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Influence of Starvation on Potential Ammonia-Oxidizing Activity and *amoA* mRNA Levels of *Nitrosospira briensis*

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The effect of short-term ammonia starvation on *Nitrosospira briensis* was investigated. The ammonia-oxidizing activity was determined in a concentrated cell suspension with a NO_x biosensor. The apparent half-saturation constant [$K_{m(app)}$] value of the NH_3 oxidation of *N. briensis* was 3 μM NH_3 for cultures grown both in continuous and batch cultures as determined by a NO_x biosensor. Cells grown on the wall of the vessel had a lower $K_{m(app)}$ value of 1.8 μM NH_3. Nonstarving cultures of *N. briensis* showed potential ammonia-oxidizing activities of between 200 to 250 μM N h^{-1}, and this activity decreased only slowly during starvation up to 10 days. Within 10 min after the addition of fresh NH_4^+, 100% activity was regained. Parallel with activity measurements, *amoA* mRNA and 16S rRNA were investigated. No changes were observed in the 16S rRNA, but a relative decrease of *amoA* mRNA was observed during the starvation period. During resuscitation, an increase in *amoA* mRNA expression was detected simultaneously. The patterns of the soluble protein fraction of a 2-week-starved culture of *N. briensis* showed only small differences in comparison to a nonstarved control. From these results we conclude that *N. briensis* cells remain in a state allowing fast recovery of ammonia-oxidizing activity after addition of NH_4^+ to a starved culture. Maintaining cells in this kind of active state could be the survival strategy of ammonia-oxidizing bacteria in nature under fluctuating NH_4^+ availability.

Chemolithoautotrophic ammonia-oxidizing bacteria (AOB) generate their energy by oxidizing ammonia (NH_3) to nitrite (NO_2^-) and fix carbon via the Calvin cycle (3, 53). The oxidation of NH_3 to NO_2^- is a two-step process, where NH_3 is first oxidized to hydroxylamine (NH_2OH) catalyzed by ammonia monooxygenase. The NH_2OH is further oxidized to NO_2^- catalyzed by the hydroxylamine oxidoreductase, which is the energy-generating step of the ammonia oxidation (3, 53). AOB often live in close proximity to nitrite-oxidizing bacteria and together they convert the most reduced form of nitrogen (NH_4^+) to the most oxidized (NO_3^-) (40).

In nature, AOB often face longer periods of NH_4^+ starvation and limitation due to low nitrogen input, low mineralization rates, or competition with other AOB (8), heterotrophic bacteria (48, 49), or plants (5, 6, 50). In order to respond rapidly when NH_4^+ becomes available, AOB must maintain their ability to oxidize NH_4^+ during these periods.

With the exception of a few marine strains within the genus *Nitrosococcus* (of the γ-subclass of the *Proteobacteria*), all known AOB belong to a distinct clade within the β-subclass of the *Proteobacteria* (13), which comprises 11 clusters (37). By using 16S rRNA gene and more recently *amoA* gene sequencing, directly from environmental samples, the distribution of the members of the different clusters of AOB has been correlated to the characteristics of the environments (29, 37). The starvation behavior of several AOB belonging to different phylogenetic groups has previously been investigated. *Nitrosomonas europaea* affiliated with *Nitrosomonas* cluster 7—a group of AOB detected in environments with high NH_4^+ availability like wastewater (36, 40, 51)—rapidly became active again after periods of starvation in batch and retentostat experiments (8, 31, 46, 52), and the marine AOB, *Nitrosomonas cryotolerans*, showed a similarly rapid response to the presence of ammonia (22, 23, 24). On the other hand, members of *Nitrosomonas* cluster 6a (*Nitrosomonas oligotropha* group), often found in freshwater environments (7, 12, 43), and *Nitrosospira briensis*, often found in terrestrial habitats, regain their activity slower than *Nitrosomonas europaea* after long-term starvation of 10 weeks or 4 months (8, 32).

Up to now, members of the *Nitrosospira* clusters have not been investigated in detail with respect to short-term ammonia starvation. Therefore, we present a detailed investigation of the starvation response of *N. briensis* on the cellular and subcellular level. The activity of the *N. briensis* was followed online using a NO_x biosensor. Additionally, we investigated the influence of starvation on both the *amoA* mRNA and protein expression patterns.

MATERIALS AND METHODS

Microorganisms. The experiments were performed with *N. briensis* ATCC 25971 and *Nitrobacter winogradskyi* ATCC 25391.

