CONCLUSIONS
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- Efficient nitrate reducers were present in all parts of DNR I & II. Nitrite was not detected at any points in the reactor and concentration of ammonia remained almost constant suggesting that respiratory denitrification was the dominant pathway in the reactor for NO$_3^-$ reduction. On an average $1.41 \times 10^5$ denitrifiers were sufficient for efficient nitrate removal in DNR of GNFC. Total 69 isolates were obtained by different isolation procedures from various habitats. Of these isolates, maximum nitrate reducers were obtained from the DNR of GNFC, and 29 isolates were confirmed as efficient nitrate reducers. However out of 29 isolates, 6 isolates being gram negative cocci were not select for bioreactor studies as most gram negative cocci are pathogenic. Only nine nitrate reducers produced gas in nitrate containing effluent within 72h retention time and hence were selected for further studies. Final screening done with gas chromatography analysis confirmed that all the nine nitrate reducers were true denitrifiers and produced more than 85% N$_2$ gas with in 48h retention time.

- Since Isolate 1-5 could reduce sulfur together with nitrate from GNFC effluent containing high amount of sulfate (800-1000mg l$^{-1}$), a consortium of two isolates (I-4 and I-5) were used to achieve maximum denitrification efficiency with successive sulfate reduction under denitrifying conditions. Gas chromatograph analysis confirmed that isolate I-4 and I-5 produced maximum, (98%) N$_2$ gas production within 48h retention time. The isolate I-4 was identified as *Pseudomonas stutzeri* and I-5 as *Comamonas testosteroni*. The consortium of these two gram negative rods was selected for further reactor studies. Maximum denitrification efficiency was observed between 30°C to 37°C. However, at low temperature denitrification process was affected adversely which was indicated by nitrite accumulation. Above 37°C the growth and denitrification rate tends to get affected. There was no major effect of pH on nitrate removal efficiency of *Pseudomonas stutzeri* and *Comamonas testosteroni*. Nitrite accumulation was significantly higher in the glucose and sucrose with less nitrate removal efficiency suggesting that *Pseudomonas stutzeri* and *Comamonas testosteroni* were able to rapidly metabolize carbohydrates. Large amount of gas
produced from sucrose under anaerobic condition proved fermentative metabolism of carbohydrates. The efficiency of denitrification with acetate, propionate, methanol, and butyrate was comparatively higher with 72h HRT under aerobic conditions. Under aerobic and anaerobic conditions methanol gave second highest nitrate removal efficiency justifying the use of fusel oil for further bioreactor studies. Denitrification efficiency was reduced at high dissolved oxygen concentration. Efficient nitrate removal with low oxygen concentration proved that the denitrifying enzymes of *Pseudomonas stutzeri* and *Comamonas testosteroni* were responsible for efficient nitrate removal and remained stable under aerobic conditions. Despite the induction of several dissimilatory enzymes in culture of *Pseudomonas stutzeri* and *Comamonas testosteroni* during inoculum preparation, it seems evident that some amount of yeast extract was required for efficient removal of nitrate in less retention time.

- Continuous bioreactors studies at 4 and 50 l showed that 4% inoculum was not sufficient for complete removal of nitrate within short period of time. Therefore 5% inoculum was used for all different bioreactor studies. At higher COD:NO₃⁻-N ratio (3.45), maximum denitrification efficiency was achieved but effluent COD concentration was not in the permissible range in both bioreactor studies. High effluent COD concentration was controlled by lowering the COD:NO₃⁻-N ratio or by decreasing the HRT. At lower COD loading (2.85; COD:NO₃⁻-N ratio), nitrate removal efficiency was not affected at 60h HRT. However, decreasing the HRT to 48h the denitrification efficiency was reduced at this particular COD:NO₃⁻-N ratio. From the performance of 50 l bioreactor at different parameters, it could be concluded that at a COD:NO₃⁻-N ratio of 3.45, maximum denitrification efficiency achieved with reasonably lower COD (100-150 ppm) in the effluent within shortest possible HRT (48h). The value of COD:NO₃⁻-N obtained in the present study is suggestive of the fact that the carbon concentration added to the reactor was just enough to support maximum denitrification at the NO₃⁻-N concentration used.

- Pilot scale reactor (1.5m³) studies showed efficient nitrate removal with higher HRTs (60h) at COD:NO₃⁻-N ratio of 2.85. Accumulation of NO₂⁻ and NH₄⁺ was not
observed implying absence of DNRA activity. To control the effluent COD concentration, HRT was reduced to 36h which decreased the denitrification efficiency to 88%. Optimization of process design parameters for nitrate removal and growth kinetics at 2.85 and 2.45 COD:NO$_3^-$ -N ratios with different nitrate loading rates were matched with continuous nitrate removal studies at 1.5 m$^3$ bioreactor level. All the optimization studies done with different COD:NO$_3^-$ -N ratios and retention times, suggested that 2.45; COD:NO$_3^-$ -N ratio and 48h HRT were ideal conditions for complete nitrate removal. The COD:NO$_3^-$ -N ratio of 3.45 adjusted in 50 l bioreactor was reduced to 2.45 for complete removal of nitrate within 48h HRT, assuming that some amount of carbon is contributed by dead organic particles. The specific denitrifying activity for *Pseudomonas stutzeri* and *Comamonas testosteroni* reported in the present investigation for suspended growth technique is 1.5 fold higher than has been reported so far in the literature, thus signifying good potential of these organisms to carry out denitrification. Complete removal of nitrite from nitrite containing effluent of Deepak Nitrite Limited within short retention time proved versatility and consistency of denitrifying activity in consortium.

- Na-alginate being relatively cheaper than the other matrix, it was used for immobilizing cells of *Pseudomonas stutzeri* and *Comamonas testosteroni*. 3% (w/v) Na-alginate gave relative stable and porous beads therefore used for developing continuous 1.5 l bioreactor with immobilized cells. Comparatively higher retention time was required for complete removal of nitrate by immobilized cells than the free cells of *Pseudomonas stutzeri* and *Comamonas testosteroni*. By increasing the amount of inoculum, complete nitrate reduction was achieved within short incubation time. Consistent denitrification efficiency till 8$^{th}$ cycles proved stability and efficiency of immobilized cells. From the observation of continuous bioreactor studies it can be concluded that the immobilized system required lower COD:NO$_3^-$ -N ratio (1.45) for complete removal of nitrate within very short retention time (14h).
- Comparative studies done with two denitrifying reactors (DNR and pilot 1.5m$^3$) concluded that the organic loading (fusel oil) was relatively higher in DNR than
required for complete nitrate removal. This leads to higher effluent COD in DNR than the pilot reactor at same NO$_3^-$ -N loading rates. Other parameters besides effluent COD and NO$_3^-$ -N which is also important for effluent treatment plant is sludge generation (or volatile suspended solids). Higher carbon addition always produce large amount of sludge and finally disposal of sludge increases the final cost of effluent treatment plant specifically for the type of DNR without sludge recycle systems. Finally effluent COD and formation of sludge in DNR can be controlled by proper maintenance of fusel oil addition and nitrate loading rates (HRT).