

TITLE: SCREENING FOR ENZYMES INVOLVED IN HETEROTROPHIC NITRIFICATION/AEROBIC DENITRIFICATION THROUGH COMPARATIVE GENOMICS

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ABSTRACT: Oil extraction generates large amounts of effluent contaminated with toxic compounds such as ammonium (NH₄⁺). Biological treatment of these effluents has been the most used by industries. The biological removal of NH₄⁺ can be done by autotrophic nitrification, in which the NH₄⁺ is oxidized to nitrite and then to nitrate under aerobiosis followed by denitrification performed by heterotrophic bacteria in anaerobiosis. Another mechanism is the heterotrophic nitrification/aerobic denitrification (HN/AD), a simultaneous process in a single microorganism through a metabolic pathway that has not yet been fully elucidated. Therefore, the main goal of this work was the genome search for possible enzymes involved in the process of NH₄⁺ removal through the HN/AD. For this purpose, 4 isolates were selected from an effluent treatment station and incubated in nitrifying specific medium. After 5 days, N₂ was detected by gas chromatography and NH₄⁺ removal by colorimetric test. DNA extraction of isolates was made, followed by amplification and sequencing of the 16S rDNA gene. Species was identified using BLAST program against the NCBI databank. For comparative genomics, the species genome and the predicted proteins from conventional nitrification and denitrification pathway were obtained from NCBI, aligned by BLASTp and Clustal Omega. To the first screening, 20% identity and 25% coverage were established as criteria for determination of possible candidates. The enzymes that met the criteria were analyzed in PFAM database to confirm domains associated to predicted function. It was detected the removal of 100% of NH₄⁺ and the production of N₂, proving that these isolates are capable of performing HN/AD. The isolates were identified as *Gordonia amicalis*, *Pseudomonas balearica*, *P. stutzeri*, and *Rhodococcus ruber*. The genus *Pseudomonas* and *Rhodococcus* are already described in the literature as capable of performing HN/AD, but no species belonging to the genera *Gordonia* were found. Through comparative genomics, only enzymes related to the assimilatory cycle of nitrogen were found, which was already expected since this stage is fundamental for the maintenance of bacterial metabolism. However, no enzyme or protein domain showed similarity with genes associated to autotrophic nitrification and conventional denitrification was found. All together, the results suggest that these microorganisms could use a completely different way to carry out this process, which still remains unknown.

Keywords: Nitrogen; Petroleum; Ammonium removal.

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