Enumeration and Inoculation of Nitrifying Bacteria in Soilless Potting Media

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ABSTRACT. Autotrophic nitrifying organisms were enumerated in soilless potting media using the most probable number (MPN) technique. Populations of NH_4^+ and NO_2^- oxidizing organisms varied widely between two soilless media—Metro-Mix 220 and 350. Estimates for NH_4^+ oxidizing organisms ranged from 0.7 to 7.8×10^5 organisms/cm³, while NO_2^- oxidizers ranged from 1.3 to 9.5×10^5 organisms/cm³. Population numbers were similar to those typically reported in soils. There was a significant effect of medium type, $\text{NH}_4^+ \text{ N} : \text{NO}_3^- \text{ N}$ fertilizer ratio, and planting on MPN counts of both groups of organisms, with significant interaction between several of the factors. Estimates of NH_4^+ oxidizers were not linearly correlated with NH_4^+ oxidizing activity, implying low counting efficiency, heterotrophic nitrification, or rate-limiting substrate NH_4^+ level. In a separate study, a soilless potting medium was inoculated with pure cultures of either *Nitrosomonas europaea* or *Nitrobacter agilis*. Rates of NH_4^+ and NO_2^- oxidation increased, respectively, as inoculum volume increased. Inoculation with nitrifying bacteria may help in the overall management of N in the rhizosphere and be feasible alternatives for the prevention of either NH_4^+ or NO_2^- phytotoxicity with fertilizers containing urea or NH_4^+ .

The form of N available to plant roots has important consequences on N uptake and use. Plants can absorb N as organic molecules, such as urea, or as NH_4^+ and NO_3^- ions. For many types of commercially produced greenhouse crops, such as annual bedding plants and flowering potted plants, NO_3^- is generally taken up in larger quantities than other forms and produces more desirable plant growth (Barker and Mills, 1980). With the widespread use of fertilizers containing urea and NH_4^+ , the transformation of N compounds in the soil or growing medium is important in the overall performance and growth of plants. The rate-limiting transformation that affects the form of N available to plants is nitrification. Nitrification is defined as the oxidation of NH_4^+ to NO_3^- and is carried out in soil predominantly by two groups of chemoautotrophic bacteria (Alexander, 1977). One group, predominantly *Nitrosomonas* sp., oxidizes NH_4^+ to NO_2^- , while largely *Nitrobacter* sp. oxidize NO_2^- to NO_3^- . The nitrification process is sensitive to pH, water, and temperature and has an absolute requirement for O_2 (Lang, 1990).

Within the last decade, there have been significant contributions to the understanding of nitrification in soilless potting media (Elliott, 1986; Elliott and Lang, 1991; Lang and Elliott, 1991; Niemiera and Wright, 1987a, 1987b). Soilless potting media are popular substrates for containerized production because of their uniformity, light mass, excellent drainage, and reproducibility. Because these media are devoid of natural soil, on a volume basis they characteristically have a lower capacity for fixing and storing nutrients (Bunt, 1988) and, consequently, are fertilized more frequently and with relatively high amounts of N. Certain environmental conditions or cultural practices, such as low temperatures, acidity, or fungicide applications, can reduce rates of nitrification

in these media (Barker and Mills, 1980; Lang and Elliott, 1991) and may lead to NH_4^+ or NO_2^- toxicity when used with fertilizers containing urea or NH_4^+ . (Goyal et al., 1982; Maynard and Barker, 1969; Tew Schrock and Goldsberry, 1982).

There has been little documented work in the scientific literature estimating populations of nitrifying organisms in soilless potting media used in commercial greenhouse production. Herlihy (1972) reported that nitrifying bacteria exist in very low numbers in uncultivated peat, but may increase after cultivation and cropping. This agrees with later work by Elliott (1986) and Lang and Elliott (1991), who found that soilless media that had been cropped had higher nitrification rates than uncropped media. Autotrophic nitrifiers are thought to be the principal agents of nitrification in the root zone. However, those microorganisms that predominate in field soils may not behave similarly in soilless media. Heterotrophic organisms that use organic compounds as their C source may also play an important role in the nitrification process, especially under acidic soil conditions (Lang and Jagnow, 1986; Stroo et al., 1986; Tate, 1977).