Medium. Mineral salt medium (MS medium) containing 3 mM $(NH_4)_2SO_4$, 10 mM NaCl, 1 mM KCl, 0.2 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CaCl_2 \cdot 2H_2O$, 0.4 mM KH_2PO_4, and 1-ml/liter trace element solution (49) in distilled water was used for all experiments. For the batch incubations, 30 mM HEPES [4-(2-hydroxy-

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ethyl) piperazine-1-ethanesulfonic acid] was added to keep the pH constant. The pH was adjusted to 7.8 with NaOH before autoclaving. The phosphate solution was autoclaved separately and added at room temperature.

Continuous culture cultivation. The continuous culture experiments were carried out in a chemostat composed of a 3-liter glass vessel, a stirrer, a pH control unit, an aeration unit, and a peristaltic pump. The cell suspension (approximately 2 liters) was kept at a temperature of 25°C. The stirrer speed was adjusted to 300 rpm and the pH was adjusted continuously to 7.5 ± 0.2 by adding 5% Na_2CO_3. The culture was aerated with 1 liter of air min^{-1}. The chemostats were inoculated with actively growing batch cultures of a coculture of *N. briensis* and *N. winogradskyi*. For 5 days the chemostats ran as batch cultures. When the cells had consumed 80 to 90% of the NH_4^+, fresh MS medium containing 5 mM NH_4^+ was pumped into the chemostats and the growth rate was adjusted to 0.014 h^{-1}. The chemostats were sampled regularly to determine the NH_4^+, NO_2^-, and NO_3^- concentrations.

Starvation and resuscitation experiments. The experiments were carried out in a 3-liter glass batch reactor, equipped with a stirrer and an aeration unit. Approximately 2 liters of culture were kept at 25°C with a stirrer speed of 200 rpm and were aerated with 200 ml of air min^{-1}. The reactors were inoculated with actively growing batch cultures of a coculture of *N. briensis* and *N. winogradskyi* and sampled daily to determine the NH_4^+, NO_2^-, and NO_3^- concentrations. Onset of starvation was defined as the time where NH_4^+ was consumed completely. To resuscitate the cultures NH_4^+ was added to a final concentration of 5 mM. During the experiments 100-ml samples were taken, centrifuged (20 min, 22,000 × *g*, 4°C), washed once in 2 ml of MS medium without NH_4^+, and resuspended in 2 ml of MS medium without NH_4^+. A 1.5-ml sample was used for activity measurement and two times 0.2 ml was frozen in liquid nitrogen and stored at −80°C for molecular analysis.

Acetylene treatment. A starved coculture of *N. briensis* and *N. winogradskyi* was incubated overnight in the presence of acetylene to inhibit ammonia monooxygenase (18). After aerating the culture for 1 h with air to remove the acetylene, fresh NH_4^+ was added. As a control, a cell suspension without acetylene treatment was used. Samples were taken and treated the same way as in the other starvation-resuscitation experiments.

Determination of the potential ammonia-oxidizing activity and the $K_{m(app)}$ value. For the determination of the apparent half-saturation constant [$K_{m(app)}$] value, a 1-liter sample was taken from the chemostat or batch cultures, centrifuged (20 min, 22,000 × *g*, 4°C), washed with NH_4^+-free MS medium, concentrated, and used within 2 h for the measurements. The $K_{m(app)}$ of the attached cells was determined from the biomass that was scrubbed off the wall and homogenized at the end of one run. A concentrated culture sample (1.5 ml) was added to 13.5 ml of MS medium without NH_4^+ (pH 7.5) to determine the $K_{m(app)}$ and the potential ammonia-oxidizing activity. The mixture was aerated for 10 min. Then, 0.15 ml of concentrated NH_4^+ solution was added. The NO_2^-/NO_3^- production was followed by using the NO_x biosensor and recorded with a strip chart recorder.

The $K_{m(app)}$ value of the NH_3 oxidation of *N. briensis* was determined by adding different concentrations of NH_4^+, corresponding to NH_3 concentrations between 0.5 and 10 μM, to the cell suspension. The $K_{m(app)}$ was calculated by nonlinear regression based on the Michaelis Menten kinetics (10).

The measurements of the potential ammonia-oxidizing activity during the starvation and resuscitation experiments were done by adding NH_4^+ to obtain a concentration of 10 μM NH_3. The high NH_4^+ concentration was chosen to ensure saturation of the ammonia oxidation.

NO_2^-/NO_3^- production measurement. The NO_2^-/NO_3^- production was determined with a NO_x biosensor (Unisense, Aarhus, Denmark). This sensor contains a denitrifying bacterial culture that reduces NO_3^- and NO_2^- to N_2O, which is then detected by an electrochemical N_2O sensor (33). Calibrations of the sensors were done at the beginning and at the end of the experiments. All experiments were done at 25°C in glass vials, which were kept dark.

