Effect of COD/N ratio on simultaneous removal of nutrients and COD from synthetic high strength waste water under anoxic conditions

Jyotsnarani Jena, Sanak Ray, Sony Pandey and Trupti Das*
CSIR-Institute of Minerals & Materials Technology, Bhubaneswar 751012, Orissa, India

Received 12 July 2012; revised 30 November 2012; accepted 03 January 2013

Simultaneous removal of nitrate, PO$_4$ and COD from synthetic wastewater containing high substrate and low COD/N ratio, under anoxic conditions has been attempted in the current study. Maximum removal of nitrate (98%), PO$_4$ (96%) and COD (93%) was achieved at a COD/N ratio of 1.62. PO$_4$ removal rate ranged between 77.6, 88.7, 90.5, 95.9, 93.1 and 93.2% at a COD/N ratio of 0.36, 0.97, 1.36, 1.62, 4.06 and 6.37 respectively. Thus PO$_4$ removal rate was more or less impervious to varying concentration of nitrate and carbon load in the system. pH measurements during the course of reaction, gave new insights into the denitrification and PO$_4$ uptake processes. Statistical analysis (ANOVA) further determined a significant variance between removal of PO$_4$, nitrate and COD with respect to time of reaction as well as in different experimental set up.

**Key words:** Biological nutrient removal, COD/N ratio, DNPAOs

**Introduction**

Nitrogen and phosphorus are important nutrients for plant growth, which when discharged in excess to the water bodies, brings about eutrophication. Attempts have been made to make apt use of the available COD for successful operation of Biological Nutrient Removal (BNR). This is considered to be a very complex phenomenon involving the metabolic pathway of anaerobic microorganisms participating in denitrification processes. Few reports throw light on factors such as, organic substrates, pH, prevailing aerobic/anaerobic conditions, C:N:P ratio, exercising significant effect on the microbial consortia apparently responsible for successful BNR operation. It has been reported that high concentration of the nitrate blocks PO$_4$ removal. However, some heterotrophs like DNPAOs (Denitrifying Phosphate Accumulating Organisms), having metabolic characteristics similar to those of Polyphosphate Accumulating Organisms, can utilise nitrate as their electron acceptor instead of oxygen and uptake PO$_4$ in anoxic condition. It brings about the removal of both the nutrients utilizing the same readily available COD. The DNPAOs generate less energy (by 40%) leading to lower cell yield (by 20-30%) and little sludge generation is an additional advantage of the process. Popularization of BNR process for the treatment of municipal sewage and industrial waste water, especially in developing countries having little resources for operating sophisticated wastewater treatment plants, would apparently provide several advantages like reduced energy consumption, sustainability, less sophistication and diminutive sludge production. Several factors need to be addressed through meticulous investigation on the basis of which, BNR can thrive as one of the most sustainable industrial/domestic waste water treatment processes.

Most of the studies carried out to achieve BNR under anaerobic/anoxic conditions, have been done with synthetic solutions resembling the municipal waste water. Areas dominated by chemical and fertilizer industries, which are growing exponentially in developing countries, are sources of nutrient rich effluents. However, there have been very few reports where BNR process is performed with high strength waste water though this issue is critical and needs to be addressed by researchers.

Keeping the above aspects in view, the focus of the current study has been to investigate the process parameters that play a key role in achieving nitrate, PO$_4$ and COD removal from high strength waste water, especially under anoxic conditions. An attempt has been made for the first time to create optimal conditions for
Materials and Methods

Experimental set-up

A 2-L closed glass reactor was used for all the experiments in batches. The experiments were carried out continuously for 21 h at room temperature (25°C-30°C) and slightly alkaline pH (7.5 and 8.5). The reactor was connected to mechanical stirrers (Spinip Magnetic stirrer) to improve contact between the microorganisms and the medium. Anoxic condition was maintained throughout by purging nitrogen gas (grade XL ~99% purity).

Synthetic waste water [1L of basic medium (1.7+0.1g/L Ca(NO₃)₂.4H₂O: as nitrate source, 0.043 g/L of KH₂PO₄: as PO₄ source, 6.86 g/L MgSO₄, 0.38 g/L peptone) and 0.3mL of nutrient solution (0.15g/L FeCl₃.6H₂O, 0.15 g/L H₃BO₃, 0.03g/L CuSO₄.5H₂O, 0.18g/L KI, 0.12 g/L MnCl₂.4H₂O, 0.06 g/L Na₂MoO₄.2H₂O, 0.12 g/L ZnSO₄.7H₂O, 0.15g/L CoCl₂.6H₂O, 10g/L EDTA)] were used as feed. Initially 0.8, 1, 1.5, 5.2, 7.8g/L of sodium acetate was added to the respective solutions (in order to maintain a desired initial COD/N ratio at 0.97, 1.36, 1.62, 4.06, and 6.37 in the synthetic waste water). Sodium acetate was absent in synthetic waste water, where the initial COD/N ratio was determined as 0.36. The initial COD in the synthetic waste water is also partially influenced by the presence of nitrate and other organic/inorganic matter.