Objectives of this study were to 1) estimate the number of autotrophic nitrifying organisms in soilless potting media previously irrigated with various fertilizers and 2) evaluate the effect of inoculation with pure cultures of nitrifying bacteria on the rate of NH_4^+ and NO_2^- oxidation in a soilless medium.

Materials and Methods

Enumeration and activity of nitrifying organisms in soilless media

GROWING CONDITIONS. Rooted cuttings of chrysanthemum [*Den-dranthema grandiflora* (Ramat.) Kitamura 'Bright Golden Anne'] were planted on 31 Oct. in 500-cm³ square pots (one plant per pot) containing either Metro-Mix 220 (MM220) or Metro-Mix 350 (MM350) (Scotts, Marysville, Ohio). Exact composition of these media is proprietary information, but MM220 contains sphagnum peat, vermiculite, and perlite, while MM350 contains sphagnum peat, vermiculite, perlite, and bark ash. Plants were grown in a glass greenhouse for 4 weeks under natural photoperiod with

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average day/night temperatures of 26/18 °C during the experimental period. Plants were fertilized at each irrigation with a nutrient solution containing (in mmol·L⁻¹) total N (15) with NH₄⁺ N : NO₃⁻ N ratios of either 1:3 [KNO₃ (3.75), NH₄H₂PO₄ (0.75), NH₄NO₃ (0.25), and (NH₄)₂SO₄ (5.5)] or 3:1 [KNO₃ (4.5), NH₄H₂PO₄ (1), NH₄NO₃ (2.75), and NaNO₃ (4)]. All solutions contained the following macronutrients (in mmol·L⁻¹): P (2.3) and K (5.4) and micronutrients (in μmol·L⁻¹): Fe (20), Mn (10), B (20), Zn (2.5), Cu (1.5), and Mo (1). Sulfur concentration varied between the two different solutions. Plants were irrigated as needed (when the medium became dry to the touch) using a plastic tube irrigation system. Pots containing medium but without plants were irrigated and fertilized similarly. Pots were arranged in a 2 × 2 × 2 factorial design (two media × two fertilizers × planting/no planting) with three pots per treatment combination. After 4 weeks, medium samples were collected, dried under a high-efficiency particulate adsorption (HEPA)-filtered air stream for 24 to 36 h at 25 to 30 °C, and sifted through a 2-mm mesh screen to remove plant roots. The pH of samples was determined in a saturated media extract, while nitrifying activity and microbial counts were determined as described below. Data were analyzed using analysis of variance.

ENUMERATION AND NITRIFYING ACTIVITY. Autotrophic NH₄⁺ oxidizers and NO₂⁻ oxidizers were enumerated by the most probable number (MPN) technique (Alexander, 1982). For ammonium oxidizers, serial dilutions from 10⁻¹ to 10⁻⁸ were prepared for each sample. A 10-cm³ (3.5- to 3.7-g) soilless medium sample was added to 90 mL of sterilized nutrient solution containing (in mmol·L⁻¹) NaCl (34.2), CaCl₂ (0.2), K₂HPO₄ (2.9), MgSO₄ (0.1), KHCO₃ (0.2), (in mol·L⁻¹) Fe-EDTA (1), CuSO₄ (0.1), CoCl₂ (0.3), MnCl₂ (1.6), NaMoO₄ (0.4), and ZnSO₄ (0.3) (Schmidt and Belser, 1982). The final pH of the solution after autoclaving was 7.8 to 8.0. The slurry was blended for 30 s on low speed with a hand-held immersion blender. A 10-mL sample was immediately transferred aseptically to a 100-mL dilution bottle containing 90 mL of fresh, sterilized nutrient solution (with no N), capped, and shaken vigorously for 1 min on a reciprocal shaker. This established the 10⁻² dilution. The sequence was repeated until a dilution of 10⁻⁸ was reached.