O_2 consumption measurement. The O_2 consumption was measured with a Clark-type O_2 sensor in a self-constructed setup (26). The samples were prepared and treated the same way as for the measurements of the NO_2^-/NO_3^- production.

Chemical analysis. Samples for measuring NH_4^+ were analyzed immediately or stored at −20°C. The NH_4^+ concentration was determined colorimetrically (25).

RNA analysis. RNA was extracted with a RNeasy Protect Bacteria kit (QIAGEN) using mechanical disruption of the cells by beat beating. The obtained RNA was treated with DNase (Ambion, Austin, Tex.). The absence of DNA contamination was tested by PCR directly using 1 μl of RNA extract as template. A two-step reverse transcription (RT)-PCR was performed: first the RNA was reverse transcribed to produce cDNA, which was then amplified by PCR in a second step. Two different primer pairs were used: an AOB-specific 16S rRNA primer pair (28) and an *amoA* primer pair, targeting the gene coding for the subunit A of the ammonia monooxygenase. *N. briensis*-specific *amoA* primers were designed based on the *amoA* sequence of *N. briensis* obtained from a public database (amoA-109F, 5′-GTT GGA ACC TAC CAC ATG CA-3′, and amoA-608R, 5′-TCT GAG TGA GCC TTG TTC GA-3′). No quantification of the RNA prior to amplification was done. The RT reactions were done in 5-μl reactions with a RT kit from Ambion or Amersham Bioscience according to the instructions of the manufacturer, by using the amoA-608R or the 16S rRNA reverse primers.

PCR using the 16S rRNA primers was done in 10-μl reactions with 25 cycles as described by Kowalchuk et al. (28). The PCR with the *amoA*-specific primers was done in 10-μl reactions containing 1.25 nmol of each dNTP, 1.5 mM Mg^{2+}, 3 μg of bovine serum albumin, and 5 pmol of each primer. Thermocycling was done with an initial step at 92°C for 1 min, 40 cycles of 92°C for 30 s of denaturation, 57°C for 30 s of annealing, and 72°C for 45 s plus 1 s/cycle extension; the last cycle had a 5-min final extension step.

All RT-PCR products were separated on 2% agarose gels containing 0.5 μg of ethidium bromide ml^{-1} and visualized by UV translumination (Gel Doc 2000; Bio-Rad, Hercules, Calif.). Digital images were acquired with a charge-coupled-device camera controlled by the software Quantity One (Bio-Rad). Quantification of band intensities was done manually by eye, and in order to evaluate the relative differences in band intensities correctly, RT-PCRs were done on undiluted, 10× and 100× diluted RNA extracts.

2D gel electrophoresis. For the analysis of the soluble protein fraction consisting of the cytoplasmatic and the periplasmatic proteins, two reactors with *N. briensis* were grown up (without *N. winogradskyi*). One reactor was harvested at the end of the logarithmic phase, the other after 2 weeks of starvation. Cells were harvested by centrifugation (22,000 × *g*, 20 min, 4°C), washed two times with sterile filtered tap water, resuspended in 1 ml of tap water, frozen in liquid nitrogen, and stored at −80°C until analysis. Sample preparation and electrophoresis were done as described by Schmidt et al. (39).

Proteins in the gel were then fixed and stained in the gels by silver staining. The digitalization of the two-dimensional (2D) protein patterns was done using a Sharp JX scanner interfaced with the Image Master 2D Elite software (Amersham Bioscience).

TABLE 1. $K_{m(app)}$ and $V_{max(app)}$ of the NH_3 oxidation of *N. briensis* grown in batch culture[a]

Determination	$K_{m(app)}$ (μM NH_3)	$V_{max(app)}$ (μM N h^{-1})
NO_2^-/NO_3^- biosensor	2.9 (±0.4)	248 (±12)
O_2 sensor	2.4 (±0.8)	303 (±42)

[a] Determined by NO_2^- production using a NO_2^-/NO_3^- biosensor and O_2 consumption using an O_2 sensor. Values in parentheses are standard errors of the nonlinear regression of the NO_2^-/NO_3^- production or O_2 consumption versus the NH_3 concentrations ($n = 7$, $r^2 > 0.93$).

