

## ANAMMOX BACTERIA DETECTION IN THE DEEP-SEA SLUDGE LAYER

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Anaerobic ammonia oxidation (ANAMMOX) with the nitrogen gas release is performed by a group of microorganisms that play certain role in the global ammonia utilization in aquatic environment anaerobic zone. They are almost impossible to isolate in pure culture. The anammox bacteria 16S rRNA ribosome research and the specific primers creation, unique sequences allocation and subsequent sequencing led to anammox bacteria classification into order Candidatus Brocadiales class Planctomycetia. After anammox bacteria discovery they were isolated around the world in different water systems and in the Black Sea in particular in 2003.

We analyzed four samples out of eighteen (№3 (80m), №4 (78m), №20 (95m), №21 (178m)), that had the inmost depths selection, to search anammox microorganisms in the deep-sea sludge. Sampling was carried out by a rotary core drilling bit at the 7 - 8 m depth from the bottom, in the area of the Danube river estuary. Samples were stored at + 4C °. To identify the *Ca. Brocadia* and *Ca. Kuenenia* anammox microorganisms Amx368F / Amx820R specific primers pair PCR was used. Brod541F / BS-820R primers pair PCR was used to identify *Ca. "Scalidua wagneri"* and *Ca. "Scalidua sorokinii"*. Fluorescent probe Amx820R-FAM was used for anammox colonies visual detection.

PCR results showed no *Ca. Brocadia*, *Ca. Kuenenia*, *Scalidua wagneri* and *Ca. Scalidua sorokinii* specific amplicon representatives. Samples total DNA electrophoretic analysis showed very low DNA concentrations. FISH microscopy allowed to determine the anammox colonies: 1 - 2 colonies per 10 µl. Thus, the data presented above indicate the anammox microorganisms presence in the deep-sea sludge layer in very small quantities, that complicates these anammox bacteria samples PCR study and requires further anammox bacteria enriching cultivation in the batch reactor.

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