Five-milliliter aliquots were then transferred from the 10⁻⁴ to 10⁻⁸ dilution bottle to 15-mL polypropylene culture tubes containing 8 mL of sterilized NH₄⁺ oxidizer medium. The NH₄⁺ oxidizer medium was similar to the MPN dilution medium listed above except that (NH₄)₂SO₄ was added to make a final concentration of 3.8 mmol·L⁻¹ NH₄⁺. Five tubes were prepared for each dilution and tubes were incubated at 25 °C in the dark.

Initial observations were made after 4 weeks by aseptically transferring a 0.1-mL sample to a spot plate and assaying for NO₂⁻ (Keeney and Nelson, 1982) and NO₃⁻ (Cataldo et al., 1975) using colorimetric procedures. Incubation with biweekly monitoring continued for an additional 6 weeks or until there was no change in the number of positive tubes (NO₂⁻ or NO₃⁻ production) for 4 consecutive weeks. The number of positive tubes in each of the appropriate end dilutions were recorded and the NH₄⁺ oxidizing population estimated from an appropriate MPN table (Alexander, 1982).

In determining MPN counts for NO₂⁻ oxidizers, counting tubes were inoculated from the same dilution series prepared for counting NH₄⁺ oxidizers. The NO₂⁻ oxidizer medium contained 1.2 mmol·L⁻¹ KNO₂ as the sole N source. Tubes were checked biweekly for NO₂⁻ production (Cataldo et al., 1975) after an initial 4-week incubation at 25 °C in the dark. The NO₂⁻ oxidizing population was estimated from an appropriate MPN table (Alexander, 1982) after there was no change in the number of positive tubes

(NO₃⁻ production) for 4 consecutive weeks.

To correlate rates of NH₄⁺ oxidation with MPN counts, short-term NH₄⁺ oxidation activity was determined on each soilless medium sample (Elliott, 1988; Lang and Elliott, 1991). Twenty milliliters of 1 mmol·L⁻¹ (NH₄)₂SO₄ and 10 mmol·L⁻¹ NaClO₃ in 1 mmol·L⁻¹ phosphate buffer (pH 7.0) were added to 15-cm³ samples. Chlorate was added to inhibit NO₂⁻ oxidation (Belser and Mays, 1980; Elliott, 1988). After incubation in the dark at 25 °C for 6 and 18 h, samples of each medium were removed, the aqueous phase was filtered, and the extracts were frozen until analyzed. Rates of NH₄⁺ oxidation were calculated from the difference in NO₂⁻ concentration between 6 and 18 h of incubation. Nitrite formation within this period is linear (Elliott, 1988) and was determined colorimetrically as described above. Data were analyzed using linear and nonlinear regression procedures.

Inoculation of soilless media with pure cultures of nitrifying bacteria

Pure cultures of *Nitrosomonas europaea*, an NH₄⁺ oxidizer (ATCC strain 19718), and *Nitrobacter agilis*, a NO₂⁻ oxidizer (ATCC strain 14123), were introduced into unplanted soilless media to test for enhanced rates of nitrification. The medium used in all inoculations was a mixture of 2 sphagnum peat : 1 no. 3 grade vermiculite : 1 horticultural grade perlite (by volume) blended by hand. The medium was limed to pH 6.5 (saturated media extract) using calcitic limestone and was immediately covered until use to reduce contamination by airborne organisms. Pure cultures of bacteria were monitored periodically for activity and N level before inoculation to prevent excessive N carryover into the assay and to help estimate expected rates of N transformations.

EXPERIMENT 1. For inoculations of *Nitrosomonas*, 0-, 2-, or 4-mL samples (0×, 2×, or 4× titer) of pure cultures were aseptically added to plastic tubes containing 15-cm³ of medium in 6 mL of 0.5 mmol·L⁻¹ (NH₄)₂SO₄ and 1 mmol·L⁻¹ phosphate buffer (pH 7.0). Sterilized distilled water was added in the 0× and 2× treatments to make a total solution content of 10 mL per tube. After incubation in the dark at 25 °C, three random samples of each inoculation treatment were removed immediately and at 4-h intervals up to 24 h. Twenty milliliters of a 1-mol·L⁻¹ KCl and 43-μmol·L⁻¹ nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) (Dow Chemical Co., Midland, Mich.) solution were added to the incubated samples, shaken for 1 h, and filtered through medium-retention qualitative filter paper (VWR Scientific, Boston). Nitrapyrin was added to inhibit autotrophic NH₄⁺ oxidation (Mills and Pokorny, 1978; Shattuck and Alexander, 1963). Ammonium concentration was determined colorimetrically on the filtered extract (Chaney and Marbach, 1962; Hinds and Lowe, 1980).

