DOI 10.18699/SBB-2022-299

Microbial anammox communities: structure, physiological and biochemical characteristics, and possibilities of directed modification

Pimenov N.V.[1]*, Kallistova A.Yu.[1], Nikolaev Yu.A.[1], Ravin N.V.[2], Mardanov A.V.[2]
[1] *Winogradsky Institute of Microbiology, FRC of Biotechnology, RAS, Moscow, Russia*
[2] *Skryabin Institute of Bioengineering, FRC of Biotechnology, RAS, Moscow, Russia*
* *npimenov@mail.ru*

Key words: anammox, microbial community, bioreactor, metagenomics, sewage treatment

Motivation and Aim: Anammox is a process of anaerobic oxidation of ammonium with nitrite, resulting in formation of gaseous nitrogen, which is carried out by a multicomponent microbial community, which is based on anammox bacteria belonging to the phylum *Planctomycetes*. According to some estimates, the anammox process is responsible for up to 80 % of the nitrogen turnover in marine ecosystems; it is also the basis of the most efficient biotechnology for nitrogen removal from wastewater. Investigation of these communities by traditional direct microbiological methods is rather difficult, since not only anammox bacteria, but also most other members of the community have not been isolated in pure cultures. The goal of the present work was to study the structure and functioning of the symbiotic anammox community (SAM) for targeted modification of the anammox process and the development of biotechnologies for nitrogen removal from wastewater.
Results and conclusions: Two laboratory anammox bioreactors for controlled cultivation of SAM were developed, assembled and launched. In the Anammox-1 sequencing batch bioreactor, the conditions were optimized for growing activated sludge capable of removal of up to 90 % of nitrogen at load of 180 mg N l^{-1} day^{-1}. The biomass of microorganisms from the Anammox-1 reactor was used to inoculate the Anammox-2 installation, which consisted of three reactors operating in parallel with complete retention of the activated sludge biomass on the non-woven plastic biomass carrier. SAM selection was carried out in the Anammox-2 installation by directed modification of optimal conditions. Successive stages of formation and development of anammox biofilms, from the initial stage of host colonization to the terminal stage of death and lysis, were studied, and the taxonomic composition of SAM was determined. High-throughput sequencing of the 16S rRNA gene fragments revealed predominance of representatives of four phyla in biofilms: *Planctomycetes, Chloroflexi, Bacteroidetes*, and *Proteobacteria*. Anammox bacteria were represented by species *Ca*. "Brocadia fulgida" and *Ca*. "Jettenia asiatica." Organotrophic members of the phyla *Chloroflexi* and *Bacteroidetes* decompose dead cellular biomass of activated sludge and the exopolymeric compounds formed by anammox bacteria. Nitrifiers of first stage in the community were mainly represented by ammonium-oxidizing bacteria of the genus *Nitrosomonas*; the share of nitrite-oxidizing bacteria was maintained at a low level (< 0.4 %). Analysis of assembled genomes of anammox bacteria revealed the absence of folate biosynthesis pathway, although this compound is important metabolite and it is necessary for carbon dioxide fixation in anammox species. A stimulating effect of folate

on the daily amount of removed nitrogen (dN) was revealed in a community enriched with anammox bacteria of the genus *Ca*. "Brocadia". Formate was also added to stimulate organotrophic growth and dissimilatory nitrate reduction of anammox bacteria. In the community dominated by anammox bacteria of the genus *Ca*. “Jettenia,” the stimulating effect of formate on dN was revealed. The results obtained support the conclusion that formate and folate can be used to improve the stability and efficiency of the operation of anammox bioreactors for wastewater treatment.
"Synthetic" SAMs were obtained by an enrichment of experimental consortium with stage I nitrifiers and different species of anammox bacteria. Bioaugmentation of the anammox community during reactor start-up increased the efficiency of ammonium removal and led to stable operating mode. However, during subsequent operation of the reactor, exogenous microorganisms were gradually replaced by more competitive autochthonous nitrifiers and anammox bacteria. The introduction of new anammox bacteria at steady state stage of the reactor operation was more effective due to better adaptation of new anammox bacteria in the autochthonous community, and therefore to an increase in the rate of ammonium removal, as well as an increase in the diversity and relative abundance of anammox bacteria in the reactor.
Acknowledgements: The study was supported by Russian Science Foundation (project No. 21-64-00019) and Ministry of Science and Higher Education of the Russian Federation.