RESULTS

$K_{m(app)}$ and $V_{max(app)}$ of NH_3 oxidation. To validate the use of the NO_2^-/NO_3^- biosensor approach to estimate $K_{m(app)}$ values, we compared obtained values with values based on the standard method of O_2 consumption measurements. The $K_{m(app)}$ and the $V_{max(app)}$ were determined based on NO_2^-/NO_3^- production and O_2 consumption, respectively (Table 1). The $K_{m(app)}$ values of the ammonia oxidation for pelagic cells grown in continuous and batch cultures were higher than the $K_{m(app)}$ for cells growing in biofilms on the vessel wall (Table 2).

Starvation experiment. The start of the starvation period was defined as the time when NH_4^+ was completely consumed in the coculture of *N. briensis* and *N. winogradskyi* and was found to be 7 days after inoculation (Fig. 1). The potential ammonia-oxidizing activity was around 200 μM N h^{-1} at the end of the growth phase and at the beginning of the starvation

TABLE 2. $K_{m(app)}$ for the NH_3 oxidation of *N. briensis* cultured under different conditions[a]

Culture conditions	$K_{m(app)}$ (μM NH_3)
Batch culture (*N. briensis*)	2.9
Continuous culture (*N. briensis* and *N. winogradskyi*)	3.2 ± 0.4[b]
Wall growth in one of the continuous cultures (*N. briensis* and *N. winogradskyi*)	1.8

[a] Determined with the NO_x biosensor.
[b] Value represents mean ± standard deviation of three independent measurements of the $K_{m(app)}$.

period but decreased to 60 μM N h^{-1} during a 2-week period of starvation.

Parallel to the potential ammonia-oxidizing activity measurements, samples for RNA extraction were taken and analyzed for the presence of *amoA* mRNA and 16S rRNA (Fig. 2). The amplification products obtained with the 16S rRNA-specific primers were used as an internal standard, as AOB are known to keep their ribosomes intact during starvation (23, 51). The band intensities of the 16S rRNA amplicons were stable, but the intensities of the *amoA* mRNA products were decreasing over time, indicating a relative decrease of the *amoA* mRNA. During the growth phase of *N. briensis*, the *amoA* mRNA level appeared constant, whereas it constantly decreased over a period of 12 days of starvation.

Resuscitation experiment. Starvation of *N. briensis* for 1 week resulted in a decrease of the potential ammonia-oxidizing activity from 200 to 150 μM N h^{-1} (Fig. 3). After the addition of fresh substrate, NH_4^+ consumption started immediately, whereas the activity remained constant on the first day and increased later. The potential ammonia-oxidizing activity data shown in Fig. 1 and 3 represent the maximum activities of the cultures after addition of fresh NH_4^+ to the samples during the potential ammonia-oxidizing activity measurement. In addition to these maximal values, we determined the ammonia-oxidizing activity minute by minute by calculating the NO_2^-/NO_3^- production rate at intervals of 1 min by taking into account the slope of the NO_2^-/NO_3^- concentration for the 2 min before and after each time point. Using this approach it was possible to track the development of the ammonia-oxidizing activity over time after the addition of fresh NH_4^+ to the concentrated cell suspensions (Fig. 4). Growing cells reached their maximum ammonia-oxidizing activity 5 min after the addition of NH_4^+. When the cells were starved for 3 and 7 days, they needed 30 and 50 min, respectively, to regain their maximum activity. However, if the culture was resuscitated just before, it took only 5 to 10 min until the maximum ammonia-oxidizing activity was regained (Fig. 4). The *amoA* mRNA concentration decreased during the starvation period (Fig. 5). After the addition of NH_4^+ to the culture, an increase in *amoA* mRNA was detectable after 10 min, but the relative amount was very low and the level of *amoA* mRNA increased slowly, regaining the level for unstarved cells by the final sample (245 min).

Protein pattern. We compared the soluble protein fraction of a growing and a 2-week-starved *N. briensis* culture by using 2D gel electrophoresis (Fig. 6). The protein pattern of the starved and growing *N. briensis* cultures showed only small changes, with five spots disappearing and two spots appearing after starvation.

Treatment with acetylene. A starved coculture of *N. briensis* and *N. winogradskyi* was incubated with acetylene to inhibit the ammonia monooxygenase and subsequently resuscitated by the addition of fresh NH_4^+ parallel with an untreated control culture (Fig. 7). NH_4^+ was consumed in the noninhibited culture immediately after the addition of fresh NH_4^+ and the potential ammonia-oxidizing activity remained at the same high level. In the acetylene-treated culture, the potential ammonia-oxidizing activity decreased to almost zero. After the addition of NH_4^+ the potential ammonia-oxidizing activity increased slowly but constantly.