For inoculations with *Nitrobacter*, 0-, 2-, or 4-mL samples (0×, 2×, or 4× titer) of pure cultures were added to tubes containing 15 cm³ of soilless medium in 6 mL of 0.13 mmol·L⁻¹ NaNO₂ and 1 mmol·L⁻¹ phosphate buffer (pH 7.0). Incubations were carried out similarly as for *Nitrosomonas*, except that the length of incubation was 16 h. After removing samples, the aqueous phase was filtered and immediately analyzed for NO₂⁻ colorimetrically. Data were analyzed using simple linear regression with paired *t* testing of slope parameters.

EXPERIMENT 2. Experiment 2 was similar to Expt. 1 except that incubations were carried out for longer durations. For inoculations with *Nitrosomonas*, pure cultures were added to tubes containing 15-cm³ of soilless medium in 6 mL of 1.0 mmol·L⁻¹ (NH₄)₂SO₄ and 1 mmol·L⁻¹ phosphate buffer (pH 7.0). After incubation in the dark at 25 °C, three random samples of each inoculation treatment were removed immediately and at 24-h intervals up to 120 h. For

Nitrobacter inoculations, pure cultures were added to tubes containing 15 cm³ of soilless medium in 6 mL of 0.52 mmol·L⁻¹ NaNO₂ and 1 mmol·L⁻¹ phosphate buffer (pH 7.0). Samples were removed immediately and at 24-h intervals up to 72 h. Filtered solutions were analyzed similarly as in Expt. 1.

Results and Discussion

ENUMERATION OF NITRIFYING ORGANISMS. Populations of NH₄⁺ and NO₂⁻ oxidizers varied widely among the different medium samples (Table 1). MPN counts of NH₄⁺ oxidizers ranged from 0.7 to 7.8 × 10⁵ organisms/cm³, while NO₂⁻ oxidizers ranged from 1.3 to 9.5 × 10⁵/cm³. Populations were generally in the range of most soils where estimates have been taken using similar MPN procedures (Ardakani et al., 1974b; Belser, 1977; Morrill and Dawson, 1967; Voltz et al., 1975). When compared with similar nontreated, unplanted media (data not shown), populations were some 1000-fold less than in the samples of cultivated media shown in Table 1. These low counts in nontreated, unplanted media agree with other studies with uncultivated peat (Herlihy, 1972).

Ammonium oxidizers were more abundant in MM220 than MM350; however, differences in MPN counts between the two media were affected by planting and fertilizer ratio (Table 1). Generally, samples that were unplanted or were fertilized with 3:1 NH₄⁺ N : NO₃⁻ N ratios had higher counts than respective samples either planted or fertilized with 1:3 ratios. In contrast, trends were different with estimates of NO₂⁻ oxidizers, with more organisms in MM350 than MM220. As with the NH₄⁺ oxidizers, relative differences between the two media depended on planting and NH₄⁺ N : NO₃⁻ N ratio. With NO₂⁻ oxidizers, unplanted samples were not as affected by NH₄⁺ N : NO₃⁻ N fertilizer ratio as planted samples. A significant three-way interaction between medium, fertilizer ratio, and planting indicated that microbial populations responded uniquely to each treatment, making broad generalizations difficult.

