musty-smelling metabolites (Table 1). Blue-green algae were suspected as early as 1883 by Farlow but were not confirmed to be producers of taste and odor metabolites until 1968 by Medsker and associates. In 1981 Kikuchi and associates determined that the fungus Chaetomium globosum was a source of taste and odor metabolites.

The actinomycetes responsible for "odor events" in municipal water supplies are Streptomyces spp., Nocardia spp., and Micromonospora spp. (Silvey and Roach 1975). The Streptomyces spp. produce the most intense odor (Romano and Safferman 1963). These genera have been isolated from mud and water of impoundments and rivers and cultured on solid and in liquid media (Table 2) (Cross 1981, Johnston and Cross 1976a,b).

Table 1. Organisms Producing Taste and Odor Metabolites.
(Adapted from Sivonen 1982 and Gerber 1979)

GEOSMIN PRODUCERS

ACTINOMYCETES

- *Microbispora rosea* 3748
- *Micromonospora* sp.
- *Nocardia* sp. SS1/1
- *Nocardia* sp. W-68
- *Nocardia* sp. I-15
- *Streptomyces alboniger* 12464
- *S. alboniger* 12462
- *S. albosporeus* (Biwako-C)
- *S. antibioticus* IMRU 3720
- *S. antibioticus* IMRU 3491
- *S. cinnamoneus*
- *S. filipinensis* (Biwako-D)
- *S. fradiae* IMRU 3535
- *S. fradiae* IMRU 3535-R7
- *S. griseoluteus* IMRU 3718
- *S. griseus* LP-16
- *S. lavendulae* 3440 1-Y
- *S. lavendulae* 3440-14

ACTINOMYCETES (cont.)

- *S. lavendulae* CBS 16245
- *S. odorifer* IMRU 3334
- *S. odorifer* ATCC 6246
- *S. resistomycificus* (Biwako-B)
- *S. sulfureas* R6678
- *S. viridochromogenes* 94
- *S. viridochromogenes* 26-L
- *Streptomyces* sp. 27-20
- *Streptomyces* sp. B-5a
- *Streptomyces* sp. B-7
- *Streptomyces* sp. CWW3
- *Streptomyces* sp. 37-12
- *Streptomyces* sp. (lavendulae group) (P-V-011)

BLUE-GREEN ALGAE

- *Symploca muscorum* 1V617
- *Oscillatoria agardhii*
- *Oscillatoria tenuis*

Table 1. Continued.

GEOSMIN PRODUCERS	
BLUE-GREEN ALGAE (cont.)	FUNGI
Amphanizominon sp.	*Basidobolus ranarum*
Anabaena circinalis	*Chaetomium globosum*
Shizothrix muelleri Nageli	
Lyngbya sp.	

METHYLISOBORNEOL PRODUCERS	
ACTINOMYCETES	ACTINOMYCETES (cont.)
Actinomadura sp. I-15	*S. odorifer*
Nocardiopsis (*Actinomadura*) *dassonvillei* IMRU 1324	*S. odorifer* ATCC 6246
Streptomyces albosporeus (Biwako-C)	*S. praecox* ATCC 3374
S. antibioticus Nr 5234	*S. resistomycificus* (Biwako-B)
S. antibioticus ATCC 5234	*Streptomyces* sp. 100-1
S. filipinensis (Biwako-D)	*Streptomyces* sp. AY-19
S. griseus ATCC 10137	*Streptomyces* sp. AY-219
S. lavendulae IMRU 3440-1 Y	*Streptomyces* sp. (lavendulae group) (P-V-011)
S. lavendulae CWW 3	BLUE-GREEN ALGAE
S. lavendulae CBS 16245	*Oscillatoria curviceps*

Table 2. Media for Isolating Actinomycetes Producing Taste and Odor Metabolites.

MEDIUM	REFERENCE
RS Medium for Isolation RS Mass Culture Medium	Romano and Safferman 1963
Yeast-Dextrose Soy Bean Meal	Gerber 1974
Pablum Medium Krainsky's Agar	Gerber and Lechevalier 1965
Chitin-Actidone Agar Starch Casein Agar	Willoughby 1969
Czapek-Dox	Yurkowski and Tabachek 1974
Enriched Broth Medium M_1B_2	Silvey and Roach 1975
Basal Salts Medium ISPM-4 Sodium Caseinate	Yagi et al. 1981

The generalized life cycle of Streptomyces sp. has been described (Higgins and Silvey 1966, Silvey and Roach 1975, Roach and Silvey 1958). It consists of two mycelial phases that are easily distinguished in liquid media. The first phase, referred to as "submerged," "vegetative," or "primary mycelial," is a facultative anaerobe with mycelia (0.2 to 0.6 um in diameter) propagating by vegetative fragmentation. The primary phase gives rise to the secondary phase through a terminal or auxiliary, bud-like structure (Higgins and Silvey 1966).

The initiation of the secondary phase, referred to as either the "aerial," the "reproductive," or the "secondary mycelial," requires a minimum temperature of 17°C and a dissolved oxygen content equal to or greater than 1.8 ppm (Silvey and Roach 1975). This secondary phase is an obligate aerobe which forms spores. The mycelia and the spores are from 0.6 to 1.2 um in diameter (Silvey and Roach 1975). The secondary phase of hydrophobic filaments is responsible for the production of taste and odor metabolites (Bentley and Meganathan 1981, Redshaw et al. 1979, Silvey and Roach 1975).

Certain species of Micromonospora and Nocardia are exceptions to this generalized life cycle. The Micromonospora species lack a secondary phase in their life cycle. The Nocardia species have primary mycelia

which can fragment into rods or coccoid elements before producing the secondary phase (Cross and Goodfellow 1973).

Factors Influencing the Growth of Actinomycetes and Production of Metabolites

The production of metabolites by actinomycetes is based upon the coincidence of the secondary growth phase and the appropriate environmental conditions. The conditions favoring the production of taste and odor metabolites generally occur during March or October in the southern United States (Silvey and Roach 1975), from September to October in southern California (McGuire et al. 1981), and from May to late October in western Canada (Yurkowski and Tabachek 1974), but are limited to either May or October in Japan (Yagi et al. 1981).

The numbers of actinomycetes present in water supplies cannot be used as an indicator for taste and odor problems. Large numbers of actinomycetes have been reported in water samples without taste and odor metabolites (Henley et al. 1965) and conversely, actinomycetes have been undetected in water samples with concentrated odor-causing metabolites (Cross 1981).

By manipulating the nutrients or the incubation temperature of a medium or both, changes can be brought

about in actinomycete growth and in metabolite production, including those responsible for taste and odor. Temperature is a primary factor in controlling the growth rate and the transition from one growth stage to another (Weete et al. 1977). A temperature coefficient (Q_{10}) of two applies in the temperature range of 10° to 36°C (Higgins and Silvey 1966).

Weete and his associates (1977) determined that optimum growth of a Streptomyces sp. on Romano and Safferman's medium occurred at 30°C and a pH between 7 and 9. Optimum growth of another Streptomyces sp. on enriched medium occurred between 32° and 35°C and at a pH range of 6.5 to 7.0 (Higgins and Silvey 1966).

The amount of phosphorus added to a medium affected the growth of streptomycetes. No growth was found in a medium lacking phosphate-phosphorus (Higgins and Silvey 1966), but increasing the amount of inorganic phosphorus from 0.0258 to 0.774 $ug \cdot ml^{-1}$ produced a linear increase in growth (Weete et al. 1977). In liquid cultures the depletion of phosphorus by several Streptomyces spp. favored sporulation (Kalatkouskii and Agre 1976). The growth of Nocardia turbata has been affected by the form of nitrogen utilized. The use of nitrate-nitrogen resulted in scanty vegetative growth, whereas ammonia-nitrogen promoted abundant vegetative

growth (Kalakoutskii and Agre 1976). The opposite effect was demonstrated in the growth of Streptomyces (isolate 33) when nitrate-nitrogen and ammonia nitrogen were used (Weete et al. 1977).

Carbon sources and specific anions can also affect the amount of growth in Streptomyces. Weete and his associates (1977) found that succinate, glycerol, and sucrose were more effective carbon sources than glucose, cellobiose, maltose yeast extract, or lactose. Silvey and Roach (1975) determined that HCO_3^- and Cl^- were essential for the growth of actinomycetes.