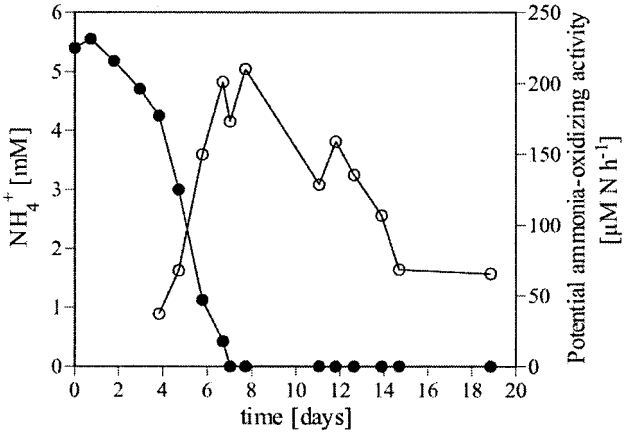

FIG. 1. NH_4^+ dynamics (●) and potential ammonia-oxidizing activity (○) of a coculture of *N. briensis* and *N. winogradskyi* during growth and starvation (reactor 1).

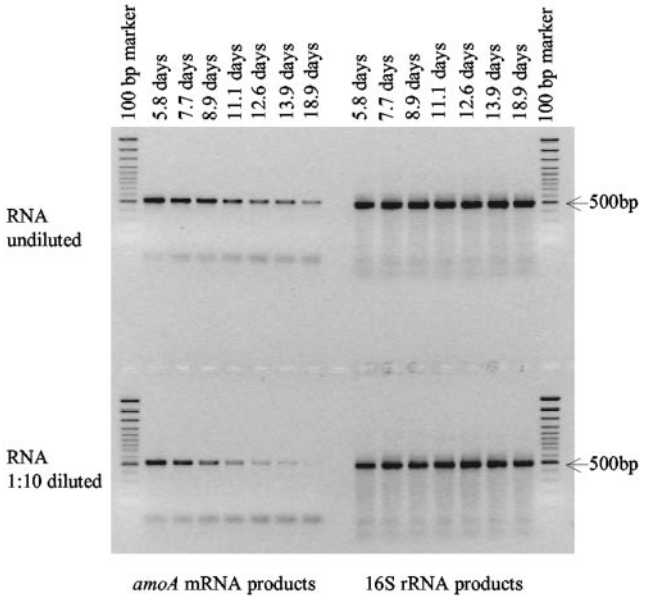

FIG. 2. *amoA* mRNA and 16S rRNA RT-PCR products of *N. briensis* from the reactor 1 during starvation for NH_4^+.

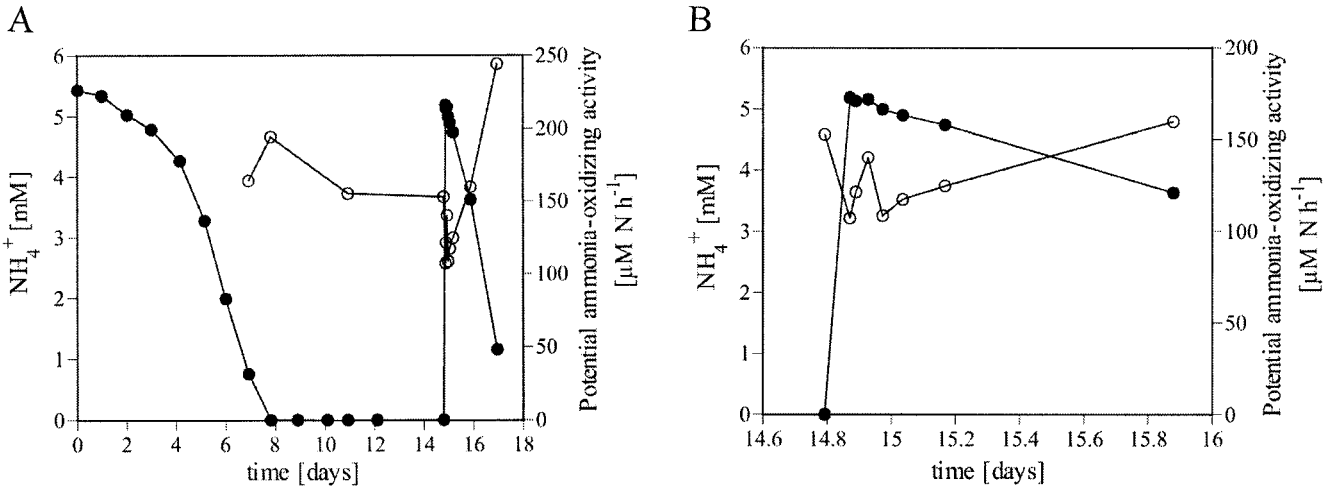

FIG. 3. NH_4^+ dynamics (●) and potential ammonia-oxidizing activity (○) of a coculture of *N. briensis* and *N. winogradskyi* during growth, starvation, and resuscitation (reactor 2). A, overall picture; B, detailed graph for the first hours of resuscitation.

