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Kinetics of nitrite oxidation in two *Nitrobacter* species grown in nitrite-limited chemostats

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Abstract. The influence of growth rate, the presence of acetate and variation in the dissolved oxygen concentration on the kinetics of nitrite oxidation was studied in suspensions of intact cells of *Nitrobacter winogradskyi* and *Nitrobacter hamburgensis*. The cells were grown in nitrite-limited chemostats at different dilution rates under chemolithotrophic and mixotrophic conditions. Growth of *N. hamburgensis* in continuous culture was dependent on the presence of acetate. Acetate hardly affected the maximal nitrite oxidation rate per cell (V_{max}), but displayed a distinctly negative effect on the saturation constants for nitrite oxidation (K_m) of both *Nitrobacter* species. This effect was reversible; when acetate was removed from the suspensions the K_m-values for nitrite oxidation returned to their original values.

A reduction of the dissolved oxygen concentration from 100% to 18% air saturation slightly decreased the V_{max} of chemolithotrophically grown *N. winogradskyi* cells, whereas a 2.3 fold increase was observed with mixotrophically grown cells of *N. hamburgensis*. It is suggested that the large variation in K_m encountered in field samples could be due to this observed phenotypic variability. The V_{max} per cell is not a constant, but apparently is dependent on growth rate and environmental conditions. This implies that potential nitrite oxidation activity and numbers of cells are not necessarily related. Considering their kinetic characteristics, it is unlikely that *N. hamburgensis* is able to compete successfully with *N. winogradskyi* for limiting amounts of nitrite under mixotrophic conditions. However, at reduced partial oxygen tensions, *N. hamburgensis* may become the better competitor.

Key words: *Nitrobacter winogradskyi* – *Nitrobacter hamburgensis* – Chemolithotrophic – Mixotrophic – V_{max} – K_m – Acetate – Oxygen

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When chemolithotrophic nitrite-oxidizing bacteria in grassland soils were enumerated by a Most Probable Number (MPN) technique, different results were obtained with the same soil sample when different concentrations of nitrite were used (Both et al. 1990b). These differences could partly be ascribed to differences in the kinetic parameters of nitrite oxidation, i.e. the maximal oxidation rate V_{max} and the saturation constant for nitrite K_m, of the different subcommunities of nitrite-oxidizing bacteria in the soil (Both et al. 1991). During a study of the characteristics of the nitrite-oxidizing community in a well drained grassland soil, V_{max} and K_m appeared to be variable in time as well as in space (Both 1990a).

Assuming constant nitrite oxidation parameters, Belser and Mays (1982) used potential ammonium-oxidizing activities of soil and sediment samples to calculate the efficiency of MPN-enumerations. A similar approach was followed by Berg and Rosswall (1986) during a 3 years study of the nitrite-oxidizing community of arable soils. Both (1990a) could not demonstrate a correlation between numbers of nitrite-oxidizing bacteria and potential nitrite-oxidizing activities in a natural, permanent grassland soil. It was hypothesized that this lack of correlation between cell number and activity was due to changing overall kinetic parameters of nitrite oxidation of the whole nitrite oxidizing community. This could be caused by changing kinetic parameters per *Nitrobacter* strain, or by a change in the community composition leading to variability in dominant nitrite oxidizers with different kinetic parameters.

In the present paper the kinetic parameters of nitrite oxidation in two different *Nitrobacter* species, *N. hamburgensis* and *N. winogradskyi*, were studied. In batch culture, *N. winogradskyi* and *N. hamburgensis* differ with respect to their optimal growth conditions: *N. winogradskyi* grows better chemolithotrophically, whereas *N. hamburgensis* prefers mixotrophic conditions (Watson et al. 1989). Since concentrations of nitrite are seldom detectable in the soil, cells of both *Nitrobacter* species, grown in a nitrite limited chemostat were used in this study. Mixotrophic conditions were achieved by addition of acetate to the growth medium.

Materials and methods

Pure cultures used

Nitrobacter winogradskyi strain ATCC 321 and *Nitrobacter hamburgensis* strain X14 were used in the experiments.