Although phosphate is essential for the growth of actinomycetes (Silvey and Roach 1975), the biosynthesis of many antibiotics and other metabolites is inhibited by inorganic phosphates present in the range of 1 to 50 ppm (Martin 1978). As the phosphate concentration is increased, the production of antibiotics is either inhibited or stopped (Martin and Demain 1977). The production of chlortetracycline by Streptomyces aureofaciens required utilization of all the inorganic phosphates in the medium. In the absence of phosphates, growth rates do not stop immediately (Vanek et al. 1973). This suggests an intracellular storage pool of inorganic phosphates (Kalakoutskii and Agre 1976, Vanek et al. 1973).

Conflicting results have been reported concerning increasing amounts of phosphorus on geosmin production. When phosphorus was added to a phosphorus-limited medium, it seemed to have little effect on the production of geosmin (on a dry weight basis) by Streptomyces isolate 33 from a lake in Auburn, Alabama (Weete et al. 1977). Yagi and his associates (1981) found that increasing the phosphates in a limited basal salts medium increased the T.O.N. of the culture of Streptomyces S-1 isolated from Lake Kasumigaura in Japan.

The Auburn study (Weete et al. 1977) reported other chemical and physical variables affecting geosmin production. Geosmin production, on a dry weight basis, was favored by the use of ammonia-nitrogen and nitrate-nitrogen in the growth medium at a temperature of 25°C, an alkaline pH (9 to 10), and either succinate, sucrose, or lactose as carbon sources. Different carbohydrate sources in a basal salts medium did not produce any quantitative changes in geosmin production, but demonstrated a qualitative change in odor production by seventeen strains of Streptomyces (Sivonen 1982).

In freshwater environments, the optimum growth temperature for actinomycetes is 25°C and this growth is enhanced at a slightly alkaline pH (Silvey and Roach 1975). The water temperature must be at least 17°C in

the Southwest (Silvey and Roach 1975) and between 18° and 20°C in the Southeast (Rashke et al. 1975) before actinomycetes begin to utilize organic carbon and then produce taste and odor metabolites. In Silverwood Lake (southern California), geosmin was found in the water column where the dissolved oxygen content was at least 8 ppm and the temperature was greater than 20°C (McGuire et al. 1981).

In lakes, organic nutrients are provided for actinomycetes by the submergent/emergent vegetation and allocthonous leaf material (Willoughby 1969), and by decomposing plant and animal matter (Cross 1981). In rivers, algae (Erdei 1969) and allocthonous leaf material (Willoughby 1969) provide the organic nutrients. The amount of nutrients washed in from soils is greater in an agricultural than a non-agricultural area because of the increased presence and use of manures and commercial fertilizers (Cross 1981).

The amount and frequency of rainfall directly affects the concentration of metabolites in lakes and rivers and, therefore, indirectly affects taste and odor episodes. Rashke et al. (1975) reported that an initial high flow rate due to rainfall would flush actinomycetes and nutrients into the rivers. Then, if

the flow rate subsided for a minimum of two weeks, mild musty odors would occur in the water system because of the concentration of metabolites. This musty odor episode would persist until high flow rates ($\geq$ 6500 cfs) occurred and diluted the metabolites.

Metabolic Pathways Generating Taste and Odor Metabolites

Secondary metabolites appear to be responsible for taste and odor generated by actinomycetes in water (Gerber 1979, Weete et al. 1979, Rosen 1970). Ideally secondary metabolites appear only in small, systematically defined groups of organisms (family, genus, species), occur as a variety of compounds belonging to different chemical groups, and serve no obvious function in the producing organism. The production of secondary metabolites can be considered part of the biochemical evolution of the actinomycete. In this type of evolution a new metabolite must not adversely affect the established functions of the organism. This allows the possibility of evolving another product of ecological advantage (Zähner 1978). Secondary metabolites can be produced in substantial quantities, especially by microorganisms, in an aqueous medium (Turner 1973).

Secondary metabolites are formed by and use the intermediates of a small number of biosynthetic

pathways (Turner 1973). In the case of actinomycetes, these pathways are the polyketide and terpenoid pathways. These biosynthetic pathways diverge from the glycolysis-tricarboxylic acid cycle metabolic pathway immediately after the production of acetyl CoA (Figure 2). The polyketide route results from a modification in the pathway between malonate and the fatty acids; the terpenoid route begins with mevalonate (which is produced by condensation of three acetate units), decarboxylation of which affords the isoprenoid precursor, isopentyl pyrophosphate.

Geosmin and MIB both appear to rise from isoprenoid precursors--geosmin by modification of a sesquiterpene, and MIB by methylation of a monoterpene. Although the possibility exists that geosmin may be produced by the polyketide route, the production of angularly substituted decalins by this pathway is infrequent (Bentley and Meganathan 1981).

The production of secondary metabolites, pigments, and aerial mycelia may be controlled by inter-organismal plasmid transferral which could increase the rate of biochemical evolution and variability (Martin 1978). Recently, this has been confirmed by the production of an aerial mycelia-negative strain of Streptomyces. The loss of one or

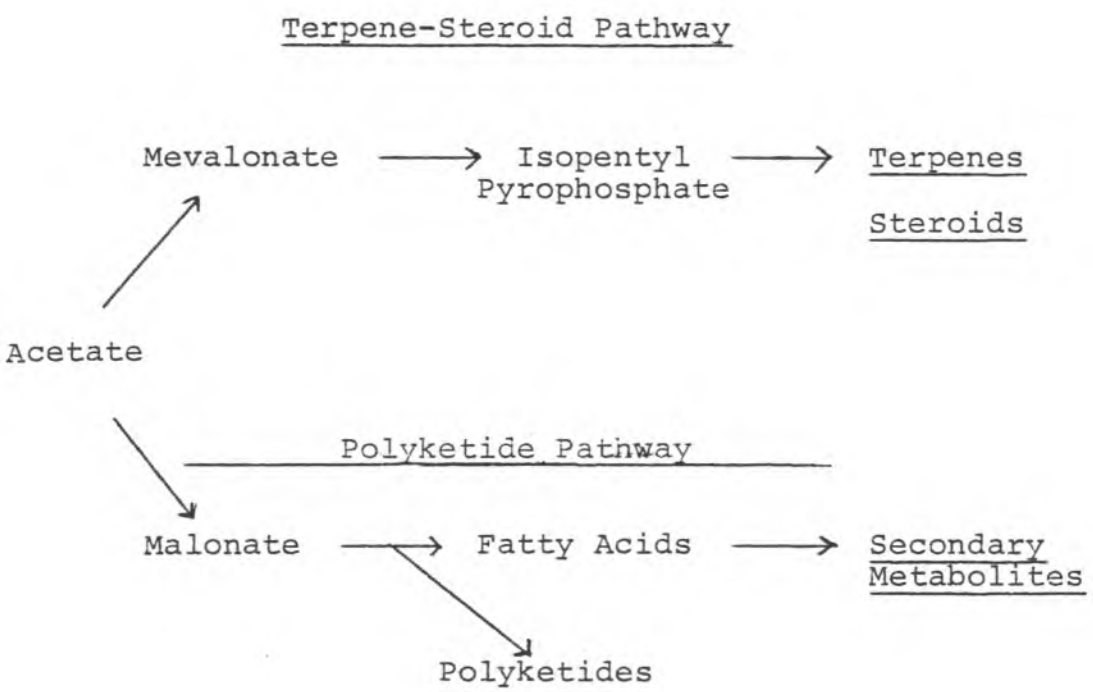


Figure 2. Abbreviated metabolic pathways for secondary metabolites. (Adapted from Turner 1973.)

more extra-chromosomal elements coding for arginino-succinate synthetase caused this strain of Streptomyces not to produce aerial mycelia or geosmin (Redshaw et al. 1979). Bentley and Meganathan (1981) suggested that their data for aerial mycelia-negative isolates and the isolates reported by Redshaw et al. (1979) could be explained by Sermonti's statement (1980) that another transposable genetic element other than a plasmid could be responsible for the growth of aerial mycelia-negative colonies.