Within each medium, there appeared to be certain treatment combinations that were favorable for maximizing cellular growth and function of NH₄⁺ and NO₂⁻ oxidizing bacteria. Cell yields and oxidation rates of nitrifying bacteria empirically determined from bacterial counts and substrate oxidation rates have been previously reported (Ardakani et al., 1974a; Belser, 1974; Voltz et al., 1975). Belser (1974) showed that ≈1 to 4 × 10⁴ cells of *Nitrobacter* are produced from the oxidation of 1 μg of NO₂⁻ N. Voltz et al. (1975) found that about three times this cell concentration is supported from an equivalent amount of NH₄⁺ N oxidized by *Nitrosomonas* in a soil column. Despite the fact that bacteria cell yields are larger from 1 mol of NH₄⁺ than from NO₂⁻, NO₂⁻ oxidizing bacteria in nature have been found to be more abundant than NH₄⁺ oxidizing bacteria (Ardakani et al., 1974b; Morrill and Dawson, 1967; Voltz et al., 1975). Morrill and Dawson (1967) observed that *Nitrosomonas* and *Nitrobacter* numbers varied depending on soil pH. In one instance, the maximum cell densities were about the same for both genera where soil was slightly acid (i.e., pH 6.4) and where no accumulation of NO₂⁻ was observed. Others have observed NH₄⁺ oxidizer populations ≈10-fold less than NO₂⁻ oxidizers in soils of pH 7.0 to 7.5 (Ardakani et al., 1974b; Voltz et al., 1975). For MM350, populations of NO₂⁻ oxidizers were 4- to 10-fold greater than NH₄⁺ oxidizers, supporting similar findings in soils (Table 1). This was not the case for MM220, however, as NH₄⁺ oxidizers were sometimes more populated than NO₂⁻ oxidizers in similarly treated media.

In this study, pH of the media did not appear to be a factor influencing the differential growth of these nitrifying bacteria (Table 1). The pH was affected only by NH₄⁺ N : NO₃⁻ N fertilizer ratio (readings significantly lower in the 3 NH₄⁺ N : 1 NO₃⁻ N ratio treatment) and did not significantly differ between the two media. Optimum growth and metabolism of nitrifying bacteria is normally in the range of pH 7.0 to 7.5 (Focht and Verstraete, 1977), where most readings were in this study. With relatively little variation in pH between samples, other environmental factors are

Table 1. Effect of medium, planting, and NH₄⁺ : NO₃⁻ fertilizer ratio on medium pH and most probable number (MPN) counts of NH₄⁺ and NO₂⁻ oxidizing bacteria.

Medium ^z	Planted ^y	NH ₄ ⁺ N : NO ₃ ⁻ N ratio ^x	Medium pH	MPN count (organisms × 10 ⁵ /cm ³)	
				NH ₄ ⁺ oxidizers	NO ₂ ⁻ oxidizers
MM220	Yes	1:3	7.28 ± 0.11 ^w	1.3 ± 0.12	1.3 ± 0.10
		3:1	7.11 ± 0.06	4.9 ± 0.87	2.2 ± 0.15
	No	1:3	7.30 ± 0.029	4.9 ± 0.87	3.3 ± 0.10
		3:1	7.19 ± 0.010	7.8 ± 0.95	3.3 ± 0.30
MM350	Yes	1:3	7.30 ± 0.10	1.3 ± 0.12	4.9 ± 0.31
		3:1	6.90 ± 0.058	1.3 ± 0.10	9.5 ± 0.35
	No	1:3	7.28 ± 0.10	2.3 ± 0.12	7.9 ± 0.23
		3:1	7.06 ± 0.087	0.7 ± 0.058	7.9 ± 0.23
Significance					
Medium (M)			NS	***	***
Planting (P)			NS	***	***
NH ₄ ⁺ : NO ₃ ⁻ ratio (NH)			**	**	***
M × P			NS	***	*
M × NH			NS	***	***
P × NH			NS	NS	***
M × P × NH			NS	NS	***

²Metro-Mix 220 or 350.

³Unplanted or planted with *Dendranthema grandiflora* (Ramat.) Kitamura 'Bright Golden Anne.'

⁴Media were irrigated for 4 weeks with a 200-mg·L⁻¹ N nutrient solution containing one of two different ratios of NH₄⁺ N : NO₃⁻ N.

^wIndicates SE of the mean (n = 3).

NS, **, *** Nonsignificant or significant at P = 0.01 or 0.001, respectively, using analysis of variance.

Fig. 1. Scatter plot of most probable number (MPN) estimates for NH_4^+ oxidizing microorganisms vs. short-term NH_4^+ oxidation rate for 24 different samples of fertilized soilless potting media. Regression equation for the plot is $y = 2.56(1 - e^{-0.6x})$, $r^2 = 0.89$.

likely influencing microbial population dynamics and nitrifying activity.

