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DESIGN OF SYNTHETIC CONSORTIA FOR BIOAUGMENTATION OF BIOLOGICAL DENITRIFICATION REACTORS TREATING GROUNDWATER FOR HUMAN CONSUMPTION.

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Nitrate pollution in groundwater is a global concern attributed to human activities, such as agriculture, and poor wastewater management. A high nitrate level in drinking water is hazardous to human health. Biological denitrification (BD) is a water treatment technology involving the transformation of nitrate into gaseous nitrogen with low operating costs and high water recovery. BD is mainly carried out in fixed-bed reactors by a denitrifying biofilm, composed of a self-assembled consortium of indigenous microorganisms naturally selected by the process conditions. We determined in a previous work the optimal conditions for the establishment of denitrifying bacterial mixed communities at the laboratory and pilot scale (Dotto 2021). An acclimation period of approximately 100 hydraulic retention times (HRT) was needed to accomplish the nitrite limits given by the Argentinean Food Code. We hypothesize that bioaugmentation with a synthetic consortium of indigenous microorganisms will reduce the start-up time. In this line, the initial step was to obtain, characterize and identify native bacterial isolates from the reactors at laboratory scale. For this purpose, plate isolates, MALDI-TOF and DGGE assays were performed to group similar isolated strains. Three of them were identified as nitrite accumulators. Genome sequencing of non-redundant isolates and metagenome analysis of bioreactors were undertaken to identify genes of interest and describe the taxonomic diversity of the former. It was determined that the majority of the isolates belong to two of the ten most abundant genera found in the biofilm of the acclimatized bioreactors, *Pseudomonas* and *Acidovorax*. Moreover, two of the three nitrite-accumulating strains were found to belong to the genus *Acidovorax*. The genomes obtained for the isolates were annotated and the genes involved in DB were identified. Nine strains with complementary metabolic pathways to the three accumulators were selected and grown on solid medium to examine the reversion of nitrite accumulation. This method allowed us to identify five isolates belonging to the genus *Pseudomonas*. Growth curves were performed in liquid medium using eight strains with complementary denitrification pathways to evaluate growth rate and biomass yield in axenic and co-culture. Significant differences were detected for three of the fifteen combinations tested, corresponding to the co-cultures of *Acidovorax* sp. with different isolates of *Pseudomonas chlororaphis* (A2, F6, and

H2). These cultures showed higher growth rates than the axenic cultures and also produced more biomass. The above combinations showed promising features for bioaugmentation of denitrifying bioreactors and lab-scale testing. It remains to study the nitrite accumulation and biofilm formation capacity of the aforementioned pairs in order to select the most suitable ones.

Palabras clave: Biological denitrification - synthetic consortia - bioaugmentation