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Development of Simple and Rapid Method for Determining Anammox Activity and Discovery of Anammox in Natural Fresh Water Environments

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Anaerobic ammonium oxidation (abbreviated as anammox) is a microbial reaction which mediates direct oxidation of ammonia with concomitant reduction of nitrite under anaerobic condition, subsequently resulting N₂ gas production.

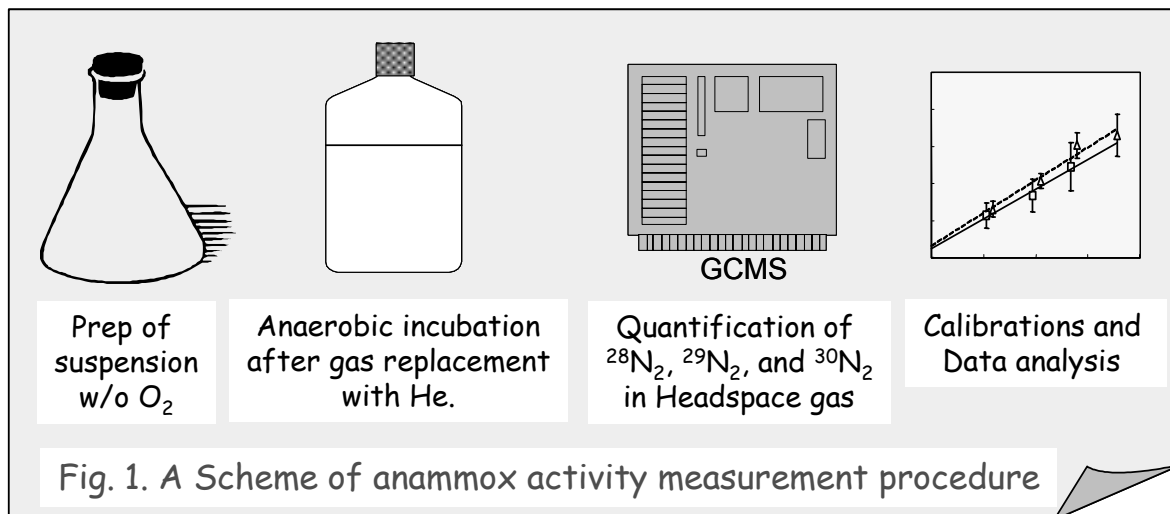


Anammox discovered in mid '90's at an anaerobic wastewater treatment process (Mulder *et al.*, 1995). Before the discovery of anammox, it had long been thought that nitrate/nitrite could be eliminated from aqueous environments only via denitrification. Anammox can be regarded a novel pathway that could short circuit the nitrogen cycle, bypassing what was previously recognized to be a critical nitrification and denitrification. This bypass in the biogeochemical N cycle has first drawn an enormous attention from environmental engineers, because this has a potential that may allow us to design an energy-saving process.

It is notable that a reaction stoichiometry similar to that described for anammox was suggested by a lot of ecological surveys and researches more than 50 years before the anammox was first reported by microbiologists in 1995. As referred in a recent review article by Engstrom *et al.* (2005), anammox-like reactions were studied rather extensively in anoxic basins of a Norwegian Fjord and the Black Sea in the mid 1960s, and indications of this novel route of nitrate/nitrite reduction by ammonium have been proposed by many marine ecologists. It had been also a big scientific interest from which natural environment anammox can be discovered and how much extent anammox contributes in biogeochemical N cycle. A few years ago, Danish marine ecologists eventually reported anammox activity in marine sediment of continent shelf off Denmark, which is the first paper reporting anammox activity in nature (Thamdrup and Dalsgaard, 2002). Anammox activity has also been discovered at

oxic-anoxic interfaces in various environments, especially where both ammonia and nitrite can be continuously supplied, such as estuary and marine sediments, and recognized its significance in nitrogen cycle. However, anammox activity in fresh water environments has remained still unclear. I and my colleagues got very much interested in this point, because our residential areas are often surrounded by freshwater environments, from which we sometimes obtain water resources.

Anammox activity can be recognized by several different methods. Among these methods, the tracer experiment has several significant advantages over others. If ammonium is labeled with ^{15}N , anammox produces unique product, $^{29}\text{N}_2$, as shown in equation 1. This method provides direct evidence for presence of anammox, and allows to determining rate constant of the activity under various conditions. However, no simple and rapid activity assay protocol for determining anammox activity has been available. We have developed a simple ‘flask’ test, which allows accurate determination of the activity, based upon tracer experiment principle (Suwa *et al.*, 2005). For tracer experiments for anammox activity detection, previous workers has mainly used so-called ‘isotope mass spectrometer (IRMS)’, which is not easy for microbiologists to access. Another advantage of our method is to employ another type of equipment, quadrupole mass spectrometer, which is much more widely used in many purposes and easily found in laboratories. Our procedure for determining the activity is straight-forward (Fig. 1).



We surveyed horizontal distribution of anammox activity in a freshwater lake, which locates north of Tokyo (Yamagishi *et al.*, 2006). Lake Kitaura, a eutrophic shallow (7.0 m) freshwater lake (ca 20 km long, 4.5 km width) receives nutrient inputs mainly from two small rivers discharging into its northern end and has an outlet at the southern end. Dissolved oxygen in water body is usually near saturation level, but it completely depletes in the sediment even at 10 mm depth, suggesting that oxic-anoxic interface would be found just beneath the sediment surface. Since it is absorbed by soil

particles in freshwater environments, ammonium as well as oxygen can be supplied for nitrification at the sediment surface. Presumably, anoxic sites continuously receiving both ammonium and nitrite can be formed in sediment just beneath the surface, possibly serving a niche for anammox.

Anammox rates were determined for eleven sediment samples obtained from various sites (100 mm depth) over the lake. Samples were amended with $^{15}\text{NH}_4\text{Cl}$ and non-labeled nitrite, and anoxically incubated in dark. Since anammox produces $^{29}\text{N}_2$ from 1 mol each of $^{15}\text{NH}_4\text{Cl}$ and ^{14}N -nitrite, potential $^{29}\text{N}_2$ production rates were monitored using GC/MS over time. Anammox activity was detected in six northern sites, but was not from five southern sites. Especially two sites showed significantly high activity, of which contribution in N_2 emission was >25%. Anammox microbes were detected from six out of seven activity-positive sites by a molecular method using a PCR technique with a specific primer set, but the remaining five gave no signal. The results demonstrate that anammox contributes to nitrogen cycle in freshwater eutrophic lake ecosystems, and activity 'hot spots' may exist. Anammox activity was detected also from water column of a fresh water lake just recently (Schubert *et al.*, 2006).

References

1. Engstrom, P., *et al.* (2005) *Geochem. Cosmochem. Acta*, 69;2057-2065.
2. Mulder, A., *et al.* (1995) *FEMS Microbiol. Ecol.*, 16;177-183.
3. Schubert, C.J., *et al.* (2006) *Environ. Microbiol.*, 8;1857-1863.
4. Suwa, Y., *et al.* (2005) *JSME21 Abstr.* p. 70.
5. Thamdrup, B. and Dalsgaard, T. (2002) *Appl. Environ. Microbiol.*, 68;1312-1318.
6. Yamagishi, T., *et al.* (2006) *ISME11 Abstr.* p. A294.