DISCUSSION

In summary, we demonstrated that *N. briensis* is able to recover rapidly after periods of starvation up to 2 weeks. The culture responded within minutes to the addition of fresh NH_4^+ and the maximum potential ammonia-oxidizing activity was reached after 30 to 60 min (Fig. 4). A fast recovery after starvation within the first weeks has been observed for other AOB, e.g., *Nitrosomonas europaea*, *Nitrosomonas cryotolerans*, and the culture G5-7, closely related to *Nitrosomonas oligotropha* (8, 17, 23, 31, 46). Recovery after longer starvation periods revealed differences between the AOB strains; *Nitrosomonas europaea* and *Nitrosomonas cryotolerans* were recovering very fast after >10 weeks of starvation, whereas the culture G5-7 and *N. briensis* showed a longer lag time before they regained their activity (8, 23, 32). Despite this recovery after longer starvation periods of *N. briensis* that was slower than with other ammonia-oxidizing bacteria, *N. briensis* showed recovery patterns similar to other AOB with respect to short-term starvation. The delay in reaching the maximum potential ammonia-oxidizing activity after starvation (Fig. 4) could be explained by the need for key molecules necessary for the metabolism. The addition of the intermediate NH_2OH to a starved culture of *Nitrosomonas europaea* reduced the time delay before reaching the maximum activity (17). As the oxidation of NH_2OH delivers electrons back to the ammonia monooxygenase in addition to electrons for ATP generation (53), the observed delay in the ammonia oxidation could be due to a lack of reducing equivalents for the ammonia monooxygenase. A similar observation was made with methanotrophic bacteria, where the addition of

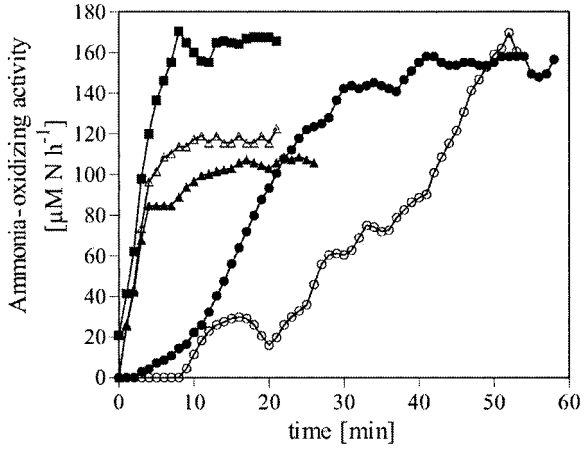

FIG. 4. Ammonia-oxidizing activity over time in the concentrated samples used to measure the potential ammonia-oxidizing activity at the different time points during growth, starvation, and resuscitation (reactor 2). The ammonia-oxidizing activity was calculated for every minute as the slope of the NO_2^-/NO_3^- production within the 2 min before and after each time point. ■, during growth; ●, 3 days starved; ○, 7 days starved; ▲, after 10 min fresh NH_4^+; △, after 4 h fresh NH_4^+.

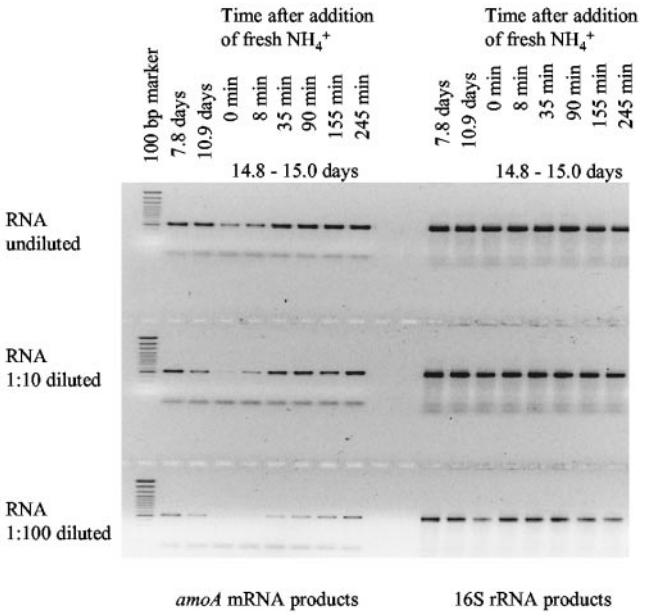

FIG. 5. *amoA* mRNA and 16S rRNA RT-PCR products of *N. briensis* from the reactor 2 during starvation and resuscitation.