Methods for Isolating and Characterizing Metabolites

The collection, confirmation, and quantification of secondary metabolites produced by actinomycetes are necessary in determining the source of tastes and odors in rivers and in impoundments. Collection of the taste and odor-causing material is accomplished by one of several methods. When activated carbon is used in the treatment or collection of these materials in vivo, the adsorbed metabolite can be extracted with dichloromethane, carbon disulfide, or ether (Kikuchi et al. 1973b, McGuire et al. 1981, Weete et al. 1977). Another in vivo method, the "closed-loop stripping process," utilizes a recirculating stream of air to remove semivolatile organic compounds from the gas

phase by a carbon filter before extraction with carbon disulfide (McGuire et al. 1981). The metabolites from in vitro isolated and/or cultivated actinomycetes can be absorbed by n-butyl phtalate, or the cultures can be distilled and the metabolite extracted with ether or dichloromethane (Weete et al. 1977, Gerber and Lechevalier 1965, Kikuchi et al. 1973b, Medsker et al. 1969, Romano and Safferman 1963, Wood and Snoeynik 1977, Vajdic 1971).

Identification of the metabolites is made by comparing their infrared (IR) spectra (Weete et al. 1977), nuclear magnetic resonance (NMR) (Gerber 1969, Weete et al. 1979), or mass spectra (MS) (Kikuchi et al. 1973a, Murray et al. 1975, Yurkowski and Tabachek 1974) against a known standard or a published spectrum. A fourth method of identification matches the retention time of the unknown compound with a sample of the known metabolite on three different gas chromatography columns (Safferman et al. 1967). Quantifying the amount of metabolite present in an extraction has involved the use of an "odor panel" (Medsker et al. 1968, Romano and Safferman 1963, Safferman et al. 1967, Yagi et al. 1981) or the use of gas chromatography with an internal standard. The use of a gas chromatograph-mass spectral analysis with an

internal standard (Kikuchi et al. 1973a, Murray et al. 1975, Tyler et al. 1978, Wood and Snoeyink 1977, Yasuhara and Keiichiro 1979, Yurkowski and Tabachek 1974, Irie et al. 1976) has also been used to quantify metabolite extracts.

INTRODUCTION

The correct form and concentration of elemental nutrients such as iron, phosphorus, nitrogen, and carbon are required for the growth and development of organisms. As a result of metabolic activity, materials, including secondary metabolites, may diffuse into the environment. It is the purpose of this study to determine what effect the form and concentration of nitrogen have on the production of actinomycete secondary metabolites which adversely affect the odor of drinking water.

The adverse effect of disagreeable tastes and odors on water quality is a widespread problem. Gerber (1979) reported that taste and odor problems have been associated with water supplies in Egypt, Russia, Argentina, and Israel. The United States had one of its first recorded taste and odor problems in 1883 (Farlow 1883). Episodes in the United States have been reported for Iowa (Morris et al. 1963), Oklahoma (Silvey and Roach 1975), South Carolina (Rashke et al. 1975), the Great Lakes Region (Teirney 1976), and southern California (McGuire et al. 1981). Odors in these episodes have been described as "fishy," "grassy,"

"yeasty," "musty," "earthy," and/or "pigpen-like" or "camphor-like" (Sivonen 1982, Allen 1960) and, because of the sensitivity of the human nose, can be detected in concentrations as small as 0.1 to 0.2 ppb.

The earthy (musty) odor was selected for this study for the following reasons: This type of odor has affected the water supply of Waco, Texas. The organic compounds responsible for the earthy odor have been identified. The organisms responsible for the production of these organic compounds have been isolated.

The organic compounds responsible for the odor episodes are secondary metabolites produced in the terpene-steroid pathway (Gerber 1979, Bentley and Meganathan 1981) (Figure 1). This is the same pathway in which the isoprene unit of natural rubber, menthol, B-carotene, and cholestrol are produced (Solomons 1980). These compounds may confer an ecological advantage on the producing organisms or be responsible for keying a sequence of events with other microorganisms (Zähner 1978).

Two of the most important of these organic compounds are geosmin (Gerber and Lechevalier 1965, Bentley and Meganathan 1981) and methylisoborneol or MIB (Gerber 1969, Medsker et al. 1969, Bentley and Meganathan 1981). Geosmin (Figure 2) is a bicylic,

twelve-carbon compound produced by blue-green algae (Medsker et al. 1968, Safferman et al. 1967), a fungus (Kikuchi et al. 1981), and actinomycetes (Gerber and Lechevalier 1965) (Table 1). Methylisoborneol is a bicyclic compound with eleven carbons (Figure 2) produced by blue-green algae (McGuire et al. 1981) and actinomycetes (Gerber 1969, Medsker et al. 1969). From the three groups of organisms capable of producing an earthy or musty odor, actinomycetes were selected based on previous studies done in the laboratories of Drs. Lind and Eldridge at Baylor University identifying these organisms as associated with local odor episodes.

Production of odor metabolites by actinomycetes is maximized by a water temperature at 25°C (Weete et al. 1977), dissolved oxygen content at least 8 ppm (McGuire et al. 1981), and pH at 7 to 9 (Weete et al. 1977). Iron (Yagi et al. 1981), succinate, sucrose, and lactose (Weete et al. 1977) have quantitative effects on the production of these metabolites. An increase in iron from 0.0 to 0.1 $mg \cdot L^{-1}$ in a basal salts medium increased the T.O.N. from 20 to 75. (Increases in T.O.N. values indicate increases in geosmin production.) The addition of succinate, sucrose, or lactose was more effective than yeast extract, glycerol, maltose, and glucose as a carbon source in the production of geosmin on a dry

weight basis. Phosphorus and nitrogen also have an effect. Yagi and associates (1981) reported an increase in T.O.N. from 5 to 80 when inorganic phosphorus was increased from 0.0 to 0.8 $mg \cdot L^{-1}$ in a basal salts medium. Weete and associates (1977) had previously come to the conclusion that inorganic phosphorus had little stimulatory effect on geosmin production. Yagi et al. (1981) found that by increasing the concentration of nitrate-nitrogen (NO_3-N) as KNO_3 from 0.0 to 1.0 $mg \cdot L^{-1}$, the T.O.N. value was increased from 5 to 60. Weete et al. (1977) recorded more geosmin production in cultures when both NO_3-N as $NaNO_3$ and ammonia-nitrogen (NH_3-N) as NH_4Cl were added as the growth medium nitrogen source than when NO_3-N and NH_3-N were added separately. However, they found that lower concentrations of either NO_3-N or NH_3-N in their standard medium produced more geosmin on a dry weight basis than higher concentrations. They also found that growth increased as NO_3-N or NH_3-N concentrations increased with NO_3-N producing more biomass. These statements suggest that both NO_3-N and NH_3-N concentrations are negatively related to geosmin production and positively related to growth. Yagi et al. (1981) suggested that increases in NO_3-N concentrations are positively related to geosmin production. Thus,

studies conflict in their reports of the effects of NO_3-N on metabolite production.

The present study attempted to resolve these differences regarding the effect of form and concentration of nitrogen on the production of geosmin by a Streptomyces sp. The forms of nitrogen used were NO_3-N as KNO_3, NH_3-N as NH_4Cl, and, unlike the previous studies, included organic nitrogen as asparagine. The different nitrogen compounds and concentrations were added to nitrogen-free Thornton's Medium (Johnson and Curl 1972). The effects of the form and concentration of nitrogen on threshold odor number (T.O.N.), biomass production (mg dry wt), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}) were examined.

METHODS AND MATERIALS

Isolation of Actinomycetes

The actinomycetes were isolated from a mud sample taken at a depth of 12 m from Lake Waco using an Ekman dredge. The mud sample was divided into two 300 ml subsamples. One subsample was used to inoculate three Petri dishes (10 cm i.d.) containing Thornton's Medium (Table 3). The other subsample was kept sealed at room temperature in case isolates were not found in the first subsample. As mycelial growth appeared on the agar plates, part of each mycelium was transferred to separate Yeast-Dextrose and Thornton's Medium slants for determining odor production, growth form, color of aerial mycelia, and pigment production.