The batch reactor was inoculated with properly washed sludge (100 mL), well acclimatized to PO₄ removal in anaerobic condition, from another 3L Sequencing Batch Reactor (SBR) (working volume of 2L) operational in the laboratory in a continuous scale for 240 days. This SBR, considered to be the parent reactor, was operated at 2 cycles/day, with Hydraulic Retention Time (HRT) of 12 h. Each cycle consisted of 6h anerobic-5h aerobic-1h settle/decant/refill. The SBR was fed with synthetic waste water having a COD/N ratio of ~10 (before initiation of anaerobic period).

Analysis

Performance of the bioreactor was monitored by hourly measurement of PO₄, nitrate (spectrophotometrically with Varian 50 Bio, UV-Visible spectrophotometer at 420nm and 880nm respectively), COD, TSS, VSS and pH as described in the standard methods. Statistical analysis

Statistical analysis was carried out here by two way ANNOVA, which was used to find significance level of various parameter using MS- office Excel -2007.

Results and discussion

Nitrate removal efficiency

Nitrate removal rate was negligible in absence of acetate (Fig. 1), due to apparent inhibition of heterotrophic denitrification. Maximum removal (98%) of nitrate was observed in COD/N ratio of 1.62 followed by 96% of removal with 2.5 times rise in the COD/N ratio (4.06). With further increase in COD/N ratio (6.37) in the medium, there was a marked decline in the rate of nitrate removal (81%), signifying the ineffectiveness of organic carbon availability to enhance the overall reaction kinetics beyond a certain threshold limit. It has been reported that COD/N ratio of waste water regulates the most critical parameters of nitrogen removal process as it has a direct correlation with the functional microorganism population including heterotrophic denitrifiers. Under anoxic conditions, the denitrifying bacterial population utilizes organic carbon as electron donor and nitrate as electron acceptor. Therefore the COD/N ratio is a critical parameter in determining the rate of nitrate utilization by the denitrifying population. However with an increase in the organic carbon load, keeping the initial nitrate concentration more or less similar, the COD/N ratio increases in the system. This might lead to dominance of heterotrophs other than DNPAOs which in turn might have a negative impact on the overall denitrification process. A similar impact on the metabolism of denitrifying bacteria can also be contemplated from the current observation and analogous results have been reported elsewhere.
PO$_4$ removal efficiency

With suspension of sludge (collected in fasting condition from the parent SBR during aerobic cycle) in fresh media, microorganisms switch to fasting condition and assimilate PO$_4$ rapidly. In spite of varying organic load and high nitrate concentration, PO$_4$ removal was achieved in all the sets (Fig. 1) and maximum (60-70%) uptake occurred within the first 3-4 hours of reaction. Anoxic PO$_4$ removal in all the experiments appears to be closely linked to the metabolic activity of DNPAOs, a denitrifying bacterial community, that are capable of PO$_4$ uptake with nitrate load in the medium$^{18,19}$. The DNPAOs present in the anoxic reactor use nitrate as electron acceptor for the uptake of external carbon source and for further PO$_4$ uptake$^{20}$. But in conventional aerobic-anaerobic reactor setup, presence of nitrate or nitrite acts as a detrimental factor for denitrification as oxygen is the electron acceptor in the first phase to uptake PO$_4$ $^{6,21}$.

In the present set up there is hardly any PO$_4$ release into the medium, which may be due to the presence of very high initial nitrate that blocks the breakdown of poly P and the subsequent release of PO$_4$ $^{19}$. Again, the carbon substrate is considered as a deciding factor in PO$_4$ uptake and release. It has been reported that in absence of nitrate, acetate present in the treatment reactor facilitates the rapid release of PO$_4$ $^{22,23}$. However, in the present setup, carbon source is unable to induce PO$_4$ release due to availability of high initial nitrate in the medium. Significant PO$_4$ uptake (77%) in the absence of organic carbon (at COD/N ratio 0.36) under anoxic conditions, can be justified by characteristic metabolic feature of the DNPAOS to utilize nitrate as an electron acceptor for phosphorus removal without any external carbon substrate but with the help of previously stored internal PHB$^{24}$.

The current observation contradicts previous reports on toxic effects of high nitrate (in presence of carbon substrate) on biological PO$_4$ uptake in conventional processes$^{6,1,25}$. The anoxic condition in the current study facilitates the growth of DNPAOs to uptake PO$_4$ using nitrate as electron acceptor in presence of optimum carbon substrate as denitrification process requires organic carbon to act as electron donor$^{16}$.

COD removal efficiency

With a gradual increase in organic carbon load (as well as COD/N ratio), there was a marked variation in COD removal efficiency. Maximum COD (~98%) removal was achieved in presence of COD/N ratio of 1.62 in the medium (Fig. 1). The trend of COD removal rate was synchronous to that of nitrate removal with respect to carbon load, suggesting the following:

- The metabolic activity of the denitrifier population is comparatively slow (at COD/N ratio is <1), resulting in lower organic carbon utilisation.
- COD concentration is higher than the stoichiometric requirement for denitrification reaction (when COD/N ratio is >5) considering the electron transmitting balance between organic substrate and nitrate$^{16}$.

