SUMMARY

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IMMOBILIZATION OF ANAMMOX BACTERIA ON AGRO-NONWOVEN FABRICS

Key words: agro-nonwoven fabrics, anammox bacteria, fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR) with NGS next generation sequencing, immobilisation.

Changes in the technological systems of wastewater treatment were the result of the development of knowledge in the field of engineering sciences, biotechnology, and the growing requirements for the protection of natural resources, especially water resources, considering sanitary aspects and the effects of urbanization and the impact of industry on the natural environment. All known modern wastewater treatment technologies have been developed over decades. An example is activated sludge technology, which is very energy intensive. The need to reduce the electricity demand of equipment installed at wastewater treatment plants has contributed to the development of ammonium nitrogen removal technology in full nitrification/anammox. Wastewater treatment plants where sludge is stabilised by methane digestion using SHARON/ANAMMOX®, ANAMMOX®, DEMON® or ANITA^{TM}MOX technology on a side stream achieve energy self-sufficiency. However, the use of anammox bacteria presents numerous difficulties due to too low a wastewater temperature in winter, fluctuations in ammonium nitrogen concentration and high variability in the composition of municipal wastewater. Another barrier to the introduction of anammox bacteria into mainstream use is their slow growth and ease of leaching anammox bacteria biomass from biological reactors. Various types of carriers have been used to eliminate these problems especially plastic carries. An alternative to such solutions may be portable modules made of non-woven fabrics with a construction analogous to the spiral modules used in membrane techniques.

The main objective of the study was to evaluate the possibility of transporting anammox bacteria immobilized on modules from biological reactors to another wastewater treatment plant. Five modules were constructed, consisting of an agro-nonwoven fabric wound over a filter that allows a generated vacuum during bacterial immobilisation. After immobilisation, the agro-nonwoven modules were transferred to laboratory reactors. Between 12.12.2017 and 16.03.2022, a series of experiments were carried out to test the impact of freezing on the biochemical activity of anammox bacteria and the removal of ammonium nitrogen by denitritation using as mineral media and municipal wastewater. An experiment

was also carried out with a linen fabric inserted between the agro-nonwoven layers. During the research, ware analyzed the physicochemical parameters, granulometric composition of the formed sediments, identification of bacteria with the FISH method (***F****luorescence* ***I****n* ***S****itu* ***H****ybridization*) and staining of bacteria with the LIVE/DEAD® method which allows obtaining information on the bacteria condition. The structure of the bacterial community on agro-nonwoven layers was periodically analyzing using PCR (***P****olymerase* ***C****hain* ***R****eaction*) NGS (***N****ext* ***G****eneration* ***S****equencing*) method.

These studies have shown that permanent immobilisation of anammox bacteria cultures is possible on agro-nonwoven modules, allowing the biomass of these bacteria to be transferred between the immobilisation site and other wastewater treatment plants. The introduction of linen fabric as a spacer between the layers of agro-nonwoven fabric on the module has a positive effect on the development of anammox bacteria in the module. The culture of anammox bacteria, after freezing and thawing was no loss of biochemical activity.

Studies have shown that the pH impacts the growth of specific species of *Candidatus* Brocadiaceae. A pH lower than 8.0 promotes the growth of *Ca.* Brocadia, while a higher pH results in the development of *Ca.* Kuenenia.

PCR NGS tests of sediments deposited on the modules allow for a better understanding of the impact of environmental factors and operation time on the populations of bacteria located on the agro-nonwoven layers. Novel modules are a promising technique in denitration systems, and their main advantages include the ease of immobilization of anammox bacteria on agro-nonwoven fabrics and protection against leaching of biomass from the system. The design of the modules allows to retain the biomass of anammox bacteria, while not eliminating the activity of other groups of bacteria involved in the transformation of nitrogen compounds during wastewater treatment.