Chemostat cultures

Cells of *N. winogradskyi* and *N. hamburgensis* were grown chemolithoautotrophically and mixotrophically in continuous culture in "Biostat M" fermentors (Braun, Melsungen, FRG). The chemolithotrophic growth medium was composed of 0.091 mM $CaCl_2$, 0.81 mM $MgSO_4$ 1 mM KH_2PO_4 30 mM $NaNO_2$ and 1 ml l^{-1} of a trace element solution described by Laanbroek and Pfennig (1981). For mixotrophic growth, 10 mM sodium acetate and 0.015 g l^{-1} yeast extract (Difco Detroit, Mich., USA) were added to the medium. The phosphate buffer was always added after autoclaving the other constituents. The pH of the growth medium was maintained at 7.3 by automatic titration with 0.25% (w/v) Na_2CO_3. The temperature was kept at 25° C and the cultures were well aerated. The culture volume was 1400 ml.

Steady state of the cultures was assumed when the optical density of the culture measured at 660 nm and the substrate concentrations in the culture vessel were constant. This condition was usually reached after five volume changes.

Nitrite and acetate determinations

Nitrite was determined colorimetrically using the Griess-Ilosvay reagents (Schmidt and Belser 1982). The detection limit for nitrite was 5 µM.

Acetate was determined with a Perkin-Elmer (Pomona, Calif., USA) 8500 gaschromatograph equipped with a flame ionisation detector and a 2m × 1/4 × 2 glass wide bore column containing Chromosorb 101 (Chrompack, Middelburg, The Netherlands) (80–100 mesh). Temperatures of injection port, column oven and detector were 230, 180 and 270 °C, respectively. The flow rate of the formic acid saturated nitrogen carrier gas was 36 ml min^{-1}. The detection limit for acetate was 0.1 mM.

Enumeration technique

Total cell counts were performed using a Bürker-Türk (Schreck, Hofheim, FRG) counting chamber. Specific activities were expressed on the basis of cell numbers, since total carbon- and/or protein-contents in a small sample volume proved to be too low for precise measurements. Moreover, changes in activities were recorded per cell.

Determination of V_{max} and K_m in a biological oxygen monitor

A 1-ml sample of a steady state culture was incubated in the reaction chamber of a Biological Oxygen Monitor (Strathkelvin Instruments Glasgow, UK, Model 781), while the temperature was maintained at 25.0 °C. The sample was flushed with an air stream during 1 min. Before substrate was added, the endogenous consumption of oxygen was determined; all values measured have been corrected for this activity. After addition of 5 µl of a sterile nitrite stock solution, the initial linear decrease in oxygen was followed in the reaction chamber with a Clark-type oxygen electrode during 6 to 12 minutes. During this period the oxygen concentration decreased linearly from about 100% to approximately 85% air saturation. Final substrate concentrations at the start of each determination ranged from 0.025 to 2.5 or 5 mM nitrite. The stoichiometry of the reaction $NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$ was confirmed, so that the oxidation rates could be expressed as pmol NO_2^- $cell^{-1}$ h^{-1}.

Before the measurements of oxygen consumption by mixotrophically grown cells were started, the cells were washed 3 times in order to remove acetate from the cultures. The medium used for washing consisted of the growth medium with 30 mM sodium nitrate replacing 30 mM sodium nitrite. In addition to nitrite, 5 µl of a sodium acetate stock solution was added to final concentrations of 0.5, 1.0 or 6 mM just before the oxygen electrode was placed in the reaction chamber. The oxygen uptake rates were corrected for endogenous respiration, which was measured in the absence of nitrite or acetate during 30 min. The effects of acetate on oxygen uptake in the absence of nitrite was measured at final acetate concentrations of 0.5, 1.0 or 6 mM. It was confirmed that the pH remained constant at 7.3, while the oxygen uptake rates were determined.