Identification of Isolates

The actinomycete isolates were separated and identified to genus using two procedures. The first separated the isolates in the mud sample based on the presence or absence of pigment and the color of the secondary mycelium when grown on Yeast-Dextrose agar or Thornton's Medium agar. In the second procedure, the

Table 3. Thornton's Standardized Medium.
(Johnson and Curl, 1972)

Ingredient	Amount
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$CaCl_2$	0.1 g
NaCl	0.1 g
$FeCl_3$	trace
KNO_3*	0.5 g
Asparagine*	0.5 g
Mannitol	1.0 g
Distilled H_20	1000.0 ml

*Used only in isolation technique and not for experimental purposes.

isolates were individually streaked on a Yeast-Dextrose agar plate. After 3 to 5 days growth, a sterile cover slip was inserted into the agar at a 45° angle adjacent to the colony. After 2 days the cover slip with attached mycelial growth was removed from the agar, secured to a microscope slide with a drop of water with the mycelial growth exposed, and viewed at 400X using a light microscope (Blevins personal communication 1982). Identification to genus was based on the structure of the sporophore of secondary mycelium (Shirling and Gottlieb 1966). Each identified isolate was grown in pure culture on a separate Thornton's Medium agar slant, then covered with sterile mineral oil, and refrigerated for reference.

Identification of Metabolites by GC-MS

From the four Streptomyces isolates and one possible Nocardia or Micromonospora isolate from the mud subsample, two Streptomyces isolates (WM1C1 and WM1) were selected for study based on their intense odor production in Thornton's Medium and on a gas chromatography screening against Environmental Protection Agency standards (Figures 3, 4, and 5). The two isolates were cultured in three, 500 ml Erlenmyer flasks. Each flask contained 200 ml of Thornton's Medium with NH_4Cl as the nitrogen

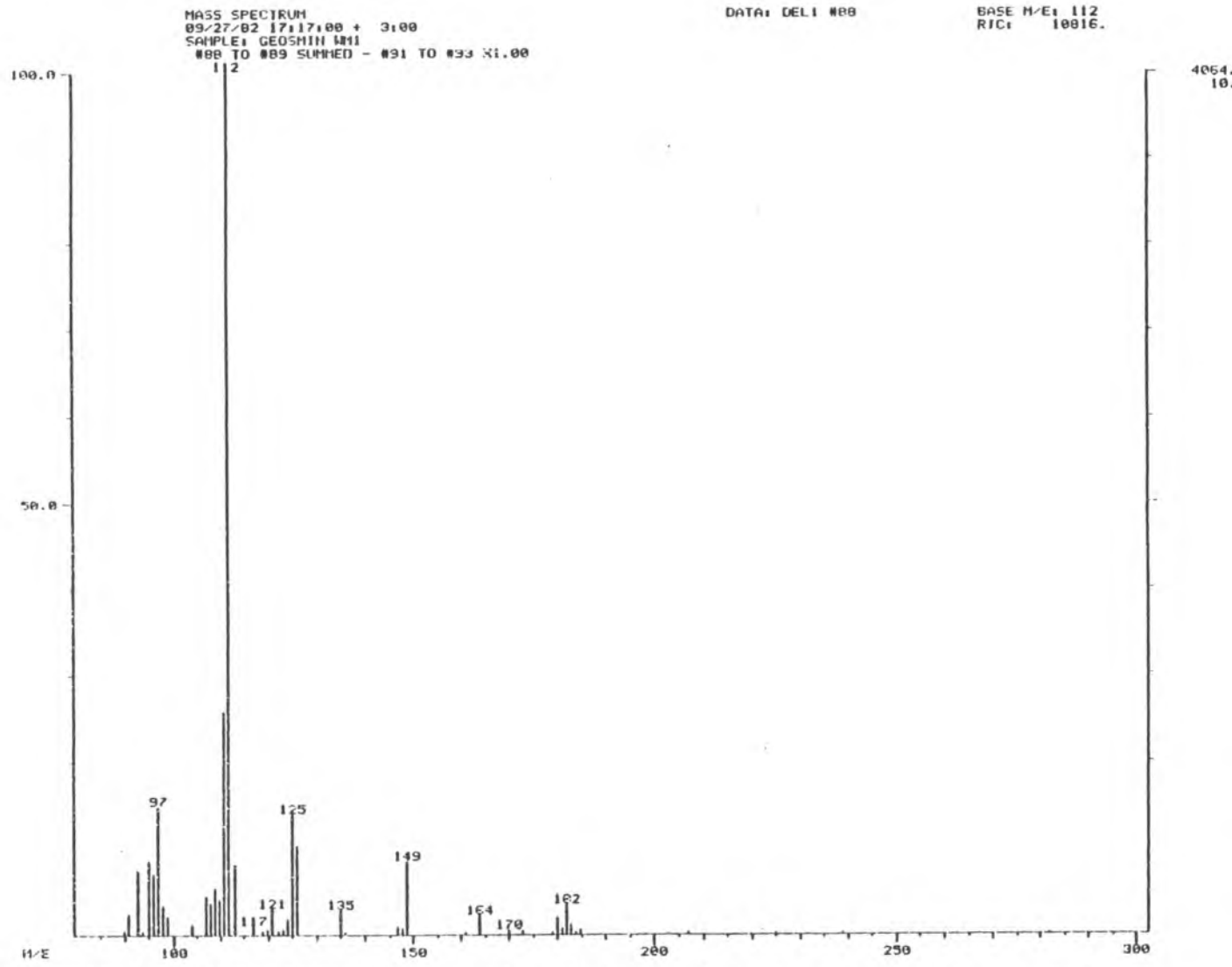


Figure 3. Mass spectrum of extract from Streptomyces Isolate WM1.

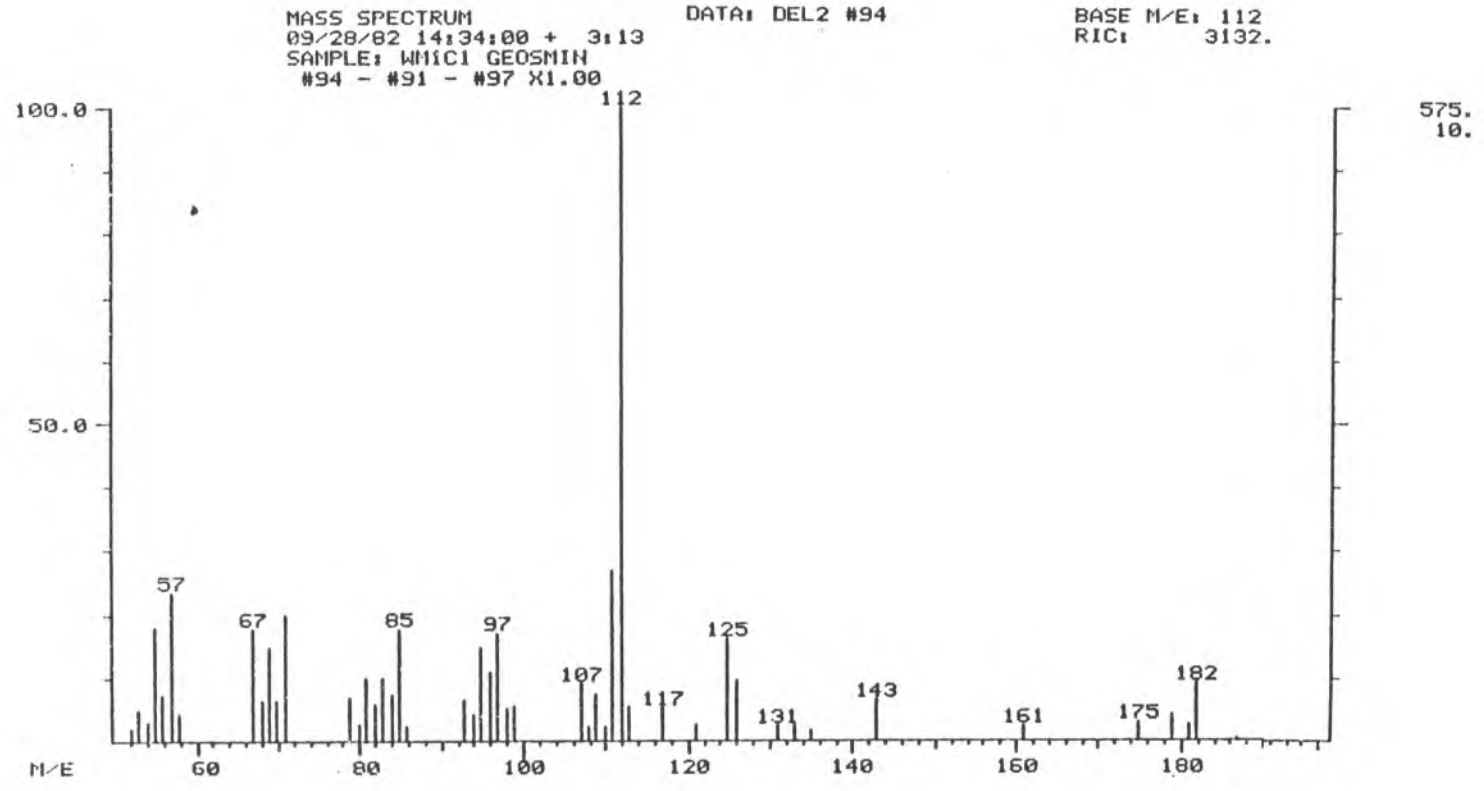


Figure 4. Mass spectrum of extract from Streptomyces isolate WM1C1.

