Chapter - 4

BIOTREATMENT OF NITRATE-RICH EFFLUENT BY SUSPENDED GROWTH TECHNIQUE IN 4 & 50 L BIOREACTOR
4. Biotreatment of nitrate-rich effluent by suspended growth technique in 4 & 50 l bioreactor.

The suspended growth technique is the most widely applied biological wastewater treatment process in the world. Success of a suspended growth technique is ultimately dependent on the functions of the constituent micro-organisms as well as the related process parameters (e.g. anaerobiosis, anoxia aerobiosis) affecting microbial growth and activity (Bux et al., 1994). It is therefore believed that inadequate control of the micro-organisms in the activated sludge/suspended growth process is responsible for many variations in process performance. A better understanding of the ecological, physiological and biochemical activities of these micro-organisms in relation to the change in physico-chemical parameters in the activated sludge/effluent is the need of the day to gain optimal control on any biological treatment process. Under anoxic conditions certain heterotrophic bacteria are stimulated into utilizing nitrates and nitrites as final electron acceptors for cellular respiration in place of oxygen. In wastewater industry uncertainty exists regarding the bacteria involved in denitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. A more intensive understanding of denitrifying heterotrophic bacteria is therefore essential as this may be one of the next steps towards optimizing efficiency of nitrogen removal.

In the biological removal of pollutants, an important factor is that of optimization of certain parameters like inoculum size, C:N ratio, dissolved oxygen and residence time to achieve the removal of the target compound within shortest possible time. Our flask level studies using GNFC effluent showed that, consortium of Pseudomonas stutzeri and Comamonas testosterone (1:1), removed all the NO$_3^-$ within shortest possible time (60h) and produced more than 90% N$_2$ gas which was confirmed by Gas Chromatographic analysis. These strains removed the NO$_3^-$ under the following range of conditions: pH between 6.5- 7.5, temperature between 25°C - 30°C and with sufficient agitation.
4.1. Nitrate removal by suspended growth technique in continuous 4-L bioreactor.

A circular bioreactor of 4-L capacity was used to optimize the operational conditions for pilot scale bioreactor studies (Fig. 4.1). Chemically treated effluent, which was used as the medium throughout the process was low in organic carbon and needs to be supplemented with external carbon source. The influent supplemented with fusel oil was inoculated with a consortium of *Pseudomonas stutzeri* and *Comamonas testosteroni* (5% (v/v) inoculum, 1# O.D. at 600 nm). Initially the reactor was run in a batch mode for a week to develop a sufficient microbial biomass, and thereafter it was maintained in a continuous mode. The effects of different COD:NO$_3$-N ratio and nitrate loading rates, on denitrification efficiency were checked. The efficiency of denitrification is reported to be dependent on the availability of a sufficient quantity of suitable organic carbon source (Anderson & Ibrahim 1978). The nature of organic carbon source is also known to control competition between denitrification and dissimilatory nitrate reduction to ammonium (Tiedje, 1994). Fusel oil added as a source of organic carbon in the present study has methanol as a main component. The different concentrations of carbon were adjusted by varying COD:NO$_3$-N ratio in the medium, fusel oil contributing to the major COD in the influent and residence time in the reactor was changed by adjusting the flow rate. The COD:NO$_3$-N ratio was adjusted to the levels of NO$_3$-N present in DNR influent used for the particular run. The significance of this is to ensure that the necessary carbon is made available for efficient denitrification, at the same time limiting the final effluent COD to an acceptable level.

In the whole study the objective was to achieve efficient denitrification at minimum hydraulic retention time (HRT) with COD remaining in the effluent at the permissible limit (100-150mg l$^{-1}$). To achieve this, parameters like COD: NO$_3$-N ratio and HRT were varied in a 4 l bioreactor being run in a continuous mode.

