Pergamon

0043-1354(95)00172-7

Wat. Res. Vol. 30, No. 3, pp. 593–600, 1996

0043-1354/96 $15.00 + 0.00

BIOLOGICAL OXIDATION OF HIGH STRENGTH NITROGENOUS WASTEWATER

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(First received December 1993; accepted in revised form June 1995)

Abstract—A nitrification study was conducted in continuous flow stirred tank reactors using high strength nitrogenous wastewater (concentrated stream from a urea plant mixed with pharmaceutical wastewater). The reactors were operated at different solids retention times (SRT = 10–62.5 d) and hydraulic retention times (HRT = 1.5–2.1 d). Pharmaceutical wastewater was used as an organic carbon source to maintain a COD/TKN ratio of 1. The nitrification achieved at different SRTs varied from 87 to 99%. The nitrogen balance data show that ammonia assimilation and denitrification accounted for 4–53% of the total nitrogen removed. The yield coefficients and decay coefficients were $Y_h = 0.5$ (COD basis), $k_{dh} = 0.07\ d^{-1}$ (COD basis) for heterotrophs and $Y_n = 0.15$ (TKN basis), $k_{dn} = 0.06\ d^{-1}$ (TKN basis) for nitrifiers respectively.

Key words—nitrification, wastewater treatment, activated sludge process, ammonia removal

INTRODUCTION

A large number of nitrogenous fertiliser industries produce mainly ammonia and urea. In the urea plant the urea solution is concentrated in vacuum concentrators. During evaporation, vapors condense to form contaminated condensate. This condensate contains urea (350 mg N/l) and ammoniacal nitrogen (650 mg N/l). This is the main source of pollution from urea plants. In ammonia plant the effluent is from process condensate containing 400–1000 mg N/l ammoniacal nitrogen depending upon the process used (Ministry of Science and Technology, India, 1992). The limit for discharging of wastewater containing ammoniacal nitrogen into aquatic environment is 50 mg N/l (Central Pollution Control Board, New Delhi, India, 1991) because of toxic and other effects on aquatic life in addition to deterioration of water quality. Thus there is need to remove ammoniacal nitrogen prior to discharge. Biological nitrification-denitrification is the process commonly used to accomplish this objective.

Nitrification occurs in most aerobic biological treatment processes when operating and environmental conditions are suitable (Sharma and Alhert, 1977; EPA, 1975). One of the important controlling variables is solids retention time (SRT) i.e. sludge age. One way of maintaining high sludge age is to recycle the settled sludge. But the nitrifiers grown alone on inorganic nitrogen may not settle properly. Heterotrophs might play a vital role in the total process stability by providing compact sludge floc at which nitrifiers may be retained (Rashed, 1987). This will result in good settling of sludge which can be recycled. Hence high SRT of 10–60 d can be maintained even at low hydraulic retention time (HRT) of 1.5–2.0 d. A suitable organic carbon source in the form of pharmaceutical wastewater has been identified in a separate study (Gupta and Sharma, 1991).

This study was conducted to determine the efficacy of biological nitrification of high strength nitrogenous wastewater after mixing it with pharmaceutical wastewater. The specific objectives of this study were to:

(i) evaluate the biological oxidation of nitrogenous wastewater by a single stage activated sludge process;

(ii) determine the kinetic coefficients for the growth of heterotrophs and nitrifiers;

(iii) determine the optimum SRT and HRT at which maximum nitrification can be achieved.

MATERIALS AND METHODS

Experimental apparatus

The single state biological nitrification unit used in this study (Fig. 1) was a completely mixed activated sludge unit which consisted of a rectangular aeration basin (10 l capacity) and a settling unit (0.9 l capacity) made from transparent acrylic sheets. The air to the aeration basin was evenly dispersed by passing compressed air through sintered glass diffusers kept at its bottom. This gave satisfactory biological floc mixing. A continuous flow of influent was maintained using a peristaltic pump (Minipuls 2, GILSON)

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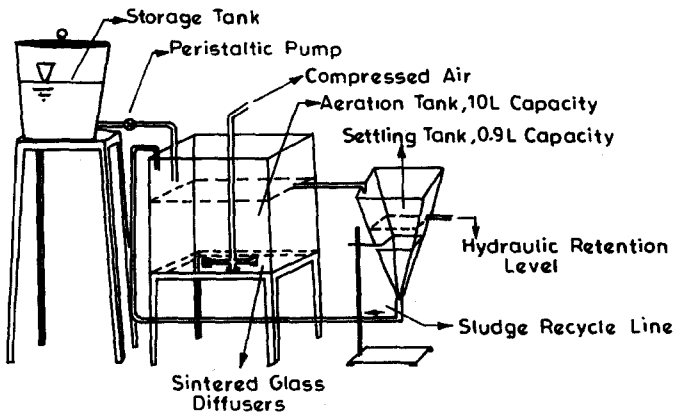

Fig. 1. Experimental set-up for bench-scale study.

and the aeration unit is referred to as a continuous flow stirred tank reactor (CSTR). The overflow from the aeration unit was sent to the settling unit having an inverted conical base with a L-joint at the bottom. With this arrangement the recirculation of settled sludge could be controlled.

Experimental design

The strategy used for studying nitrification of nitrogenous wastewater is given in Fig. 2. SRTs were selected on the basis of literature review recommendation (EPA, 1975). SRT was maintained by wasting appropriate amounts of mixed liquor from the aeration basin. Suspended solids (SS) in the effluent were also taken into account while calculating SRT.