The significant interaction between medium type, planting, and fertilizer ratio (Table 1) indicate that no single factor is strongly influencing the growth of either group of bacteria. Certain combinations of medium type, planting, and NH_4^+ N : NO_3^- N fertilizer ratio that led to greater populations of NH_4^+ oxidizers did not always lead to greater populations of NO_2^- oxidizers. *Nitrosomonas* bacteria have been shown to be less sensitive than *Nitrobacter* to high NH_4^+ concentrations (Nakos and Wolcott, 1972). Perhaps this fact, coupled with the differences in physical and chemical characteristics between MM220 and MM350, can partially explain population differences between the two genera. Elliott (1986) showed that unplanted, nontreated, commercial soilless media vary considerably in nitrifying activity, although populations were not reported in that study. Within each genera, conditions adverse to population growth and maintenance, such as moisture stress and low oxygen tensions (Belser, 1979), are undoubtedly affected by planting and medium type. For this reason, we are not surprised to see the ranges of organism populations reported in this study.

Nitrite formation in the presence of chlorate of all 24 samples ranged from 0.7 to 2.7 $\text{nmol} \cdot \text{cm}^{-3} \cdot \text{h}^{-1}$ (Fig. 1). While there was not a significant linear correlation between MPN counts of NH_4^+ oxidizers and NH_4^+ oxidation activity ($r^2 = 0.15$), there was an exponential relationship ($r^2 = 0.89$). The exponential plot shows that, at MPN counts >5 to 6×10^5 organisms/ cm^3 , NH_4^+ oxidation rate levels

off. This appears to indicate that there may have been a rate limiting substrate NH_4^+ concentration used in the assay procedure. However, while one would expect a close relationship between nitrification and nitrifying organisms, noncorrelation has been observed in other studies in which MPN counts and nitrifying potentials have been measured (Belser and Mays, 1982; Sarathchandra, 1978; Steele et al., 1980). Many samples of media had relatively high rates of NH_4^+ oxidation, but had low MPN estimates. Studies have shown that low autotrophic nitrifier counts indicate the presence of heterotrophic nitrification (Schimel et al., 1984; Tate, 1977). With the abundance of organic matter (peat and bark) in MM220 and MM350, this is a possibility. However, heterotrophic nitrifiers proliferate more in organic soils where the pH is below 5.5 (Lang and Jagnow, 1986; Stroo et al., 1986; Tate, 1977), and pH of our samples ranged from 6.8 to 7.5 with the assay conducted in a buffered solution.

The MPN method may underestimate nitrifier populations because different genera and strains of NH_4^+ oxidizers are not able to grow equally well in the same selective medium or because of inefficient counting (Belser and Mays, 1982). Counting efficiency can be calculated making a theoretical estimate of the population necessary to produce a given activity. The MPN count as a percentage of this theoretical population can then be used as an index of counting efficiency. Using a theoretical rate constant of 0.023 μmol of NH_4^+ oxidized/cell/h (Belser and Mays, 1982), counting efficiencies in this study were all 100% or better, suggesting the procedure was valid. Calculations from data of other studies show counting efficiencies for NH_4^+ oxidizers to be anywhere from 5% (Belser and Mays, 1982) to 266% (Steele et al., 1980). Efficiency calculations are complicated by the fact that the genus of NH_4^+ oxidizer being counted in a particular habitat is not

Fig. 2. Effect of inoculation with pure cultures of *Nitrosomonas europaea* on NH_4^+ oxidation in an unplanted peat-lite medium. Incubation for 24 h; regression equations are (●) $y = 188.35 - 1.50x$, ($r^2 = 0.93$); (□) $y = 190.44 - 2.12x$, ($r^2 = 0.97$); and (■) $y = 193.36 - 3.08x$, ($r^2 = 0.98$). Incubation for 120 h; regression equations are (●) $y = 393.70 - 1.43x$, ($r^2 = 0.96$); (□) $y = 400.98 - 2.34x$, ($r^2 = 0.98$); and (■) $y = 394.33 - 2.98x$, ($r^2 = 0.98$). Inoculant titer represents the relative number of microorganisms added to each sample of media. Each point represents the mean of three replicates. Error bars indicate $\pm$ SE when larger than the symbols.

generally known. Consequently, theoretical estimates of population from observed activity may not be accurate if rate constants were calculated from a pure culture of a single strain of NH_4^+ oxidizer.