The stoichiometric ratio of heterotrophic denitrification reported is 2.85mg COD to reduce 1mg NO$_3$-N to N$_2$ (Anderson & Ibrahim 1978). Generally this value was corrected to 3.45, as some of the carbon is used for cell mass biosynthesis. Using this available information as basis, the work was started with COD: NO$_3$-N ratio of 3.45.
4.1. Performance of the bioreactor at different HRTs (96h & 66h) with same COD: NO₃⁻-N ratio (3.45)

Denitrification efficiency was checked at 96h HRT (1.0 l d⁻¹ flow rate), keeping COD: NO₃⁻-N ratio at 3.45. Samples were withdrawn from different parts of reactor and checked for NO₃⁻, NO₂⁻, NH₄⁺ and COD accumulation. Nitrite and ammonium concentrations remained between 20-45mg l⁻¹ and (30-55mg l⁻¹) respectively. Almost 90-95% denitrification efficiency was observed, but the effluent COD concentration remained between 225-400mg l⁻¹ (Fig. 4.2) which was higher than the permissible limit. Therefore extra COD could be used for more nitrate reduction by reducing the HRT.
Fig. 4.2. Denitrification efficiency at 96h HRT with 3.45 COD:NO₃⁻N ratio.

The effect of nitrogen loading rates on nitrate removal had been determined by reducing the HRT to 64h. COD and NO₃⁻N loading applied in range from 1.24 to 1.71g COD l⁻¹ d⁻¹ and 0.4 to 0.48g NO₃⁻N l⁻¹ d⁻¹ respectively. Effluent NOₓ⁻N concentration was observed between 25-40mg l⁻¹ and denitrification efficiency remained in range of 92-95% (Fig. 4.3). The effluent COD concentration still remained higher than the acceptable value. Therefore HRT and COD:NO₃⁻N ratio used were higher than the required to bring down the effluent COD within permissible range.
4.1.2. Performance of the bioreactor at lower COD:NO$_3^-$-N ratio (2.85).

A separate run with 2.85 COD:NO$_3^-$-N was performed to check its effect on effluent COD concentration. The process of nitrate removal, at 96h HRT (flow rate 1.0 l d$^{-1}$) occurred without accumulation of NO$_2^-$ and NH$_4^+$ in the reactor. Nitrate removal efficiency remained in the range of 90-94%. The effluent COD concentration was quite near to acceptable value as shown in Fig. 4.4.
When the HRT was reduced to 64h and checked its effect on nitrate removal efficiency with same COD:NO$_3^-$-N 2.85 ratio, the denitrification efficiency reduced to 85-88% (Fig. 4.5). Here carbon might be the limiting factor to complete the process because effluent NO$_x$-N concentration was found in the range of 45-80mg l$^{-1}$. At this particular NO$_3$ loading rate (0.52mg l$^{-1}$ d$^{-1}$) with 64h retention time the actual carbon was not sufficient to complete the denitrification with 64h retention time the actual carbon was not sufficient to complete the denitrification.

However with 96h HRT, COD removal was affected at both COD: NO$_3$-N ratio, which was achieved within acceptable limits (50-100mg l$^{-1}$) at 64h-HRT with COD: NO$_3$-N as 2.85. The value of COD:NO$_3$-N obtained in the present study is close to the value reported by Bernet et al., (1996), suggesting that the carbon concentration added to the reactor was just enough to support maximum denitrification at the NO$_3$-N concentration used. Addition of carbon at a concentration higher than required should be avoided as it favors dissimilatory
nitrate reduction to ammonium (Tiedje 1994), and leads to higher biomass and hence more sludge formation. Many groups have used industrial wastes such as volatile fatty acids from some effluent, solid waste containing organic matter and wine distillery effluents, as an electron donor for denitrification (Bernet et al., 1996).

\[\text{Fig. 4.5. Effect of lower COD:NO}_3^- -N at 64h HRT.}\]

### 4.2. Scale-up of the system: Removal of nitrate in a continuous reactor at 50 l.

In order to validate the reduction in nitrate content obtained in the 4-L bioreactor, the parameters were scaled up to 50-L continuous system and monitored for nitrate removal efficiency (Fig. 4.6 & 4.7a, b, c). The required microbial biomass was developed in the reactor by seeding with isolates *Pseudomonas stutzeri* and *Comamonas testosteroni*, by feeding with methanol and yeast extract (0.01%w/v), for providing essential growth factors. Complete removal of nitrate was achieved within 5 days and the biomass reached to 0.30g l\(^{-1}\).
The continuous process was then started with HRT varying from 100h to 48h. For each test run, the feed COD:NO₃-N ratio varied and steady state conditions assumed to be reached when the effluent COD and NO₃⁻ level were in the range of 100mg l⁻¹ and 10mg l⁻¹ respectively. Retention time for each run was varied by adjustment of flow as well as concentration of nitrate in the influent.