Oxygen uptake rates at decreased concentrations of oxygen in the reaction chamber were established with steady state cultures of chemolithotrophically grown *N. winogradskyi* and mixotrophically grown *N. hamburgensis*. The activities were determined after the suspensions had been oxygenated with a gasmixture of 20% air and 80% nitrogen. The linear decrease in oxygen concentration at different nitrite concentrations was recorded during a few minutes while the oxygen concentration decreased from about 18% to 12% of air saturation. The oxygen uptake rates were compared to the rates determined at 100% air saturation.

Calculations of V_{max} and K_m

For the estimation of the kinetic parameters of nitrite oxidation from the data obtained by the methods described above, the "Direct Linear" method described by Eisenthal and Cornish-Bowden (1974) was applied. The application of this method also gives confidence limits of 68% for the kinetic parameters V_{max} and K_m.

Results

General features of chemolitho- and mixo-trophically grown cells

Data obtained with steady state cultures of chemolithotrophically and mixotrophically grown *Nitrobacter winogradskyi* and *N. hamburgensis* are presented in Table 1. Attempts to grow *N. hamburgensis* chemolithotrophically in continuous culture failed. The steady state nitrite concentrations of the two chemolithotrophic cultures of *N. winogradskyi* were 0.010 and 0.013 mM for the dilution rates of 0.011 and 0.017 h^{-1}, respectively. The steady state nitrite concentrations for the mixotrophic *N. winogradskyi* fluctuated between 0.3 to 0.4 mM, while about 0.5 mM acetate was consumed. The steady state nitrite concentrations of the mixotrophic *N. hamburgensis* cultures fluctuated between 0.08 and 0.2 mM. Under these conditions, 3 to 4 mM acetate was consumed in both cultures. When judged on the basis of residual nitrite concentrations, a steady state was not reached with the mixotrophic cultures.

After increasing the dilution rate of the chemostat with the chemolithotrophically growing *N. winogradskyi* from 0.011 to 0.017 h^{-1}, the optical density and the total cell number hardly changed (Table 1). The optical density of the mixotrophically grown *N. winogradskyi* cells was about three times higher compared to the chemolithotrophically grown culture, whereas the cell numbers did not

Table 1. General features of *Nitrobacter winogradskyi* and *N. hamburgensis* grown in a nitrite limited chemostat in the absence or presence of excess acetate at 25 °C and 100% air saturation. Cells were enumerated microscopically using a Bürker-Türk counting chamber. Optical density was determined at 660 nm

Organism	*N. winogradskyi*		*N. hamburgensis*		
Dilution rate (h^{-1})	0.011	0.017	0.008	0.008	0.011
Generation time (h)	63	41	87	84	63
Presence of acetate	–	–	+	+	+
Log. of total cell number ml^{-1}	8.38 ± 0.08	8.18 ± 0.07	8.25 ± 0.05	8.60 ± 0.05	8.68 ± 0.02
Optical density	0.020	0.024	0.060	0.080	0.124

differ significantly. This could be explained by the presence of large numbers of granules of reserve material, probably PHB, in mixotrophically grown *N. winogradskyi* cells, as was demonstrated by transmission electron microscopy (not shown). Formation of large amounts of reserve material in the presence of acetate in the growth medium, was also shown by Smith and Hoare (1968) in *Nitrobacter agilis* and by Gay et al. (1983) in two *Nitrobacter* serotypes.

The presence of acetate in the growth medium of *N. hamburgensis* seemed to be obligatory for growth in continuous culture. Although *N. hamburgensis* is able to grow chemolithotrophically in batch culture, the maximal growth rate is very low compared to growth rates in mixotrophic media (Sundermeyer and Bock 1981; Watson et al. 1989). The lowest dilution rate applied in the chemostat, 0.006 h^{-1}, might have been too high for chemolithotrophic *N. hamburgensis*. When the dilution rate of the mixotrophically growing *N. hamburgensis* culture was increased from 0.080 to 0.124 h^{-1}, the optical density increased but not the total cell number. Similar to mixotrophically grown *N. winogradskyi* cells, transmission electron microscopy demonstrated large numbers of granules of reserve material in the mixotrophically grown *N. hamburgensis* cells (not shown).