Influence of COD/N ratio on denitrification and PO$_4$ removal rate

COD/N ratio was <10 (0.36-6.37) to uphold denitrification as the only route of organic matter consumption $^{17}$.

Rate of denitrification and PO$_4$ uptake in the reaction medium was determined from the following equation:

\[ r_{\text{denitrification}} = \frac{Q_{\text{in}}([NO_3^-]_{\text{in}} - [NO_3^-]_{\text{out}})}{V_{\text{reactor}} \cdot \text{VSS}_{\text{reactor}}} \]  
\[ r_{\text{PO}_4 \text{ removal}} = \frac{Q_{\text{in}}([PO_4^-]_{\text{in}} - [PO_4^-]_{\text{out}})}{V_{\text{reactor}} \cdot \text{VSS}_{\text{reactor}}} \]

Where: \( r_{\text{denitrification}} \) - Rate of denitrification, \( Q_{\text{in}} \) - Influent Flux (L)  
\([NO_3^-]_{\text{in}} \) - Concentration of Nitrate-N in the influent (mg/L)  
\([NO_3^-]_{\text{out}} \) - Concentration of Nitrate-N in effluent (mg/L)  
\([PO_4^-]_{\text{in}} \) - Concentration of PO$_4$ in influent (mg/L)  
\([PO_4^-]_{\text{out}} \) - Concentration of PO$_4$ in effluent (mg/L)  
\( V_{\text{reactor}} \) - Volume of reactor (L) and \( \text{VSS}_{\text{reactor}} \) - Volatile suspended solid in the experimental reactor (mg/L)

The inlet ammonium and nitrite ion concentrations in the medium were negligible. Hence the inlet nitrate has been considered as the total nitrogen concentration in the system. Rate of denitrification as well as PO$_4$ uptake
(Fig. 2) was influenced by the COD/N ratio. Nitrate uptake rate ranged between 0.029 to 0.40 mg NO$_3$-N/mg VSS day$^{-1}$, while PO$_4$ uptake rate ranged between 0.007 to 0.014 mgPO$_4$-P/mg VSS day$^{-1}$. A simultaneous increase in the rate of denitrification and PO$_4$ uptake was observed with increase in COD/N ratio from 0.36 to 1.62. At this point, the COD/N ratio was increased almost 2.5 times (4.06) of the previous set (1.62), which resulted in a fall in the denitrification rate (0.38 mgNO$_3$-N/mgVSS.day$^{-1}$) that was almost 5% less than the maximum rate (0.4 mgNO$_3$-N/mgVSS.day$^{-1}$). PO$_4$ uptake rate was also negatively affected with ~8% decrease than the previous set. With further increase in COD/N ratio (6.37), there was significant decrease in the denitrification rate. However, PO$_4$ removal rate remained more or less similar to the previous set. The results suggest that the impact of COD/N ratio is more significant on denitrification compared to PO$_4$ removal rate.

**pH Trend**

pH of the system performing simultaneous nitrate and PO$_4$ removal has a profound effect on the overall process$^{26,27,28}$. Table 1 indicates the change of pH in various sets. It can be observed that in the absence of acetate (COD/N ratio 0.36), there was little change in the pH of the medium even after 5h of the experimental run due to inhibition of denitrification. Both PO$_4$ uptake and a marked rise in the denitrification rate followed by supply of external carbon source resulted in a rise in pH to >8.0 within 1 hour of reaction. These results are at par with previous studies$^{29,30}$. However, with increase in pH in the range 8.0-8.45, PO$_4$ concentration never went below 2mg/L, which contradicts previous reports$^{31}$, where the authors indicated that there might be a chance of PO$_4$ precipitation beyond pH 8.0.

**Statistical analysis**

It can be seen from the ANNOVA table (Table 2) that the variance between different experimental sets, having different COD/N ratios, is significant. The results of the analysis (Table 2) indicated that the trend of removal of PO$_4$, nitrate and COD are completely different in different sets. Considering time as a factor, statistically it was determined that there is a significant variance between the sets and each set showed a distinct rate of removal for each parameter (PO$_4$, nitrate and COD).

**Conclusion**

Simultaneous removal of PO$_4$, nitrate and COD was achieved under anoxic conditions in a batch scale reactor system containing high strength waste water. COD/N ratio played a crucial role in determining the denitrification rate. Maximum denitrification rate (0.4 mg NO$_3$-N/mgVSS.day$^{-1}$) was achieved at a COD/N ratio of 1.62 and with further increase in the ratio the rate of denitrification declined. Variation of COD/N ratio had little impact on the rate of PO$_4$ removal which ranged
between 0.007-0.014 mgPO$_4$/mgVSS.day$^{-1}$. Statistical analysis further confirmed a significant variance between various parameters (PO$_4$, nitrate and COD) studied in different experimental sets.

**Acknowledgement**
Authors are thankful to the Director, CSIR-IMMT, and the Head, Environment & Sustainability Department, CSIR-IMMT, Bhubaneswar for their encouragement and to DST, New Delhi for financial support.

**References**


DAS et al: EFFECT OF COD/N RATIO ON REMOVAL OF NUTRIENTS AND COD