Fig. 4.6. Schematic diagram of the continuous bioreactor system.
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Fig. 4.7a. Different stages (viz. a,b) of continuous denitrifying reactor.

Fig. 4.7b.
4.2.1 Nitrate removal at flow rate 100h HRT (12 l d$^{-1}$) with COD:NO$_3^-$-N; 3.45

The reactor was run on a continuous mode with 100h HRT for 20 days. Almost 97% nitrate removal was achieved with 100h HRT with COD:NO$_3$-N ratio at 3.45 (Fig. 4.8), but effluent COD remained between 250 to 300 mg l$^{-1}$. No accumulation of nitrite was found in the reactor, and ammonium remains constant throughout the process indicating absence of the DNRA pathway (Dissimilatory Nitrate Reduction to Ammonia), in the reactor.

![Flow rate 12 l d$^{-1}$, COD:NO$_3^-$-N 3.45](image)

Fig. 4.8. Relationship between influent NO$_3^-$-N and effluent NO$_3^-$-N, COD concentrations.

The Specific Denitrification Activity (SDA) achieved was between 0.36-0.42g NO$_3$-N gVSS$^{-1}$ d$^{-1}$ at 0.16-0.19gNO$_3$-N l$^{-1}$ d$^{-1}$ loading rates (Fig. 4.9). Since effluent COD level remained high, HRT was decreased to 66h and the nitrate removal efficiency was examined with same COD:NO$_3$-N(3.45) ratio.
Fig. 4.9. Effect of NO$_3^-$ -N loading rates on specific denitrification activity.

4.2.2. Denitrification efficiency at 66h HRT (flow rate 18 l d$^{-1}$) and 50h HRT (24 l d$^{-1}$) with COD:NO$_3^-$N:3.45.

Satisfactory operating conditions were those that allowed 99% reduction in the initial nitrogen and carbon source with no accumulation of nitrite or ammonium. The process was adjusted and run at 66h HRT for 20 days. The process of nitrate removal at 66h HRT occurred without accumulation of NO$_2^-$ and NH$_4^+$ in the reactor. This represents a significant advantage over denitrifying microorganisms able to reduce NO$_3^-$ at similar high concentration (1200mg l$^{-1}$). Performance of denitrifying reactor at 60h HRT is shown in Fig. 4.10.

COD and nitrate nitrogen loading applied in range from 1.02 to 1.11g COD l$^{-1}$ d$^{-1}$ and 0.34 to 0.42g NO$_3^-$N l$^{-1}$ d$^{-1}$ respectively. Denitrification efficiency remained in range of 88 – 95%. The effluent COD remaining still higher than the acceptable value (180 – 240mg l$^{-1}$), therefore, it can be concluded that the COD:NO$_3^-$N ratio is still higher than the actual ratio required to bring down the effluent COD level in the range of 100mg l$^{-1}$. The specific
denitrification activity achieved was between 0.9-1.1 g NO₃-N gVSS⁻¹ d⁻¹ at 0.36-0.42 g NO₃-N l⁻¹ d⁻¹ loading rates (Fig. 4.11).

Flow rate 18 ld⁻¹, COD: NO₃-N: 3.45

Fig. 4.10. Influent and effluent NO₃⁻-N and COD concentration at 66h retention time.
Flow rate 18 ld⁻¹, COD: NO₃-N: 3.45