Compared to *N. winogradskyi*, about twice as much *N. hamburgensis* cells were formed at 30 mM nitrite and 10 mM acetate which could mean that *N. hamburgensis* cells were using part of the consumed acetate as a carbon source for growth. Smith and Hoare (1968) measured also an active incorporation of ^{14}C from labelled acetate in all fractions of biomass of *N. agilis*. As will be shown later, acetate is probably not involved in the generation of energy.

Effect of growth rate on kinetic parameters of nitrite oxidation

The maximal nitrite oxidation rate (V_{max}) per cell of chemolithotrophically growing *N. winogradskyi* cells increased as the growth rate increased, while the apparent saturation constant for nitrite oxidation (K_m) also increased (Table 2). The ratio V_{max}/K_m, which might be used as an indication of efficiency of nitrite oxidation (Healy 1980; Button 1983), was not influenced by the growth rate.

The actual nitrite-oxidizing activity per cell in the chemostat, as calculated from nitrite consumption rates, increased as the dilution rate increased, but was always lower than V_{max}. In relation to the actual nitrite oxidation rates, the steady state cultures appeared to have an overcapacity of nitrite oxidation of 136 and 123% at dilution rates of 0.011 and 0.017 h^{-1}, respectively.

Table 2. Calculated maximal nitrite oxidation rates (V_{max}) and saturation constants (K_m) of *Nitrobacter winogradskyi* and *N. hamburgensis* grown separately in nitrite limited chemostats in the absence or presence of excess acetate at 25 °C and 100% air saturation. Nitrite oxidation rates were calculated from oxygen consumption rates determined in a Biological Oxygen Monitor and are expressed as fmol $cell^{-1}$ h^{-1}. Significant differences ($p < 0.32$) between species and growth conditions are indicated by different letters

Organism	*N. winogradskyi*			*N. hamburgensis*	
Dilution rate (h^{-1})	0.011	0.017	0.008	0.008	0.011
Presence of acetate in growth medium	–	–	+	+	+
V_{max}	1.9a	3.7b	2.5c	3.3b	1.0d
K_m (μM nitrite)	36v	69w	260x	1370y	540z
Activity per cell in culture vessel	1.4	3.0	1.3	0.6	0.7

The maximal nitrite oxidation rate (V_{max}) per cell of mixotrophically growing *N. hamburgensis* cells as well as the apparent saturation constant decreased as the growth rate increased, whereas the V_{max}/K_m ratio hardly changed with changing dilution rate. The actual nitrite-oxidizing activity per cell in the chemostat was almost independent of the growth rate. In relation to the actual nitrite oxidation rates, the steady state cultures of *N. hamburgensis* appeared to have an overcapacity of the nitrite oxidation of 550% and 143% at dilution rates of 0.008 and 0.124 h^{-1}, respectively.

Effect of acetate on kinetic parameters of nitrite oxidation

The effect of the presence of acetate in the growth medium of *N. winogradskyi* is shown in Table 2. The V_{max} values were about the same for the chemolithotrophically and mixotrophically grown cells. However, the K_m values for nitrite oxidation were significantly higher under mixotrophic conditions, which was also reflected by the high steady state nitrite concentrations in the culture vessels in the presence of acetate.

Table 3 shows that addition of acetate had a different effect on the V_{max} values of the different suspensions. With chemolithotrophically or mixotrophically grown *N. winogradskyi* cells, addition of acetate had almost no effect on V_{max}. Only the addition of 6 mM acetate to mixotrophically grown cells increased V_{max} significantly. With mixotrophically grown cells of *N. hamburgensis*, addition

Table 3. Effect of addition of acetate on the maximal nitrite oxidation activity of washed cells of *Nitrobacter winogradskyi* and *N. hamburgensis* grown under nitrite limitation in the absence or presence of excess acetate at 25 °C and 100% air saturation. Significant differences ($p < 0.32$) between data are indicated by different letters