INOCULATION OF SOILLESS MEDIA WITH NITRIFYING BACTERIA. The inoculation of an unplanted peat-vermiculite-perlite medium with *Nitrosomonas europaea* increased NH_4^+ oxidation rate over a 24-h and 5-d period compared to the uninoculated medium (Fig. 2). Paired *t* tests of the slope parameters indicated significant rate differences between all treatments within each experiment (data not shown).

Samples inoculated with *Nitrobacter agilis* showed similar trends with a significant increase in the oxidation of NO_2^- over a 16-h period (Fig. 3). Incubation for 72 h with higher concentrations of NO_2^- substrate also resulted in significant linear rate increases for inoculated samples, although there was no significant difference between the 2 $\times$ and 4 $\times$ treatments (Fig. 3).

Inoculation of nitrifying organisms into soilless potting media could potentially reduce supraoptimal levels of NH_4^+ or NO_2^- . This may have important implications in the prevention of NH_4^+ or NO_2^- toxicity in susceptible plants. Paul and Polle (1964) showed that the inoculation of *Nitrobacter* into an alkaline soil that tended to accumulate NO_2^- , both reduced toxic levels of NO_2^- and dramatically improved the growth of *Lactuca sativa* L. Although their study was carried out over a period of days, results in our study indicate that relatively high levels of either NH_4^+ or NO_2^- can be decreased very quickly through inoculation. Nitrite concentrations $>1 \text{ mmol}\cdot\text{L}^{-1}$ have been observed in saturated media extracts of soilless potting media (G.C. Elliott, unpublished data). Acidophilic nitrifying organisms have been isolated from extremely acidic environments (Hankinson and Schmidt, 1988) and may provide an

inoculum source for acidic soilless media. Actual results from inoculation that may occur in the field or greenhouse, however, would probably be different than highly controlled *in vitro* studies. Samples in this study were incubated under ideal conditions for autotrophic nitrifier activity. Less favorable conditions, such as lower pH or lower O_2 potentials, would probably yield lower activities.

Managing the rhizosphere by controlling biological or biochemical processes has received considerable attention. Applying nitrapyrin to soils to reduce N leaching or inoculating legume crops with N-fixing bacteria are two examples. One should be aware, however, that extended and overuse of inoculants of nitrifying bacteria in potting media or fertilizer solutions could result in increased NO_3^- leaching. For those production facilities that do not collect irrigation runoff, inoculation could possibly lead to environmental pollution of ground and surface waters. On the other hand, under certain situations where NH_4^+ or NO_2^- levels in soilless potting media could potentially become toxic to plants, if the specific microorganisms responsible for nitrification could be introduced into the media, crop growth might be improved. Inoculants of nitrifying bacteria are currently being marketed for freshwater aquarium management (Fritz Chemical Co., Dallas, Texas). With the current technology of greenhouse subirrigation systems and recycled fertilizer runoff, nitrifier inoculants for greenhouse media warrant particular attention.

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Fig. 3. Effect of inoculation with pure cultures of *Nitrobacter agilis* on NO_2^- oxidation in an unplanted peat-lite medium. Incubation for 16 h; regression equations are (●) $y = 51.32 - 1.54x$, ($r^2 = 0.97$); (□) $y = 51.58 - 2.34x$, ($r^2 = 0.97$); and (■) $y = 50.77 - 2.93x$, ($r^2 = 0.98$). Incubation for 72 h; regression equations are (●) $y = 204.85 - 1.37x$, ($r^2 = 0.95$); (□) $y = 209.33 - 2.36x$, ($r^2 = 0.95$); and (■) $y = 204.11 - 2.67x$, ($r^2 = 0.97$). Inoculant titer represents the relative number of microorganisms added to each sample of media. Each point represents the mean of three replicates. Error bars indicate $\pm \text{SE}$ when larger than the symbols.

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