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Effects of Aerobic and Microaerobic Conditions on Anaerobic Ammonium-Oxidizing (Anammox) Sludge

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The anaerobic ammonium oxidation (Anammox) process is a promising novel option for removing nitrogen from wastewater. In this study it was shown that the Anammox process was inhibited reversibly by the presence of oxygen. Furthermore, aerobic nitrifiers were shown not to play an important role in the Anammox process.

Recently, a new process was discovered in which ammonium was converted to dinitrogen gas under anaerobic conditions with nitrite as the electron acceptor (7, 8). This anaerobic ammonium oxidation (Anammox) process is a promising opportunity for low-cost ammonium removal from concentrated wastewaters (6). From an application point of view it is important to know the influence of oxygen on the Anammox process, because in practice Anammox will be combined with a preceding partial-nitrification step (3). The first aim of this study was to investigate the influence of oxygen on the Anammox process. This is particularly interesting since recent studies have indicated that aerobic nitrifiers such as *Nitrosomonas* spp. are capable of anaerobic ammonium oxidation (1, 2). Therefore, the second aim of this study was to investigate whether aerobic nitrifying bacteria such as *Nitrosomonas* might be involved in the Anammox process.

Anammox under alternating aerobic and anaerobic conditions. An intermittently aerobic and anaerobic reactor was inoculated with 0.86 g of volatile suspended solids (VSS) of granular anaerobic ammonium-oxidizing sludge from a denitrifying fluidized bed reactor (5). The reactor (volume, 2 liters; temperature, 32 ± 2°C; pH, 7.8 ± 3; stirrer speed, 150 to 200 rpm) was made aerobic by sparging with air and anaerobic by sparging with argon. Each aerobic period lasted 2 h, and each anaerobic period lasted 2 h. The reactor was intermittently fed with medium (8) containing 6 mM NH_4^+ and 8 mM NO_2^- for 20 days. The (hydraulic) dilution rate was maintained at 0.010 h^{-1} throughout the experiment.

The aerobic and anaerobic regime was maintained for 20 days, and the ammonium, nitrite, and oxygen concentrations were monitored during 22 aerobic and 22 anaerobic periods. Fig. 1 shows two typical concentration profiles. Figure 1A shows that ammonium was oxidized anaerobically but gives little information on aerobic ammonium oxidation. However, from Fig. 1B it is clear that no ammonium was oxidized during the aerobic periods. The Anammox activity in the anaerobic periods remained constant (2 μmol of NH_4^+/g of VSS/min) throughout the experiments, indicating that the oxygen inhibition was reversible. During 20 days, no aerobic ammonium oxidation was observed.

The sensitivity of this culture to oxygen was further investigated under microaerobic conditions. In four consecutive experiments, the oxygen tension was decreased stepwise (partial O_2 pressure, 2, 1, 0.5, and 0% of air saturation). No ammonium was oxidized in the presence of 2, 1, and 0.5% air. After these three experiments, the reactor was flushed with argon until it was completely anaerobic (0% O_2). In this case, ammonium and nitrite were consumed as before. This is shown in Fig. 2

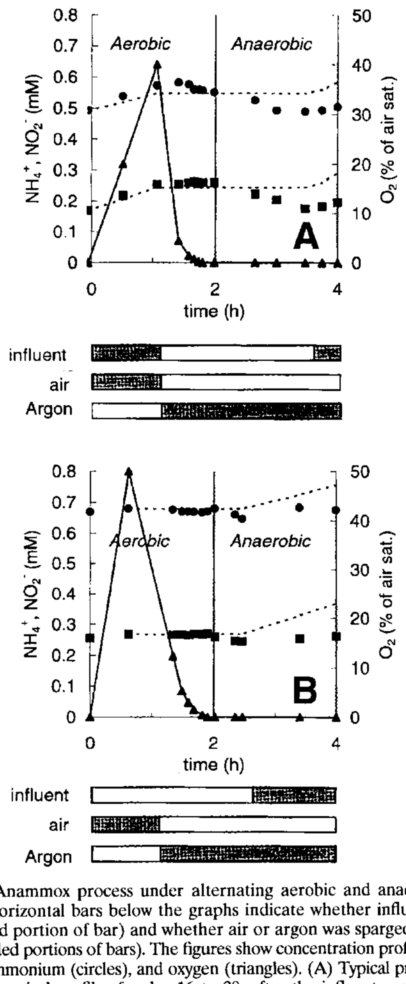

FIG. 1. Anammox process under alternating aerobic and anaerobic conditions. The horizontal bars below the graphs indicate whether influent was supplied (shaded portion of bar) and whether air or argon was sparged through the reactor (shaded portions of bars). The figures show concentration profiles for nitrite (squares), ammonium (circles), and oxygen (triangles). (A) Typical profiles for day 1 to 15; (B) typical profiles for day 16 to 20, after the influent supply had been changed (see horizontal bar). The dotted lines show the ammonium and nitrite profiles that were expected when no Anammox activity occurred. sat., saturation.

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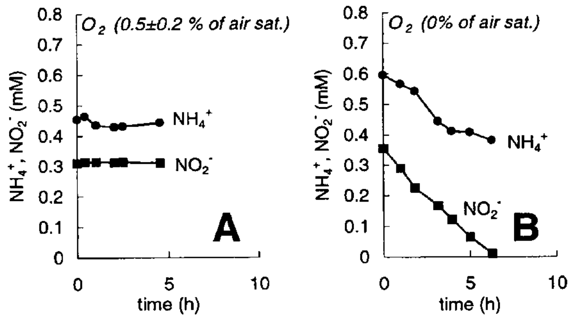

FIG. 2. Anammox activity at air saturations (sat.) of 0.5% ± 0.2% (A) and 0% (B). Only when no oxygen was present (B) was Anammox activity observed.