Organism	Acetate in growth medium	Dilution rate (h^{-1})	V_{max} (fmol NO_2^- cell^{-1} h^{-1})			
			0 mM	0.5 mM	1.0 mM	6 mM acetate
N. winogradskyi	–	0.017	3.7a	–	2.8a	2.7ab
	+	0.008	1.7c	1.7c	–	1.9d
N. hamburgensis	+	0.008	0.66e	0.88e	–	3.5a
	+	0.011	1.9d	–	1.9d	2.2d

Table 4. Effect of addition of acetate on the saturation constant of nitrite oxidation of washed cells of *Nitrobacter winogradskyi* and *N. hamburgensis* grown under nitrite limitation in the absence or presence of excess acetate at 25 °C and 100% air saturation. Significant differences ($p < 0.32$) between data are indicated by different letters

Organism	Acetate in growth medium	Dilution rate (h^{-1})	Saturation constant K_m (μM NO_2^-)			
			0 mM	0.5 mM	1.0 mM	6 mM acetate
N. winogradskyi	–	0.017	69a	–	56ab	118b
	+	0.008	95b	76ab	–	322c
N. hamburgensis	+	0.008	278abcd	194bcd	–	1420e
	+	0.011	363d	–	355cd	655d

of 6 mM acetate to the incubation medium increased V_{max} significantly, whereas lower concentrations of acetate had no significant effect on the maximal nitrite oxidation rate.

The K_m of both chemolithotrophically and mixotrophically grown cells of *N. winogradskyi* and *N. hamburgensis* increased considerably in the presence of 6 mM acetate (Table 4).The V_{max}/K_m ratio of *N. hamburgensis* was not affected by addition of acetate, whereas this ratio in *N. winogradskyi* was decreased in the presence of 6 mM acetate. This might indicate that the efficiency of the nitrite oxidation by *N. winogradskyi* is negatively affected by acetate, whereas the nitrite oxidation by *N. hamburgensis* was not influenced by acetate.

Above we have shown that the presence of acetate in the growth medium of the chemostat increased the apparent K_m value for nitrite oxidation by *N. winogradskyi*, whereas V_{max} was more or less not affected (Table 2). This effect of acetate on K_m appeared to be reversible. Chemolithotrophically and mixotrophically grown cells of *N. winogradskyi* incubated in a medium free of acetate, showed a much lower K_m that is comparable with the steady state value of this species (Table 2, 4), whereas the K_m in the presence of 6 mM acetate in the incubation medium was high and almost similar to the K_m value of mixotrophically grown cells.

Removal of acetate from mixotrophically grown *N. hamburgensis* cells at D = 0.008 by washing, resulted also in a decrease of K_m (Table 2, 4). This effect on the kinetic parameters of nitrite oxidation was less pronounced in the culture grown at the dilution rate of 0.011 and addition of acetate to the incubation medium used for determining oxygen consumption rates, had also no significant effect on K_m (Table 4).

Endogenous respiraton rates were not affected by the presence of acetate at concentrations ranging from 0.1 to 10 mM. Obviously, acetate was not respired by either *Nitrobacter* species, although it was established that all mixotrophic cultures did consume acetate in the chemostat. As was discussed above, acetate was probably only involved in the production of cell carbon including reserve material. The results obtained with *N. winogradskyi* and *N. hamburgensis* grown under nitrite limitation in continuous culture are at variance with the results of Smith and Hoare (1968), obtained with *N. agilis* grown with excess nitrite in batch cultures. These authors observed a stimulation of the endogenous respiration by addition of acetate, whereas no effect of 10 mM acetate was observed on nitrite oxidation and growth rates.

Effect of oxygen tension on nitrite oxidation

To study the effect of oxygen concentration on the kinetic parameters of nitrite oxidation, experiments were performed at different oxygen tensions, i.e. 100% and 12–18% air saturation (Figs. 1, 2). Chemolithotrophically grown *N. winogradskyi* cells and mixotrophically grown *N. hamburgensis* cells were taken from the chemostats run at 100% air saturation and dilution rates of 0.017 and 0.011 h^{-1}, respectively. The kinetic parameters determined are presented in Table 5. Nitrite oxidation by *N. hamburgensis* is apparently repressed by high oxygen tensions, while the V_{max} of *N. winogradskyi* is hardly affected by the oxygen tension. Expressed as