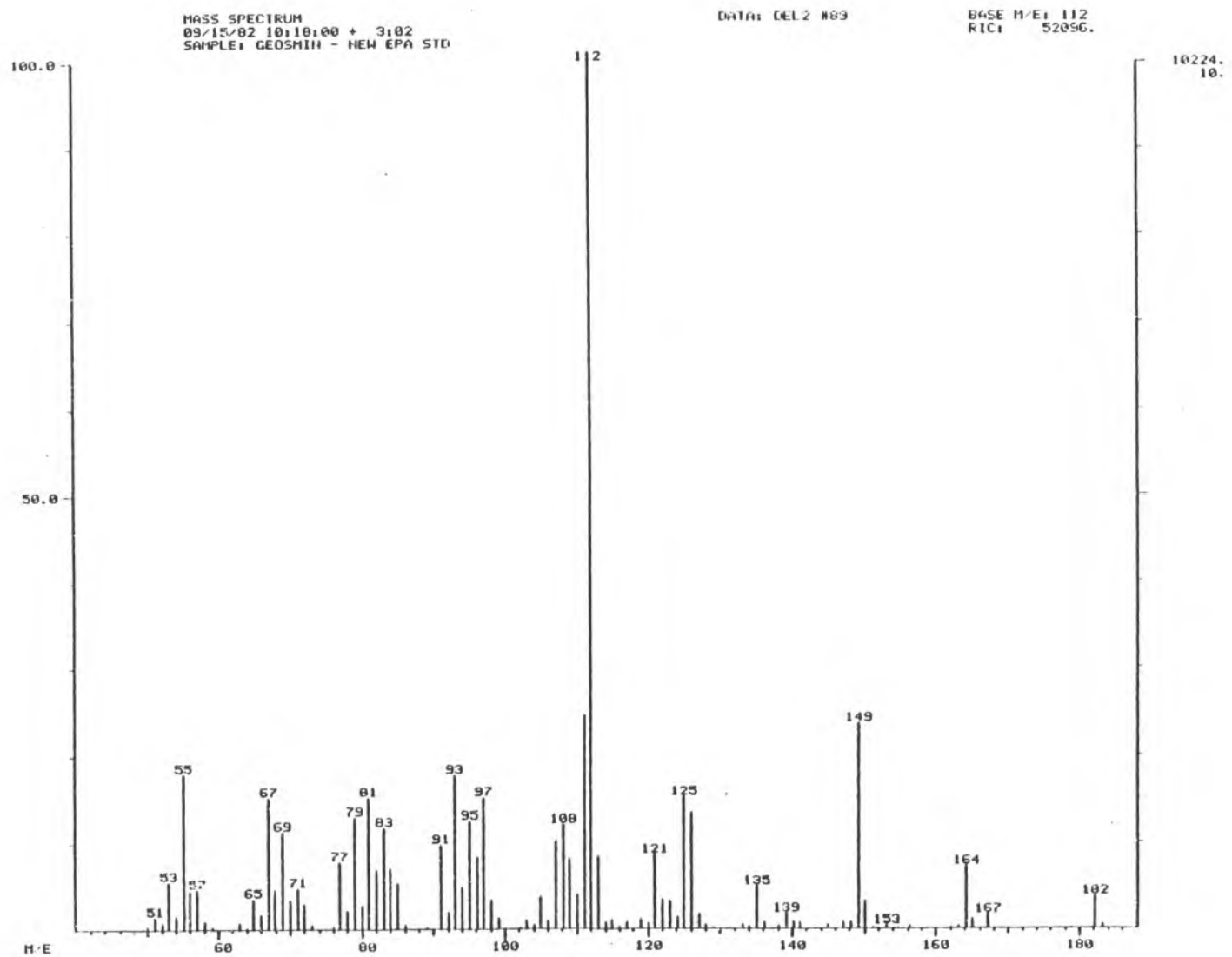


Figure 5. Mass spectrum of EPA geosmin standard.

source. The flasks were placed on a rotary shaker at 215 rpm at 29°C. After 48 hours the three flasks of each isolate were pooled, equal amounts (600 ml each) were poured into two 2800 ml Fernbach flasks, and enough Thornton's Medium was added to bring the final volume to 1500 ml. The Fernbach flasks were placed on a reciprocal shaker at 80 spm at room temperature (21.6° to 25.5°C) for 5 days. This speed ensured that the mycelia and spores would be kept in suspension to increase both growth rate and odor development (Romano and Safferman 1963).

Distillation and Extraction of Metabolites

The 1500 ml of medium and growth suspension were distilled in glass to 300 ml. The distillate was extracted twice with 60 ml of dichloromethane (CH_2Cl_2), the extracts placed in a 250 ml Erlenmeyer flask in a 30°C water bath, and the contents reduced to approximately 2 ml. The 2 ml of reduced extract were rinsed with dichloromethane into a preweighed test tube, returned to the 30°C water bath, and the volume reduced to less than 1 ml of material. This volume was determined by reweighing the test tube and its contents, subtracting the weight of the test tube and dividing the

remainder by 1.3266 (density of dichloromethane) (Handbook of Chemistry and Physics 1981) for the final volume determination. A Finnegan GC-MS with a 3% OV-1, 6' column was used to match the retention time and the mass spectrum of the sample against EPA-supplied standards for both geosmin and MIB.

Experimental Design for Odor and Biomass Production

Liquid nitrogen-free Thornton's Medium was used as the basic medium. After preliminary study of $NaNO_3$, KNO_3, NH_4NO_3, NH_4Cl, $Ca(NO_3)_2$, and $(NH_4)_2SO_4$ as nitrogen sources, KNO_3 and NH_4Cl were selected as the NO_3-N and NH_3-N sources respectively. This selection was based on production of odor and growth by the isolate WM1C1. Asparagine was the organic nitrogen source because it was one of the nitrogen sources in the Thornton's Medium. Five concentrations of each nitrogen source (0.000 $mg \cdot L^{-1}$, 0.075 $mg \cdot L^{-1}$, 0.150 $mg \cdot L^{-1}$, 0.300 $mg \cdot L^{-1}$, and 0.600 $mg \cdot L^{-1}$), and three replicates per concentration were used.

The inoculum was prepared by pipetting 50 ml of the mycelia/spore suspension from a 1500 ml stock culture of strain WM1C1 into a sterile container. The 50 ml of suspension were filtered using a Gelman type A/E glass fiber filter. The filtered cells were resuspended and

rinsed four times with 50 ml of sterilized nitrogen-free Thornton's Medium. The washed mycelia and spores were resuspended in 50 ml of sterilized nitrogen-free Thornton's Medium.

Each growth culture consisted of 1 ml of washed isolate WM1C1 mycelia/spore inoculum added to 200 ml of Thornton's Medium with the appropriate experimental nitrogen source in a 500 ml Erlenmyer flask. The pH was previously adjusted to 7.0 to 7.2 before autoclaving at 121.5°C and 15 psi for 20 min. The inoculated flasks were placed on a reciprocal shaker set at 80 spm at room temperature (21.6° to 25.5°C) for incubating. The growth was terminated after 14 days by refrigerating the flasks at 4°C. Growth ceases at 7°C (Silvey and Roach 1975).

Dry weights were determined by filtering the contents of each flask through a predried and preweighed type A/E glass fiber filter. Each filter was rinsed first with 50 ml of 5% HCl to remove any alkaline deposits and then with 50 ml of distilled water to remove the acid residue. The filters were oven-dried at 103°C until a constant weight (to the nearest milligram) was attained (at least 24 hrs.).