Fig. 4.11. Effect of NO₃⁻-N loading rates on specific denitrification activity at 66h HRT.
To bring the COD value in the range of 100 mg l\(^{-1}\), HRT was further decreased to 48h for 20 days keeping same COD:NO\(_3\)-N (3.45). It could be observed that at 0.56g NO\(_3\)-N l\(^{-1}\) d\(^{-1}\) loading, more than 90% nitrate removal efficiency was achieved (Fig. 4.12). The biomass of the effluent obtained from the overflow was around 250 – 450mg VSS l\(^{-1}\). Nitrate and nitrite concentrations decreased to 10–30 mg l\(^{-1}\) and 50mg l\(^{-1}\) respectively. At this HRT 48 h (flow rate 25l d\(^{-1}\)), organic load was in the range of 1.40 to 1.59g COD l\(^{-1}\) d\(^{-1}\) and the COD level in the effluent was almost near to acceptable value as shown in Fig. 16. The specific denitrification activity achieved was between 0.8-1.1g NO\(_3\)-N gVSS\(^{-1}\) d\(^{-1}\) at 0.45-0.52gNO\(_3\)-N l\(^{-1}\) d\(^{-1}\) loading rates (Fig. 4.13).

**Fig. 4.12.** Influent and effluent NO\(_3\)-N, NO\(_2\)-N and COD concentration at 50h retention time.
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Flow rate 25 l d$^{-1}$, COD NO$_3$-N 3.45

![Graph showing the effect of NO$_3$-N loading rates on specific denitrification activity at 48h HRT.]

**Fig. 4.13.** Effect of NO$_3$-N loading rates on specific denitrification activity at 48h HRT.

Flow rate 27 l d$^{-1}$ and COD NO$_3$-N 3.45

![Graph showing influent and effluent NO$_3$-N, NO$_2$-N and COD concentration at 44h HRT.]

**Fig. 4.14.** Influent and effluent NO$_3$-N, NO$_2$-N and COD concentration at 44h HRT.

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4.2.3 Denitrification efficiency at retention time of 44h with COD:NO$_3$-N; 3.45 (flow rate 271 d$^{-1}$).

Denitrifying reactor was subsequently fed with 271 d$^{-1}$ nitrate containing effluent by decreasing the HRT to 44h for 20 days at the same COD:NO$_3$-N ratio. It was observed that, at COD loading of 1.73 to 1.80g COD l$^{-1}$ d$^{-1}$, nitrate removal efficiency decreases to 70-80 % (Fig. 4.15) with NO$_x$-N accumulated in the reactor (>300mg l$^{-1}$) (Fig 4.14). Nitrate was not completely reduced to nitrous oxide or molecular N$_2$. Here, carbon may be the limiting factor to complete the process. The effluent COD was quite low, remained between 20–30mg l$^{-1}$ (Fig 4.14), which was not sufficient to complete the denitrification. Ammonium concentration remained constant therefore; dissimilatory nitrate reduction did not take place under carbon limiting condition. The specific denitrifying activity achieved was between 0.7-1.03g NO$_3$-N gVSS$^{-1}$ d$^{-1}$ at 0.45-0.58gNO$_3$-N l$^{-1}$ d$^{-1}$ loading rates (Fig. 4.15).

Flow rate 271 d$^{-1}$ and COD:NO$_3$-N:3.45

![Graph showing denitrification efficiency and NO$_3$-N loading rate over days]

**Fig. 4.15.** Effect of NO$_3^-$-N loading rates on specific denitrification activity at 44h HRT.
At COD:NO$_3$-N ratio of 3.45 the effluent COD (200-250mg l$^{-1}$) was remained higher than the permissible limit at 66h HRT, therefore the denitrification efficiency was checked with same HRT (66h) by decreasing the COD:NO$_3$-N ratio to 2.85.

4.3. Effect of lower organic load (COD:NO$_3$-N;2.85) on denitrification with various flow rates (18 l d$^{-1}$ and 25 l d$^{-1}$).

The developed microflora was acclimatized to methanol (fusel oil) and stoichiometric quantity of nitrate and the system was operated on the continuous basis at (COD:NO$_3$-N;2.85). Fusel oil (COD) loading was gradually decreased with nitrate by keeping COD:NO$_3$-N to 2.85.

