



Oxygen contamination during anoxic incubations

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The effect of oxygen present in very low concentrations (nanomolar range) on the performance of anaerobic bacteria is largely unknown. Therefore, it is essential to preserve the anoxic state of water during experiments aimed at investigating oxygen sensitive microbial and chemical processes. Containers for the incubation of anoxic water are carefully chosen for their ability to keep oxygen out. However, the materials out of which the containers are made can contain oxygen which can slowly be released upon contact with the anoxic water.

Glass containers with butyl rubber septa are typically used for the measurement of anaerobic processes in natural waters, for instance of nitrogen metabolisms such as the anaerobic oxidation of ammonium and denitrification. We measured the degree of oxygen contamination in anoxic water incubated in Labco[®] exetainers, glass tubes closed with butyl rubber septa, using ultrasensitive STOX sensors (detection limit < 10 nM). The O₂ concentration of helium-purged water increased by 100's of nanomolars after transfer into Labco[®] exetainers, while O₂ concentrations did not increase in exetainers with rubber septa that were pre-treated to remove oxygen. In addition, significant oxygen contamination occurred while filling the exetainers, as more oxygen was found in exetainers filled in air compared to exetainers filled in an anoxic environment such as a glove bag. The introduction of a helium headspace in exetainers substantially reduced the degree of oxygen contamination by allowing any oxygen that was introduced while handling the anoxic water to concentrate in the headspace.

Our results apply qualitatively to incubations in other types of vials and demonstrate that results of anoxic incubations in containers made of materials that can absorb oxygen have to be interpreted with care.