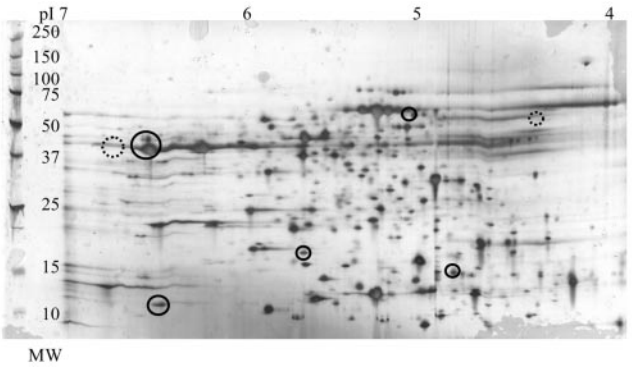

FIG. 6. Comparison of the protein pattern of the soluble protein fraction of a growing and a 2-week-starved culture of *N. briensis*. Circles with solid lines indicate protein spots disappearing and circles with dashed lines indicate spots where protein spots are appearing during starvation. MW, molecular weight.

the intermediate methanol was found to improve CH_4 uptake (20) due to provision of energy derived from the oxidation of methanol.

In contrast to many other bacteria, the rRNA content of AOB is kept at a high level during starvation (23, 51). Furthermore, our results indicate that not only ribosomes are retained but that there is a general retention of functionality by the organisms even during starvation periods. We found that *amoA* mRNA was still present after a starvation period of 12 days, although the concentration was much lower than in a growing cultures (Fig. 2 and 5). In contrast, Sayavedra-Soto et al. (38) did not detect *amoA* mRNA in *Nitrosomonas europaea* when using Northern blot hybridization after 8 to 12 h of starvation. This might, however, be explained by differences in the sensitivity of the detection of mRNA, as Northern blot hybridization has no PCR amplification step prior to detection. Although also possible, differences in the stability of the mRNA molecules for *Nitrosomonas europaea* and *N. briensis* are unlikely as both AOB show the same pattern in the response to short-term starvation.

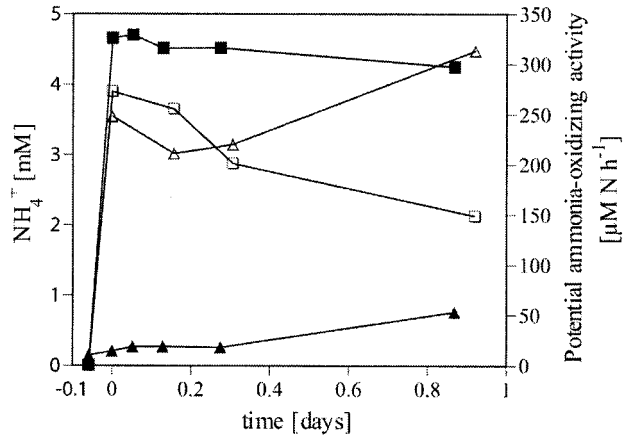

FIG. 7. NH_4^+ dynamics and potential ammonia-oxidizing activity of a coculture of *N. briensis* and *N. winogradskyi* during resuscitation after starvation treated with and without acetylene before addition of fresh NH_4^+ (reactor 3). □, NH_4^+ (without acetylene treatment); ■, NH_4^+ (after acetylene treatment); △, potential ammonia-oxidizing activity (without acetylene treatment); ▲, potential ammonia-oxidizing activity (after acetylene treatment).

The detection of *amoA* mRNA has been used to measure the activity of AOB (11), but the possibility that *amoA* mRNA would still be detectable after 12 days of starvation makes this approach questionable and care should be taken in order to make a direct correlation between mRNA detection and in situ activity of the cells.

The half-life of mRNA in most bacteria has an average of 3 min (0.5 to 50 min) (45). In the heterotrophic bacteria *Vibrio angustum* S14 (1, 2) and *Rhizobium leguminosarum* (47), starvation leads to an increase in mRNA half-life. Thus, it could be speculated that starvation leads to a stabilization of the *amoA* mRNA in AOB as well.