Threshold odor numbers (T.O.N.) were used to compare relative concentrations of the odor metabolites

(American Public Health Association 1981). First, a "screening" panel of two judges determined if there was an odor produced in a growth flask. The flasks with odor were characterized as "faint," "mild," or "definite." This characterization determined the dilution series for the T.O.N. determinations. The dilution series in T.O.N. for the flasks labeled "faint" was 4, 8, 16, 32, and 64. The series for the flasks labeled "mild" was 16, 32, 64, 128, and 256. The dilution series for the flasks labeled "definite" was 32, 64, 128, 256, 512. Each dilution in a series was arbitrarily labeled with a letter and three digits (e.g. A123). The growth flasks without odor (as determined by the screening panel) were submitted to the "analytical" panel (9 members). Those flasks determined to have odor by this latter panel were assigned a T.O.N. of 2. Those without odor were given a T.O.N. of 0.

Before presentation of the dilution series to the "analytical" panel, the members were given an opportunity to smell the odor in a high and a low concentration so that they could recognize the odor to which they were to respond. These concentrations and all dilution series were equilibrated at 40°C in a water bath to enhance the odor before presentation to the panel. The panel members were read the following instructions before their initial trial:

> There are five samples in each series to be tested for the presence or absence of odor. You do not need to describe the odor, just write down the letter and three digits of the first flask in a series in which you detect an odor. After detecting an odor in one series of flasks, count to thirty before going to the next series.

The panel members were then read this procedure for sampling the flasks:

> Remove a flask from the water bath and turn away from the water bath. Shake the flask, remove the glass top, and briefly sniff the mouth of the flask. Replace the glass top, return the flask to the water bath, record the letter and three digits of the flask if you detected an odor. If no odor was detected, continue this procedure with the next flask in the series until you do detect an odor.

Data Analyses

To determine the effects of the form and concentration of nitrogen, these data were subjected to an analysis of variance and a regression analysis. A two-way analysis of variance was used to determine significant relationships between the different concentrations of nitrogen, between the different forms of

nitrogen, and between the interactions of the concentrations and forms of nitrogen with the dependent variables of T.O.N., biomass, or Specific T.O.N. The Bonferroni multiple comparison method was utilized to find the significantly different factor means or interaction treatments (Neter and Wasserman 1974).

A regression analysis was used to determine the nature of the statistical relationship between the concentrations of each nitrogen form and the dependent variables of T.O.N., biomass, or Specific T.O.N. The Pearson's Product Moment Correlation Coefficient (r_p) and a plot of the residuals were used to determine the significance of these relationships. If the relationships did not fit a simple linear regression model, the Spearman's Rank Correlation, a nonparametric test, was used to determine the relationship between the dependent and independent variables. A significant correlation coefficient (r_{sp}) indicated that the relationship was either monotone (large values of X are associated with large values of Y or large values of X are associated with small values of Y) increasing or decreasing. All results were considered significant at the 0.95 level of confidence.

RESULTS

Identification of Isolates

Five different isolates were distinguished based on the different growth forms, color of the secondary mycelia, and pigment production when grown on Yeast-Dextrose Agar or in Thornton's Medium with NH_4Cl as the nitrogen source (Table 4). The isolates were designated WM1C1, WM3C1, WM1A, WM1, and WM23. Four isolates (WM1C1, WM3C1, WM1A, and WM1) were assigned to the genus Streptomyces based on the sporophore structure of either retinaculum apertum or spira produced by the secondary mycelia (Shirling and Gottlieb 1966). The identification procedure suggested that one isolate (WM23) which formed no secondary sporophores on Yeast-Dextrose Agar was a strain of Nocardia or Micromonospora.

Identification of Metabolites by GC-MS

Even though all five isolates produced odors, only two (WM1 and WM1C1) produced geosmin. The mass spectra of these isolates contained the parent ion and accompanying fragment ions matching those produced by

Table 4. Growth Characteristics of Five Actinomycete Isolates Grown on Yeast-Dextrose Agar (YD) and in Thornton's Medium (TM) with NH_4Cl.

	YD			TM		
Name	Growth Form	Color	Pigment	Growth Form	Color	Pigment
*WM1C1	Mycelium	Blue-Gray	+	Mycelium	White	-
*WM1A	Mycelium	Gray-Black	+	Mycelium	White	+
*WM1	Mycelium	Gray	+	Fragmented Colony	White	-
*WM3C1	Mycelium	White	-	Mycelium	Darkens w/age	-
WM23	Rod-Shaped Fragments	White	-	Mycelium	Darkens w/age	+

+ = pigment present

- = pigment absent

* = Streptomyces spp.

the EPA geosmin standard (Figures 3, 4, and 5). Also the retention times for the WM1 and WM1Cl extracts matched the EPA geosmin standard (90 scans ± 2).

Effect of the Form and Concentration of Nitrogen on Specific T.O.N.

The form of nitrogen had a significant effect on Specific T.O.N. (T.O.N.·mg dry wt^{-1}) (ANOVA $p < 0.05$, Table 5). NH_3-N produced a significantly higher T.O.N. on a dry weight per flask basis than NO_3-N ($p < 0.05$), but not significantly higher than ORG-N (Tables 5 and 6). Because of the large variation of data across nitrogen forms and the nonlinear relationship of nitrogen concentration to Specific T.O.N., neither the ANOVA nor the simple linear regression analysis, respectively, showed a significant relationship between nitrogen concentration and Specific T.O.N.

However, a rank correlation analysis (r_{sp}) did demonstrate that large values of Specific T.O.N. are significantly associated with large values of nitrogen concentration for NH_3-N and NO_3-N ($p < 0.05$, Table 7). The rank correlation results do not contradict those of the ANOVA nor the regression analyses because the rank correlations do not indicate in any way a significant difference (decrease or increase) between Specific T.O.N. over the nitrogen concentrations, nor does it

Table 5. Two-Way ANOVA Mean Square Values for Concentration of Nitrogen (MS_c), Form of Nitrogen (MS_f), Interaction of Concentration and Form (MS_{cf}), and Mean Square Error (MS_e); Degrees of Freedom (d.f.); F-test Values; and Confidence Intervals for Significant F-test Values for Specific T.O.N. (T.O.N.·mg dry wt^{-1})

Mean Square		d.f.	F-test
MS_c	5.6	4	2.0
MS_f	10.6	2	3.8*
MS_{cf}	3.4	8	1.2
MS_e	2.8	27	

Mean Value Confidence Intervals for Nitrogen Form

NO_3-N	NH_3-N	ORG-N

*F = 3.36 is significant at $p < 0.05$ based on d.f. = (2,27).

__ = no significant difference at $p < 0.05$ based on Bonferroni Multiple Comparison Method.

Table 6. Two-Way ANOVA Mean Values of T.O.N. (threshold odor number), Biomass (mg dry wt^{-1}), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}) for Nitrogen Forms and Concentrations ($mg \cdot L^{-1}$)

	T.O.N.	Biomass	Specific T.O.N.
Form			
NH_3-N	19.2	6.3	4.3
NO_3-N	11.9	7.3	1.6
ORG-N	10.3	6.4	2.0
Conc.			
0.000	6.2	9.0	0.4
0.075	10.3	2.7	4.1
0.150	12.3	4.8	3.0
0.300	10.9	6.9	2.7
0.600	29.4	10.0	3.0

Table 7. Spearman's Rank Correlation Coefficient (r_{sp}) for the Relationship Between the Concentration ($mg \cdot L^{-1}$) of Each Nitrogen Form and T.O.N. (threshold odor number), Biomass (mg dry wt^{-1}), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}) Not Fitting a Simple Linear Regression Model. ($r_{sp} = 0.458$ is significant at $p < 0.05$ based on a Z score)

Nitrogen Form	Conc. vs T.O.N.	Conc. vs Biomass	Conc. vs Specific T.O.N.
NO_3-N	0.65	----	0.47
NH_3-N	----	0.31	0.54
ORG-N	0.26	----	0.13

---- = fit a simple linear regression model.

imply that the relationship between Specific T.O.N. and nitrogen concentration is linear.

Effect of the Form and Concentration of Nitrogen on T.O.N.