Table 5. Effect of oxygen tension in the incubation medium on the kinetic parameters of nitrite oxidation of *Nitrobacter winogradskyi* and *N. hamburgensis* grown under nitrite limitation in the absence or presence of excess acetate, respectively, at 25 °C and 100% air saturation. *N. winogradskyi* and *N. hamburgensis* were grown at dilution rates of 0.017 and 0.011 h^{-1}, respectively. Significant differences ($p < 0.32$) between species and experimental conditions are indicated by different letters. The maximal nitrite oxidation rate (V_{max}) is expressed as fmol nitrite cell^{-1} h^{-1} and the saturation constant (K_m) as μM nitrite.

Nitrobacter species	100% Air saturation		16% Air saturation	
	V_{max}	K_m	V_{max}	K_m
N. winogradskyi	3.68a	69x	2.98b	26y
N. hamburgensis	1.00c	540z	2.34d	730z

V_{max} cell^{-1}, the nitrite oxidation activity of *N. hamburgensis* at reduced oxygen tension was almost similar to the values of *N. winogradskyi*. However, at high oxygen tension, the V_{max} per cell of *N. hamburgensis* was significantly lower than the value of the *N. winogradskyi* culture. In contrast to acetate, no significant effect of the oxygen tension was observed on K_m for nitrite oxidation. The specific affinity of *N. winogradskyi* increased slightly at reduced oxygen tension whereas that of *N. hamburgensis* remained the same.

Another interesting feature revealed by the study of nitrite oxidation at reduced oxygen tension was the nitrite toxicity. Nitrite concentrations over 2.5 mM inhibited nitrite oxidation of *N. winogradskyi* at 100% air saturation, whereas at 18% air saturation nitrite concentrations in excess of 0.5 mM almost completely blocked nitrite oxidation. No toxic effects of nitrite up to 7.5 mM were observed with *N. hamburgensis* cultures at both oxygen tensions.

Actual nitrite oxidation activities compared to potential activities

As discussed above, the cells had an overcapacity for nitrite oxidation, as was calculated by comparing steady state nitrite oxidation activities to the potential activities (V_{max}). The overcapacity of both species even increased as the dilution- or growth-rates decreased. This implies that determinations of potential activities can not be used for the determination of actual in situ activities in field studies nor for measuring the efficiency of MPN-enumerations as was proposed by Belser and Mays (1982). Schmidt and Belser (1982) reported V_{max}-values of 0.012 and 0.009 pmol cell^{-1} h^{-1} for *N. winogradskyi* and *N. agilis*, respectively. Recently, both *Nitrobacter* species are classified as *N. winogradskyi* (Watson et al. 1989). The values mentioned by Schmidt and Belser (1982),

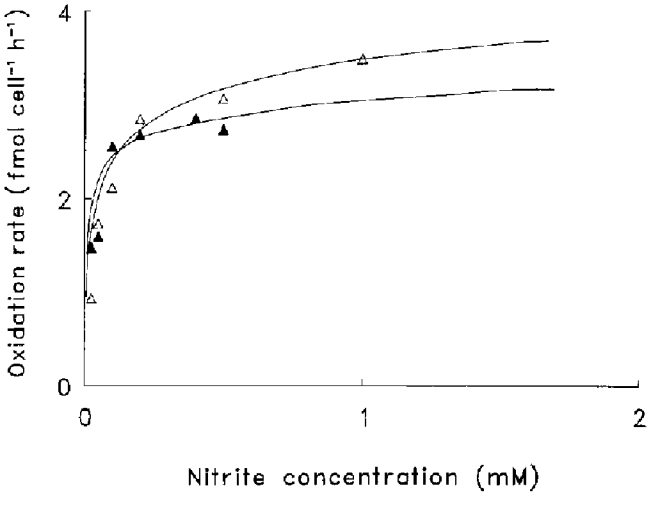

Fig. 1. The effect of oxygen tension on nitrite oxidation of *Nitrobacter winogradskyi* cells grown chemolithotrophically under nitrite limitation at a dilution rate of 0.017 h^{-1}. *Open symbols*: nitrite oxidation rates at 100% air saturation. *Closed symbols*: nitrite oxidation rates at 12–18% air saturation

