

Growth and metabolism of Anammox Bacteria

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Project description

The anoxic ammonium oxidation (anammox) process is the conversion of nitrite and ammonium under anoxic conditions- to form dinitrogen gas. The process is performed by deep-branching Planctomycetes. The start-up of the first full-scale anammox reactor in the world was described. The described full scale reactor was a granular sludge reactor which was optimized for biomass retention. The reactor was scaled up directly from lab-scale to full-scale without the intermediate step of a pilot plant- and the step from lab-scale to full-scale took three years. In the first phase of the start-up, quantification of the number of anammox bacteria, which were present in the reactor by quantitative polymerase chain reaction (Q-PCR) was a reliable indicator of growth of the anammox bacteria. The volumetric conversion of 10 kg N/m³/day is high compared to lab-scale systems. Anammox bacteria were grown as free suspended (planktonic) cells. Even at a Sludge Retention Time (SRT) of 12 days (doubling time 8.3 days) stable operation was possible. The purity of the biomass was estimated to be 97.6%, which was the highest level of enrichment ever achieved for anammox reactors.



The full-scale anammox reactor in Rotterdam (NL) converting over 500 kg-N/day.



Membrane bioreactor producing planktonic anammox cells.

The addition of hydroxylamine and the subsequent transient production of hydrazine can be regarded as a benchmark for the anammox process. The kinetics of the conversion were studied in detail for "*Kuenenia stuttgartiensis*". Hydrazine accumulated slightly after addition of hydroxylamine and remained low until near completion of the hydroxylamine. At that moment, the hydrazine level suddenly raised to ca. 100 μ M, after which it gradually disappeared. The overall reaction was a disproportionation of hydroxylamine into ammonium and dinitrogen gas. The observed sudden accumulation of hydrazine could only be explained by assuming that hydrazine was an intermediate in this process. Two simple mathematical models, based on the continuous turn over of hydrazine during hydroxylamine conversion, were capable of quantitatively explaining the observed phenomena.

The production of nitric oxide, another potential intermediate in the anammox process, was studied in combination with the emission of greenhouse gas nitrous oxide (N₂O) in a full scale two reactor nitrification anammox process. The NO and N₂O emissions in the nitrification reactor were 0.2% and 1.7% of the nitrogen load respectively and 0.003% and

0.6% for the anammox reactor. The NO emission in the nitrification reactor was higher at higher aeration flows and NO seemed to be produced mainly in the period when the nitrification reactor was aerated. The N₂O emission on the other hand seemed to be mainly produced during anoxic periods. Anammox bacteria have a unique cell plan consisting of several membrane-surrounded compartments. In the main compartment, the anammoxosome, the anammox catabolism is hypothesized to take place. Therefore, also the proton motive force is probably generated between the anammoxosome and the riboplasm by which the anammoxosome is surrounded. ³¹P nuclear magnetic resonance (NMR) spectroscopy was used to evaluate the pH difference over the anammoxosome membrane of "Kuenenia stuttgartiensis" in vivo. Two compartments with stable pH values of 6.3 and 7.3 respectively were found in actively converting cells. The pH values were independent of the external pH and were visible already upon exposing the cells to anoxic conditions. The lower pH value was assigned to the anammoxosome, whereas the pH of 7.3 was assigned to the riboplasm. The stability of the pH in both compartments is a strong indication that the anammoxosome is the locus of the catabolism and thus functionally resembles the eukaryotic mitochondrion.

Dissertation

Growth and metabolism of anammox bacteria. Wouter van der Star, 15 April 2008.

References

Startup on full scale

Van der Star WRL, Abma WR, Blommers D, Mulder JW, Tokutomi T, Strous M, Picioreanu C and Van Loosdrecht MCM. 2007. Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. Chapter 2. Water Res 41(18) 4149-4163.

Growth as suspended cells

Van der Star WRL, Miclea AI, Van Dongen L, Muyzer G, Picioreanu C, Van Loosdrecht MCM. 2008. The membrane bioreactor: a novel tool to grow anammox bacteria as free cells. Chapter 3. Biotechnol Bioeng, published online, doi: 10.1002/bit.21891.

Emission of NO and N₂O

Kampschreur MJ, Van der Star WRL, Wielders HA, Mulder JW, Jetten MSM and Van Loosdrecht MCM. 2008. Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment. Chapter 5. Water Res 42(3) 812-826.

Internet

www.anammox.com

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