T.O.N., although not affected by nitrogen form, was significantly affected by nitrogen concentration (ANOVA $p < 0.05$, Table 8). Mean T.O.N. values for 0.600 $mg \cdot L^{-1}$ were significantly higher than the mean values for 0.000 $mg \cdot L^{-1}$ but not for other concentrations ($p < 0.05$, Tables 6 and 8).

Increases in NO_3-N and NH_3-N concentrations produced linear increases in T.O.N. values (Table 9). However, a Spearman's rho was needed to confirm the regression analysis for NO_3-N because the error variance of T.O.N. residuals increased with increased concentrations (r_{sp} $p < 0.05$, Table 7).

Effect of the Form and Concentration of Nitrogen on Biomass Production

Biomass production was not affected by the form, but was significantly affected by the concentration of nitrogen (ANOVA $p < 0.05$, Table 10). The mean biomass production decreased significantly when the concentration of nitrogen was increased from 0.000 $mg \cdot L^{-1}$ to 0.075 $mg \cdot L^{-1}$ and continued to be significantly lower at

Table 8. Two-Way ANOVA Mean Square Values for Concentration ($mg \cdot L^{-1}$) of Nitrogen (MS_c), Form of Nitrogen (MS_f), Interaction of Concentration and Form (MS_{cf}), and Mean Square Error (MS_e); Degrees of Freedom (d.f.); F-test Values; and Confidence Intervals for Significant F-test Values for Threshold Odor Number (T.O.N.)

Mean Square		d.f.	F-test
MS_c	242.6	4	4.5*
MS_f	113.9	3	2.1
MS_{cf}	17.8	8	0.3
MS_e	53.6	27	

Mean Value Confidence Intervals for Nitrogen Concentration

0.000 0.075 0.150 0.300 0.600

*F = 2.725 is significant at $p < 0.05$ based on d.f. = (4,27)

__ = no significant difference at $p < 0.05$ based on Bonferroni Multiple Comparison Method

Table 9. Equations and Pearson's Product Moment Correlation Coefficients (r_{sp})* for the Simple Linear Regressions Between the Concentrations ($mg \cdot L^{-1}$) of Each Nitrogen Form and T.O.N. (threshold odor number), Biomass (mg dry wt), or Specific T.O.N. ($T.O.N. \cdot mg\ dry\ wt^{-1}$)

Nitrogen Form	Conc. vs T.O.N.	r_p Value	Conc. vs Biomass	r_p Value	Conc. vs. Specific T.O.N.	r_p Value
NO_3-N	Y = 44.1X + 2.5	0.8	Y = 9.9X + 4.8	0.6	Y = 17.1X + 1.0	0.5
NH_3-N	Y = 42.1X + 9.9	0.6	Y = 5.4X + 4.8	0.3	Y = 3.6X + 3.3	0.2
ORG-N	Y = 25.7X + 5.5	0.5	Y = 8.9X + 4.1	0.6	Y = -0.3X + 2.2	0.0

*$r_p = 0.532$ is significant at $p < 0.05$ based on d.f. = 12

Table 10. Two-Way ANOVA Mean Square Values for Concentration ($mg \cdot L^{-1}$) of Nitrogen (MS_c), Form of Nitrogen (MS_f), Interaction of Concentration and Form (MS_{cf}), and Mean Square Error (MS_e); Degrees of Freedom (d.f.); F-test Values; and Confidence Intervals for Significant F-test Values for Biomass Production

Mean Square		d.f.	F-test
MS_c	27.1	4	16.4
MS_f	1.7	2	1.0
MS_{cf}	1.3	8	0.8
MS_e	1.6	27	

Mean Value Confidence Intervals for Nitrogen Concentration

0.000	0.075	0.150	0.300	0.600

*F = 2.275 is significant at $p < 0.05$ based on d.f. = (4,27)

__ = no significant difference at $p < 0.05$ based on Bonferroni Multiple Comparison Method

0.150 $mg \cdot L^{-1}$ ($p < 0.05$, Tables 6 and 10). Although mean biomass production began to increase after 0.075 $mg \cdot L^{-1}$, no concentration (0.075 $mg \cdot L^{-1}$ to 0.600 $mg \cdot L^{-1}$) produced significantly more biomass than 0.000 $mg \cdot L^{-1}$ (Table 10). Increases in biomass production were linear corresponding to increases in NO_3-N and ORG-N concentrations (Table 9).

DISCUSSION

Effect of the Form and Concentration of Nitrogen on Specific T.O.N. (T.O.N.·mg dry wt^{-1})

Although Specific T.O.N. varies with increases in nitrogen concentration, the form of nitrogen is the significant controlling factor for Specific T.O.N. These results demonstrate that metabolite production varies based on the availability of a specific nitrogen source. Streptomyces isolate WM1C1 produced more Specific T.O.N. (geosmin on a dry weight basis since no other taste and odor metabolites were found) utilizing NH_3-N than either NO_3-N or ORG-N. These results disagree with the findings reported by Weete et al. (1977) for Streptomyces isolate 33L which produced more geosmin on a dry weight basis with NO_3-N than NH_3-N. These data indicate that separate isolates can differ in nitrogen utilization or that the opposing results may be due to different experimental techniques since the experimental methods were not identical.

The positive linear regression and the significant rank correlations of NO_3-N and NH_3-N with Specific T.O.N.

indicate that the production of geosmin on a dry weight basis by Streptomyces isolate WM1C1 increased with increasing concentrations of nitrogen. These findings contradict Weete et al. (1977) who found that lower concentrations of NO_3-N and NH_3-N initiated more geosmin production on a dry weight basis by Streptomyces isolate 33L. Again these differences could be due to the isolate used or experimental methods.

Effect of the Form and Concentration of Nitrogen on Biomass Production

The addition of a small amount, 0.075 $mg \cdot L^{-1}$, of any form of nitrogen (NO_3-N, NH_3-N, or ORG-N) to the culture medium seems to be inadequate to prompt the growth of Streptomyces isolate WM1C1. This suggests an inhibitory effect by a small amount of nitrogen due to the significant decrease in mycelial production between 0.000 $mg \cdot L^{-1}$ and 0.075 $mg \cdot L^{-1}$. The positive linear relationship between biomass production and nitrogen concentration when the production value for 0.000 $mg \cdot L^{-1}$ was deleted (Table 11) suggests that the 0.075 $mg \cdot L^{-1}$ concentration does not prompt growth but, as nitrogen concentrations are increased, there is an increase in growth.

The growth of Streptomyces isolate WM1C1 showed no significant difference among NO_3-N, NH_3-N, or ORG-N

Table 11. Equations and Pearson's Product Moment Correlation Coefficients (r_p)* for the Simple Linear Regression Between the Concentrations ($mg \cdot L^{-1}$) of Each Nitrogen Form (0.075 $mg \cdot L^{-1}$ to 0.600 $mg \cdot L^{-1}$) and Biomass

Nitrogen Form	Conc. vs. Biomass (mg dry wt)	r_p value
NO_3-N	Y = 14.8 X + 2.8	0.828
NH_3-N	Y = 10.3 X + 2.7	0.643
ORG-N	Y = 14.6 X + 1.6	0.910

*r_p = 0.576 is significant at $p < 0.05$ based on d.f. = 10

sources. This contradicts Weete et al. (1977) who reported that NO_3-N, when compared with NH_3-N, was the best nitrogen growth source for Streptomyces isolate 33L. Again this may be due to the isolate used or experimental technique.

Effect of the Form and Concentration of Nitrogen on T.O.N.

The concentration of nitrogen affects total geosmin production measured as T.O.N. As the concentration of any nitrogen form increases, there is an increase in the corresponding T.O.N. values. The NO_3-N T.O.N. versus concentration results agree with Yagi et al. (1981) who demonstrated an increase in T.O.N. as the concentration of NO_3-N was increased in a basal salts medium.

The results of the ORG-N concentrations versus T.O.N. suggests that a concentration of 0.300 $mg \cdot L^{-1}$ is critical to the production of geosmin by Streptomyces isolate WM1C1 (Table 12). The average T.O.N. value increases from 0.000 $mg \cdot L^{-1}$ to 0.150 $mg \cdot L^{-1}$, decreases slightly at 0.300 $mg \cdot L^{-1}$, and begins to increase as the concentration of nitrogen approaches 0.600 $mg \cdot L^{-1}$.