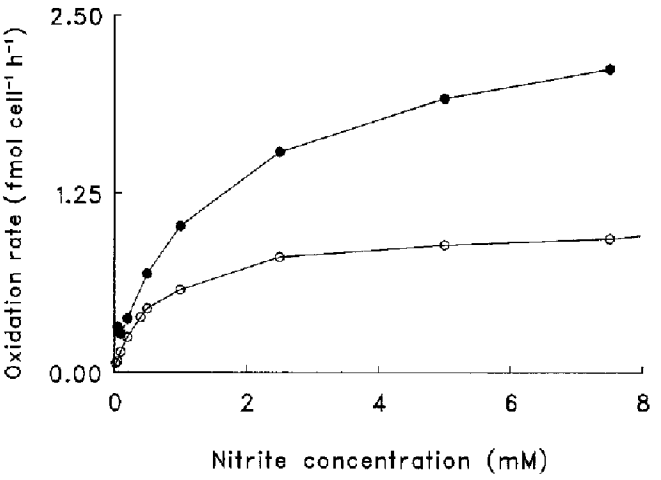

Fig. 2. The effect of oxygen tension on nitrite oxidation of *N. hamburgensis* cells grown mixotrophically under nitrite limitation in the presence of excess acetate at a dilution rate of 0.011 h^{-1}. *Open symbols:* nitrite oxidation rates at 100% air saturation. *Closed symbols:* nitrite oxidation rates at 12–18% air saturation

were determined using exponentially grown cells with excess substrate, whereas our data refer to cells that were grown under substrate limitation and at low dilution rates. The V_{max} values obtained from the exponentially grown cells are a factor 3 to 12 higher than those of the cells grown in continuous culture. The variation in V_{max} could indicate a dependency of the V_{max} on substrate availability and thus on growth rate. Moreover, V_{max} varies between species. This could explain why both parameters measured in field samples do often not correlate.

Niche differentiation

The coexistence was shown of *N. hamburgensis* and two serotypes of *N. winogradskyi* in 14 soil samples of 500 g taken at the same moment from a field plot (Both 1991). It was concluded that niche differentiation between the two species must have occurred within this 500 g of soil. As can be deduced from the results presented in this paper, *N. winogradskyi* will outcompete *N. hamburgensis* with respect to nitrite oxidation in well aerated soils under nitrite-limiting conditions. Both under autotrophic and mixotrophic conditions, *N. winogradskyi* has the lowest K_m for nitrite. If the ratio V_{max}/K_m is taken as a measure for nutrient sequestering ability, also *N. winogradskyi* is the better competitor for nitrite. The K_m values of both *Nitrobacter* species show a similar variation as was found in a field study of the kinetic parameters of nitrifying bacteria in soil and are in the range of values given by different authors (Both 1990a; Both and Laanbroek 1991).

The increased presence of simple organic substrates is one of the many features that distinguishes the rhizosphere from the surrounding soil. If acetate is a representative model substrate, the mixotroph *N. hamburgensis* is not better adapted to growth in the rhizosphere than *N. winogradskyi*. However, the maximal nitrite oxidation

activity of *N. hamburgensis* increased when the oxygen tension decreased, which may indicate a niche differentiation due to the oxygen tension in the soil rather than the availability of organic substrates supporting mixotrophic growth. More experiments need to be done on the behaviour of these *Nitrobacter* species under truely microaerophilic conditions and on the spatial distribution of nitrifying bacteria along several types of gradients to get a better insight in the possible niche differentiation of *Nitrobacter* species. The observations presented in this paper, the occurrence of large numbers of nitrite oxidizers in a water-saturated peat soil (Both et al. 1992) together with the finding that *Nitrobacter* species can also denitrify (Bock et al. 1988) suggest that niche differentiation between *Nitrobacter* species could be at least partly, related to oxygen availability.

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