(0.5% air [Fig. 2A] and 0% air [Fig. 2B]). Thus, no Anammox was observed under microaerobic conditions.

Presence of aerobic nitrifiers. Although it was shown previously (8) that aerobic nitrifiers were present in the sludge inoculum (10^4 cells/ml of sludge), the experiment described above showed that enrichment of aerobic nitrifiers from the Anammox sludge under alternating aerobic and anaerobic conditions was not possible. To (i) verify the presence of aerobic nitrifiers and to (ii) investigate the possibility that the Anammox population was in fact facultatively aerobic but did not manage to switch to aerobic metabolism in a 2-h period, the following experiment was conducted. Enriched Anammox sludge from an Anammox repeated fed batch reactor (0.028 g [VSS]) (4) was continuously sparged with air (stirrer speed, 200 rpm; partial oxygen pressure, 50 to 80% of air saturation; pH 8.0). Under these continuously aerobic conditions, an aerobic ammonium-oxidizing population was enriched (Fig. 3). In 12 days, 27 mM ammonium disappeared with the appearance of 20 mM nitrite. Nitrite was not further oxidized, since no nitrate could be detected. The aerobic nitrifying population grew exponentially, with a doubling time of 1.2 days. To investigate whether this aerobic ammonium-oxidizing culture could still oxidize ammonium anaerobically, the culture was exposed to the alternating aerobic and anaerobic regime for 20 days as described before. In this case, anaerobiosis was achieved not by sparging with argon but by suffocation (2). When the air supply was shut down, the active nitrifying population consumed all oxygen present in the reactor within 5 min.

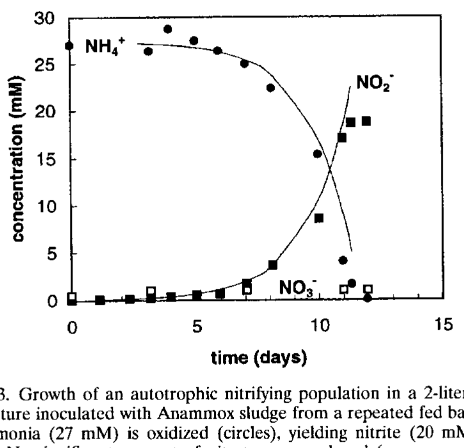

FIG. 3. Growth of an autotrophic nitrifying population in a 2-liter aerobic batch culture inoculated with Anammox sludge from a repeated fed batch reactor. Ammonia (27 mM) is oxidized (circles), yielding nitrite (20 mM) (filled squares). No significant amount of nitrate was produced (open squares). The lines represent the concentration profiles that would be expected when the growing nitrifiers have a doubling time of 1.2 days.

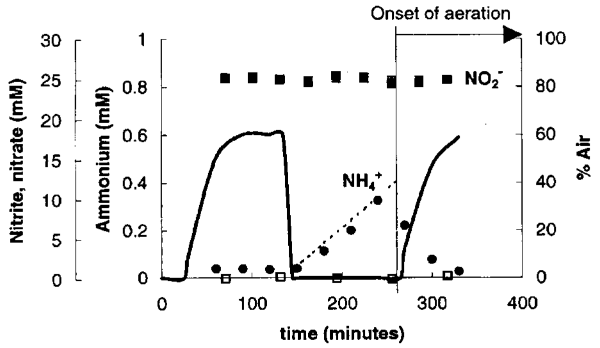

FIG. 4. Typical concentration profiles of ammonium (circles), air saturation (thick line), nitrite (filled squares), and nitrate (open squares) in a mixed culture of aerobic nitrifiers (influent ammonium concentration, 30 mM) under alternating aerobic and anaerobic conditions. The dotted line shows the ammonium concentration profile that was expected when no Anammox activity occurred. Error bars, standard errors of the means.

During 20 days, 30 mM influent NH_4^+ (D = 0.0075 h^{-1}) disappeared in the reactor with the appearance of 25 mM NO_2^- (Fig. 4). No formation of nitrate was observed. Thus, in the N balance the fate of 5 mM (16%) of the total 30 mM of ammonium was not known. This missing ammonium could have disappeared either in the aerobic periods or in the anaerobic periods or in both. To resolve this issue, Fig. 4 contains a dashed line that shows the concentration profile of ammonium that would have been expected during the anaerobic periods, when no anaerobic ammonium oxidation would have occurred. The experimentally observed ammonium concentration profile matched this line. This indicates that the anaerobic ammonium oxidation activity of the aerobic enrichment culture was less than 5% of the activity in the aerobic periods. Since 19% would be required to make up for the missing nitrogen, it was concluded that the nitrogen disappeared mainly during the aerobic periods. Ammonium and nitrite may have been converted to N_2O, as reported for *Nitrosomonas* under oxygen limitation (2).

To remove ammonium from wastewater, the Anammox process has to be supplied with nitrite as the electron acceptor. Therefore, complete nitrogen removal can only be achieved when part of the ammonium is first nitrified to nitrite in a preceding partial-nitrification step (3). The reversibility of the oxygen inhibition observed in this study favors the application of the Anammox process, since it makes possible the combination of partial nitrification and anaerobic ammonium oxidation in one vessel, yielding fully autotrophic nitrogen removal in a single step. This will be the subject of further research.

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