In the presence of acetylene, the activity is almost zero but increases slowly after the addition of NH_4^+, as the inhibition is irreversible and new ammonia monooxygenase has to be synthesized to regain activity (Fig. 7). In the starved and resuscitated cultures without acetylene treatment, the ammonia-oxidizing activity reached a maximum almost immediately (Fig. 3 and 7). The pattern of the soluble protein fraction of actively growing cells and 2-week-starved cells showed only small differences (Fig. 6), indicating that the overall change to function of the cells is not great and that they maintain much of their metabolic machinery. The observed lack in major changes in the protein pattern of *N. briensis* is in contrast to the stress and/or starvation response of other bacteria investigated. In *Bacillus subtilis*, the induction of many different proteins occurs during starvation, including proteins involved in sporulation (14). Several other comparative studies with *Vibrio* sp. strain S14, *Salmonella enterica* serovar Typhimurium, and *Escherichia coli* also demonstrated the induction of proteins necessary for survival during starvation periods (34, 35, 41, 42). In *E. coli* and other gram-negative bacteria, the stationary-phase response is regulated by the expression of the *rpoS* gene coding for the σ^s factor (15, 16). *Nitrosomonas europaea* lacks *rpoS*-like genes completely (9), and though the absence of *rpoS* in *N. briensis* was not confirmed, the observed induction of new proteins during starvation being less than that for other bacteria indicates that *N. briensis* might have a different response pattern to starvation stress.

***$K_{m(app)}$* values of the NH_3 oxidation.** The ammonia-oxidizing activity and the $K_{m(app)}$ values of the NH_3 oxidation have often been determined by measuring the O_2 uptake in the presence of NH_4^+ (19, 30, 44). This method has some disadvantages, particularly as O_2 is also the substrate of all other oxic processes and the $K_{m(app)}$ can only be measured using this method in pure cultures. In mixed cultures the O_2 consumption by non-AOB has to be otherwise inhibited or excluded. A method to measure the ammonia-oxidizing activity and the kinetic parameters of the ammonia oxidation with a NO_x biosensor was therefore developed. The comparison of the $K_{m(app)}$ and the V_{max} determined with both methods showed differences of approximately 20% (Table 1). The reproducibility of the newly developed method was determined by the threefold determination of the $K_{m(app)}$ value of the NH_3 oxidation (Table 2). The standard deviation of the threefold determination indicates that a mistake of 10 to 15% can be expected, and we thus find

the difference between the two methods within an expectable range.

The $K_{m(app)}$ value for NH_3 oxidation in *N. briensis* was 3 μM NH_3 for cultures grown in continuous and batch cultures, respectively. So the growth mode of *N. briensis* had no influence on the affinity for NH_3. However, the $K_{m(app)}$ values were quite low compared to values of other *Nitrosospira* species (21). We measured the NO_2^-/NO_3^- production rate within 15 min after the addition of NH_4^+ to the concentrated culture. Jiang and Bakken (21) calculated the starting activity from a long-term experiment and used different initial pH values to determine the different initial NH_3 concentrations. Hence, the conditions for the determination of the $K_{m(app)}$ values were very different and could have caused the differences in the results. The $K_{m(app)}$ value was also lower than values determined for members of the *Nitrosomonas* cluster 7 (relatives of *Nitrosomonas europaea*), *Nitrosomonas* cluster 6b (relatives of *Nitrosomonas marina*), and *Nitrosomonas* cluster 8 (relatives of *Nitrosomonas communis)*, but they were in the same range as the $K_{m(app)}$ values of members of *Nitrosomonas* cluster 6a (relatives of *Nitrosomonas oligotropha*) (27).

The cells from wall growth were found to have a $K_{m(app)}$ value of only 1.8 μM NH_3 (Table 2), showing that AOB growing in biofilms have a higher affinity for NH_3 than the bacteria in the culture liquid. Biofilm cells of *Nitrosomonas europaea* have been reported to recover faster from starvation than cells in liquid culture (4, 46), and a lower $K_{m(app)}$ value and better recovery after starvation indicate better competitive abilities of biofilm cells compared to cells in liquid culture.

Conclusion. The comparison of our observations with published data from studies of several heterotrophic bacteria indicates that AOB may have a unique mechanism to cope with nutrient starvation. They are able to keep their cells in a state where it is possible to start oxidizing NH_4^+ almost immediately and with the maximum rate after shorter starvation periods, and they are thereby able to respond rapidly to changing environmental conditions. This ability could represent a survival strategy for the chemolithoautotrophic AOB to enable them to be better competitors in the environment. The questions remain whether this is a more widely distributed mechanism among other groups of slow-growing bacteria and how the mechanism behind the ability to maintain this rapidly responsive state might be regulated.

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