There is no previous research to support these findings. However, if certain organic nitrogen

Table 12. Mean Values and Standard Deviations of T.O.N., Biomass (mg dry wt), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}) for Nitrogen Form and Concentration

	Concentration				
	0.000	0.075	0.150	0.300	0.600
T.O.N.					
NO_3-N	6.2 ± 7.3	4.8 ± 4.8	9.1 ± 6.5	7.7 ± 8.1	31.8 ± 13.6
NH_3-N	6.2 ± 7.3	17.5 ± 13.6	16.8 ± 20.7	20.7 ± 16.1	35.1 ± 17.6
ORG-N	6.2 ± 7.3	8.5 ± 5.9	11.0 ± 10.0	4.4 ± 3.4	21.2 ± 18.5
Biomass					
NO_3-N	9.0 ± 2.8	3.7 ± 2.1	5.3 ± 1.2	7.0 ± 2.0	11.7 ± 3.8
NH_3-N	9.0 ± 2.8	1.3 ± 0.6	5.7 ± 2.9	7.3 ± 3.5	8.0 ± 1.0
ORG-N	9.0 ± 2.8	3.0 ± 1.7	3.3 ± 0.6	6.3 ± 1.5	10.3 ± 2.1

Table 12. Continued.

	Concentration				
	0.000	0.075	0.150	0.300	0.600
Specific T.O.N.					
NO_3-N	0.4 ± 0.3	1.6 ± 1.6	1.9 ± 1.7	1.5 ± 1.8	2.7 ± 0.3
NH_3-N	0.4 ± 0.3	6.7 ± 6.6	4.1 ± 5.3	6.2 ± 4.8	4.3 ± 1.9
ORG-N	0.4 ± 0.3	4.0 ± 3.3	3.1 ± 2.4	0.6 ± 0.4	2.1 ± 1.6

concentrations inhibit metabolite production in the terpene-steroid pathway (e.g. inorganic phosphates in the polyketide-polypropionate pathway inhibit chlortetracycline production [Martin 1978] and increases growth [Kalatkouski and Agre 1976]), then the same hypothesis affects the terpene-steroid pathway. The data for ORG-N demonstrate that as organic nitrogen concentrations are increased in the range of 0.000 $mg \cdot L^{-1}$ to 0.600 $mg \cdot L^{-1}$, geosmin production at 0.300 $mg \cdot L^{-1}$ drops and biomass production continues to increase.

SUMMARY

The significant effects of the form of nitrogen and the concentration of nitrogen on T.O.N. (threshold odor number), biomass production (mg dry wt), and Specific T.O.N. (T.O.N.·mg dry wt^{-1}) of Streptomyces isolate WM1C1 are:

1. Specific T.O.N. increases as concentrations of NH_3-N and NO_3-N increase.
2. More geosmin on a dry weight basis is produced on NH_3-N-enriched media than on media containing NO_3-N sources.
3. T.O.N. increases as concentrations of NO_3-N and NH_3-N increase.
4. An intermediate amount of ORG-N (0.300 $mg \cdot L^{-1}$) inhibits T.O.N. production.
5. Low concentrations of NH_3-N, NO_3-N, and ORG-N tend to inhibit or not promote significant growth.
6. Actinomycete biomass production values increase as concentrations of NO_3-N and ORG-N increase.

APPENDICES

APPENDIX A

TASTES AND ODORS IN LAKE WACO

Organisms Responsible for Tastes and Odors

The two actinomycetes (WM1 and WM1C1) isolated from Lake Waco and confirmed as geosmin producers may be responsible for taste and odor problems. Raschke and associates (1975) stated that actinomycetes were the sole organisms responsible for the tastes and odors in a South Carolina water supply. Lin (1977) reported the same to be true for reservoirs in the Southwest United States.

However, metabolites from other biotic sources may be interacting to produce disagreeable taste and odors in the lake. Algal blooms in the North Bosque River supplying Lake Waco accompany taste and odor episodes (Meadows personal communication 1982). This compounding effect of two different types of organisms, possibly producing the same metabolite, has been reported by Kikuchi et al. (1973b). The quantity of metabolites produced by actinomycetes and blue-green algae may contribute equally to the taste and odor problem or,

as Persson (1979) and Tabachek and Yurkowski (1976) reported, the quantity of taste and odor metabolites produced by the actinomycetes may be insignificant in comparison to the amount produced by specific blue-green algae.

Abatement Alternatives

Presently a taste and odor episode is determined by complaints from consumers (Lin 1977). In response, the water plant engineer determines the intensity of the offending taste and odor metabolites by a threshold odor number (T.O.N.) (American Public Health Assoc. 1981, Suffet and Segall 1971), and proceeds to oxidize the taste and odor by the addition of chlorine or $KMnO_4$ to the water supply or includes activated carbon in the filtering process to absorb the disagreeable compound(s) (Silvey and Roach 1975).

From this study and others (Cross 1981, Yagi et al. 1981, Weete et al. 1977) there are available alternatives to the water plant engineer for treating taste and odor problems. One is to monitor the nitrogen levels in the streams and lakes that supply the water source. As nitrogen concentrations increase, abatement measures can be initiated in advance of consumer

complaints. Another alternative is to directly affect production of blue-green algae, the main source of nitrogen in many lakes and streams. By holding and gradually releasing runoff water, especially in an agricultural watershed, the nitrogen concentration of the water and the blue-green algal blooms can be controlled and, thus, reduce geosmin production.

APPENDIX B

STATISTICAL ANALYSES

Regression Analysis

Regression analysis is a statistical tool which describes the relation between two or more quantitative variables and by utilizing the mean response of the dependent variable describes the nature of this relationship. For example if one knows the relationship between mean T.O.N. values and nitrogen concentration, one can predict T.O.N. values by means of regression analysis. The regression model used in this experiment is "simple," "linear in parameter," and "linear in the independent variable." It is "simple" in that there is only one independent variable (X), "linear in parameter" because no parameter appears as an exponent or is multiplied or divided by another parameter (B_0 and B_1), and "linear in the independent variable" because the variable appears only in the first power (Y). The model is $Y = B_0 + B_1X + E_i$. "E_i" is the random error term (Neter and Wasserman 1974).

Pearson's Product Moment Correlation Coefficient

This correlation coefficient (r_p) is a numerical measure of the relationship between dependent and independent variables. A significant r_p value ($p < 0.05$) indicates that there is a tight clustering of data points around the regression line (Bhattacharyya and Johnson 1977).

Spearman's Rank Correlation Coefficient

This correlation coefficient (r_{sp}) is a non-parametric measure (not based on the shape of the underlying population distribution) of the relationship of the rank of the dependent and independent variables. A significant r_{sp} ($p < 0.05$) indicates that the relationship between the variables is either monotone (large values of X are associated with large values of Y; large values of X are associated with small values of Y) increasing or decreasing, but does not imply the nature of this relationship is either linear or nonlinear (Bhattacharyya and Johnson (1977).

Analysis of Variance

Like the regression analysis, analysis of variance (ANOVA) is concerned with the statistical relationship between one or more independent variables

and a dependent variable but unlike the regression analysis model, the ANOVA makes no assumption about the nature of the statistical relation between the dependent variable and one or more independent variables. This analysis compares the variation about means of each dependent variable (T.O.N., biomass, or Specific T.O.N.) by partitioning the total variation present in a data set into components. Each component is attributed to an identifiable source of variation (nitrogen form [α_i] or concentration [β_j], the interaction of identifiable variation sources [γ_{ij}], and the variation due to uncontrolled factors [random error in response measurements]). The equation for the ANOVA is $Y = u + \alpha_i + \beta_j + \gamma_{ij}$ + Error (Bhattacharyya and Johnson 1977). If the test statistic (F-test) indicates that the mean response for a factor (MS_c, MS_f, or MS_{cf}) is significant ($p < 0.05$), it is necessary to determine which factor level (nitrogen form, nitrogen concentration or interaction of nitrogen form and concentration) differ significantly by a multiple comparison method. This study utilized the Bonferroni method of multiple comparison to guarantee the 0.95 level of confidence for each pairwise comparison of mean values and because the replicates at each factor level varied (either 2 or 3) (Neter and Wasserman 1974).

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