COD and nitrate nitrogen loading applied to the reactor at 66h HRT was in the range of 0.81 to 0.91g COD l$^{-1}$ d$^{-1}$ and 0.32 to 0.37g NO$_3$-N l$^{-1}$ d$^{-1}$ respectively. It could be observed from Fig. 4.16, that nitrate removal efficiency was 92%. At lower COD loading, denitrification efficiency was not affected at 66h HRT; biomass concentration remained in the range of 350 – 550 mg l$^{-1}$ and effluent COD was also in the permissible range. This nitrate to carbon ratio also is favorable conditions for the denitrifying flora. The SDA was achieved between 0.8-1.12g NO$_3$-N gVSS$^{-1}$ d$^{-1}$ at 0.4gNO$_3$-N l$^{-1}$ d$^{-1}$ loading rates (Fig. 4.17).
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Flow rate 181 d⁻¹, COD: NO₃⁻N: 2.85

**Fig. 4.16.** Performance of denitrifying reactor at various influent NO₃⁻-N concentration.

Flow rate 181 d⁻¹, COD: NO₃⁻N: 2.85

**Fig. 4.17.** Relationship between denitrification efficiency and SDA at 66h HRT.

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Though the effluent COD was in the permissible range but it was further confirmed by reducing the HRT to 48h. Denitrification efficiency was reduced to 77-84% at COD:NO₃⁻ - N ratio of 2.85 with 48h HRT. It must be noted from the effluent COD that the incoming methanol was the exclusive electron donor for denitrification. The COD level remained in the range of 30-40mg l⁻¹ (Fig. 4.18). At this particular retention time NO₂-N was accumulated between 70-120mg l⁻¹ concentrations. Ammonium remained constant throughout the process. Specific denitrification rate calculated between 54.5 to 72.08 mg NO₃-N g VSS⁻¹ h⁻¹ (or 1.31 to 1.73 g NO₃-N gVSS⁻¹ d⁻¹) (Fig. 4.19), and sludge concentration was 550mg VSS l⁻¹.

Flow rate 241 d⁻¹, COD NO₃⁻-N, 2.85

Fig. 4.18. Performance of denitrifying reactor at various influent NO₃⁻-N concentration.
Fig. 4.19. Relationship between denitrification efficiency and SDA at 48h HRT.

The overall performance of denitrifying reactor for 6 months is depicted in Fig. 4.20. More than 90% nitrate removal efficiency was achieved with 100h, 66h and 48h HRT (flow rate, 12 to 25 l d⁻¹) with COD:NO₃-N:3.45, but a sharp decrease in the efficiency at 46h HRT (flow rate 26 l d⁻¹). Moreover, the denitrification efficiency was not affected with lower COD:NO₃-N ratio to 2.85 at 66h HRT (flow rate 18 l d⁻¹), but it was marginally affected by decreasing the HRT to 48h (flow rate, 25 l d⁻¹). The average specific denitrifying activity for the consortium was calculated for different COD:N0₃-N ratios with different retention times. At COD:NO₃-N ratio of 2.85, the average VSS concentration in the denitrifying reactor (50-L) being 0.346 g l⁻¹, the specific denitrifying activity was 51.33 mg NOₓ-N gVSS⁻¹ h⁻¹ (or 1.23 g NOₓ-N gVSS⁻¹ d⁻¹), while at COD:NO₃-N of 3.45, the average VSS concentration in the denitrifying reactor being 0.364 g l⁻¹, the specific denitrifying activity was 43.75 mg NOₓ-N gVSS⁻¹ h⁻¹ (or 1.05 g NOₓ-N gVSS⁻¹ d⁻¹). The specific denitrification activity reported by Bernet et al., (1986) was 35.2 mgNOₓ-N gVSS⁻¹ h⁻¹ (or 0.846 g NOₓ-N gVSS⁻¹ d⁻¹), while that reported by Bode et al., (1987), was in the range of 0.324 g NOₓ-N gVSS⁻¹ d⁻¹). The specific denitrifying activity for Pseudomonas stutzeri and Comamonas
*testosteroni* reported in the present investigation is 1.5 fold higher than has been reported so far in the literature, signifying good potential of these organisms to carry out denitrification.

**Fig. 4.20.** Specific denitrification activity and denitrification efficiency profile at different COD:NO$_3^-$-N ratios and NO$_3^-$-N loading rates.