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Water resources are at high risk of pollution due to various anthropogenic activities. Nitrate is one of the major pollutant in various ecosystems, which causes various health issues in living organisms. To address this issue biofilm based Moving Bed Biofilm Reactor (MBBR) is widely used. However, its usage has been limited due to slow-start up and poor reactor performance, due to lack of functional bacteria. To increase the efficiency of MBBR in this study denitrifying consortium was developed. This denitrifying consortium termed DC5 comprised five specially selected isolates after the screening of 33 bacterial isolates based on their denitrification efficiency and biofilm forming ability. The five isolates were identified on the basis of their 16S rRNA gene sequencing, as *Diaphorobacter* sp. R4, *Pannonibacter* sp. V5, *Thauera* sp. V9, *Pseudomonas* sp. V11 and *Thauera* sp.V14. Flask level denitrification studies and gas chromatographic analysis suggested that all the isolates were denitrifiers and showed synergistic effect on each other as well as enhanced denitrification efficiency when inoculated together. Various factors affecting the biofilm forming ability and denitrification efficiency of consortium DC5 were optimized to enhance their performance in MBBR. They were 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.5 mM K_2HPO_4, 1 % inoculum and 0.6 % sodium acetate as a carbon source. Further, continuous nitrate removal studies with synthetic effluent were carried out in denitrifying MBBR (dMBBR) where the parameters affecting nitrate and COD removal were optimized viz. 0.3 C/N ratio, HRT 3 h, nitrate loading up to 2400 ppm, pall ring carriers (among carrier types) and filling ratio of 20 %. Comparative studies of dMBBR developed with consortium DC5, suspended growth dMBBR developed with consortium DC5 and control MBBR (i.e. without inoculum) revealed that dMBBR inoculated with consortium DC5 showed the highest nitrate removal efficiency (i.e., 100 %) in biofilm reactor. Biotreatability studies revealed that dMBBR developed with the consortium DC5 was also able to remove 75 % nitrate and 60 % COD from dye industry effluent, 85 % nitrate and 60 % COD from pharma industry 1 effluent, 100 % nitrate and 60 % COD from pharma industry 2 effluent, 76 % nitrate and 69 % COD from pharma industry 3 effluent and 80 % nitrate and 80 % COD from domestic wastewater spiked with 200 mg L^{-1} of nitrate. Next Generation Sequencing (NGS) approach was used to characterize the community

structure and functional potential of biofilm associated with the carriers in long term operated dMBBR. Whole metagenomic sequencing of biofilm developed in dMBBR after 300 days operation revealed Proteobacteria as the most abundant in it. *Thauera*, *Thauera humireducens* and *Thauera* sp. MZ1T were found to be the most abundant among all the genera and species in the carrier biofilm. Principle component analysis showed that all the denitrifying organisms are positively correlated with each other. The metabolic potential of the developed biofilm showed that the denitrification process may be linked to carbon metabolism, including the degradation of amino acids, fatty acids, and carbohydrates that also produce electron donors for denitrification. Sequences were assigned to metabolic pathways like nitrogen, sulfur, methane and xenobiotic biodegradation of aromatic compounds that increased the performance of dMBBR. As shown in NGS studies, *Thauera* was found to be the most dominant and major contributing organism in dMBBR. Therefore, further nitrate removal studies were carried out with one of the isolates namely *Thauera* sp.V14 to check its potential role in nitrate removal. *Thauera* sp.V14 showed strong auto-aggregation and hydrophobicity potential with high denitrification efficiency and biofilm forming ability. Various factors affecting its denitrification and biofilm formation like peptone, yeast extract, purified amyloid, DMSO, ethanol, NaCl, $MgCl_2$ and $CaCl_2$ were optimized using Plackett-Burman analysis. Results of this analysis suggested that ethanol, $CaCl_2$, peptone, DMSO and yeast extract significantly affected biofilm formation and denitrification efficiency of *Thauera* sp.V14. Further, results of Response Surface Methodology (RSM) suggested that the response yielded a linear model as there was no interaction seen among the components for biofilm formation and denitrification efficiency of *Thauera* sp.V14. DMSO and $CaCl_2$ were found to be the most significant components influencing biofilm and denitrification of *Thauera* sp.V14. Continuous nitrate removal studies in dMBBR with *Thauera* sp.V14 suggested that it was able to reduce nitrate up to initial concentration of 2400 ppm. SEM analysis of biofilms developed inside dMBBR showed the presence of bacterial cells on the carrier material while the FTIR analysis showed that the EPS of biofilm comprised of protein, carbohydrates, fatty acids and humic acid as major components. Overall, the study suggested that the MBBR developed with special seed of biofilm forming denitrifying bacteria (consortium DC5) enhanced the performance of dMBBR. Therefore,

it can be concluded that bioaugmentation of functional bacteria as seed enhances reactor performance. Moreover, further studies on *Thauera* asserted that the most persistent bacterium in long term operated, acetate-fed dMBBR was possibly the main contributor to nitrate removal. It is suggested here that bioaugmented dMBBR is one of the best suitable and effective approach for removal of nitrate from wastewater.