



## Distant electric coupling between nitrate reduction and sulphide oxidation investigated by an improved nitrate microscale biosensor

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Bacteria are apparently able to transmit electrons to other bacteria (Summers et al. 2010) or to electrodes (Malvankar et al. 2011) by some kind of nanowires (Reguera et al. 2005, Gorbi et al. 2006). Lately it has been shown that such transfer may occur over distances of centimetres in sediments, thereby coupling sulphide oxidation in deeper layers with oxygen reduction near the surface (Nielsen 2011). The finding of these long-distance electrical connections originated from analysis of  $O_2$ ,  $H_2S$ , and pH profiles measured with microsensors. Nitrate is thermodynamically almost as good an electron acceptor as  $O_2$ , and we therefore set up an experiment to investigate whether long-distance electron transfer also happens with  $NO_3^-$ .

Aquaria were filled with sulphidic marine sediment from Aarhus Bay that was previously used to show long-distance electron transfer to  $O_2$ . The aquaria were equipped with a lid so that they could be completely filled without a gas phase. Anoxic seawater with  $300 \mu M NO_3^-$  was supplied at a constant rate resulting in a steady state concentration in the aquatic phase of  $250 \mu M NO_3^-$ . The reservoir with the nitrate-containing water was kept anoxic by bubbling it with a  $N_2/CO_2$  mixture and was kept at an elevated temperature. The water was cooled on the way to the aquaria to keep the water in the aquaria undersaturated with gasses, so that bubble formation by denitrification in the sediment could be minimised. Profiles of  $NO_3^-$ ,  $H_2S$ , and pH were measured as a function of time (2 months) applying commercial sensors for  $H_2S$  and pH and an improved microscale  $NO_3^-$  biosensor developed in our laboratory.

The penetration of  $NO_3^-$  in the sediment was 4-5 mm after 2 months, whereas sulphide only could be detected below 8-9 mm depth. The electron acceptor and electron donor were thus separated by 4-5 mm, indicating long distance electron transfer. A pH maximum of about 8.6 pH units at the  $NO_3^-$  reduction zone similar to a pH maximum observed in the  $O_2$  reduction zone of electro-active sediments could be observed. This pH maximum was the strongest evidence for long-distance electron transfer in oxic sediments, but cannot be taken as proof in denitrifying sediments as conventional denitrification may also produce elevated pH. We are now searching for the  $NO_3^-$  reducing bacteria that may be active in long-distance electron transfer in our sediment.

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