The nitrogenous wastewater (Table 1) containing high levels of ammonia and urea was brought from a concentrated stream of a local urea plant and stored under refrigeration before using. A concentrated stream was selected to reduce the frequency of wastewater collection. This wastewater was mixed with actual pharmaceutical wastewater (Table 1) in appropriate quantities and diluted with tap water (if necessary) to get final ammoniacal and urea nitrogen concentration of about 500 mg N/l each (urea was added if required) and chemical oxygen demand (COD) of about 1000 mg/l. Sodium bicarbonate and potassium dihydrogen phosphate were also added into the feed as sources of alkalinity and phosphorus respectively. The characteristics of the mixed feed to various CSTRs are shown in Table 2.

Analytical methods

All the measurements were done according to Standard Methods (APHA, 1985) except for urea which was determined by spectrometry after reaction with p-dimethylaminobenzaldehyde solution in 95% alcohol at 440 nm (Hoseney and Finney, 1964).

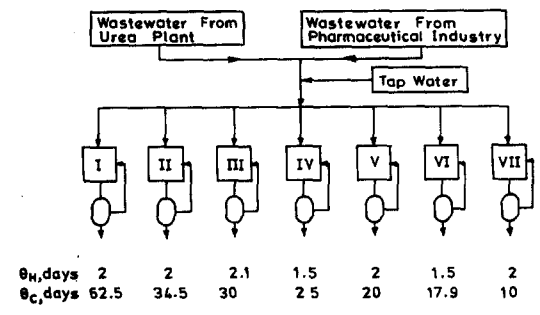

Fig. 2. Experimental design for biological nitrification studies.

Sludge acclimation and operation

Biomass was acclimated gradually from 250 mg N/l of ammoniacal and urea nitrogen each to about 500 mg N/l of ammoniacal and urea nitrogen each in a stepwise manner. The influent TKN was increased gradually from 520 to 600 mg N/l and kept at this concentration for 4 d. Subsequently the concentration of TKN was increased to 660 mg N/l and the reactor was operated for 4 d at this concentration. After this the concentration of TKN was changed to 750, 850 and 1000 mg N/l and the reactors were operated at these concentrations for 5, 7 and 10 d respectively. The acclimation from 500 to 1000 mg N/l of TKN took about 30 d. HRT was maintained at 2 d and mixed liquor suspended solids (MLSS) between 3500 and 4000 mg N/l. As the influent TKN was increased, the alkalinity in the influent was also increased by addition of sodium hydrogen carbonate to maintain the pH of the mixed liquor in the CSTRs between 7.5 and 8.0. The COD/TKN ratio was maintained in the range of 1.0–1.5. The pH and dissolved oxygen (DO) was monitored 2–3 times a day during acclimation period.

Table 1. Characteristics of wastewater from urea plant and pharmaceutical industry

Parameter	Urea plant[a] values (range)	Pharmaceutical industry, values (range)
pH	11.0–12.5	5.0–8.0
COD (mg/l)	3520–4850	1100–5500
NH_4^+–N (mg N/l)	38000–45000	30–50
Urea–N (mg N/l)	1860–2380	—
PO_4^{3-}–P (mg P/l)	0.7–1.0	2.8–14.4
Alkalinity (g $CaCO_3$/l)	1.005–1.010	0.30–2.00

[a]A concentrated stream from urea plant.

Table 2. Characteristics of the feed after mixing the urea plant wastewater with pharmaceutical wastewater

Parameter	Values (range)
pH	7.0–9.0
COD (mg/l)	1010–1290
NH_4^+–N (mg N/l)	500–550
Urea–N (mg N/l)	500
TKN[a] (mg N/l)	1016–1062
PO_4^{3-}–P (mg P/l)	22.8
Alkalinity (mg $CaCO_3$/l)	4400–5450

[a]TKN = total Kjeldahl nitrogen.

Table 3. Average influent and effluent characteristics and performance factor for nitrification studies

Operating parameter	Reactor Number I	II	III	IV	V	VI	VII	Reactor I Inf.	Reactor I Eff.	Reactor II Inf.	Reactor II Eff.	Reactor III Inf.	Reactor III Eff.	Reactor IV Inf.	Reactor IV Eff.	Reactor V Inf.	Reactor V Eff.	Reactor VI Inf.	Reactor VI Eff.	Reactor VII Inf.	Reactor VII Eff.
	..... Mixed Liquors																				
SRT (d)	62.5	34.5	30	25	20	17.9	10														
HRT (d)	2	2	2.1	1.5	2	1.5	2														
MLSS (mg/l)	4000	3500	3580	4050	2680	3500	1890														
MLVSS (mg/l)	3777	3323	3365	3809	2535	3319	1770														
F/M-COD (kg COD/kg MLVSS·d)	0.135	0.154	0.192	0.177	0.215	0.203	0.291														
F/M-TKN (kg TKN/kg MLVSS·d)	0.135	0.153	0.157	0.179	0.204	0.205	0.300														
SVI (ml/g MLSS)	78	75	65	64	67	60	56														
pH								7–9	7–8	7–9	7–8	7–9	7–8	7–9	7–8.5	7–9	7–8.1	7–9	7–8.5	7–9	7–8.1
TKN (mg/l)								1016	9.94	1016	13.8	1055	15.2	1020	23.2	1034	126.1	1020	38.2	1062	132.8
NH_4^+–N (mg/l)								500	9.94	500	13.8	550	15.2	500	23.2	510	126.1	505	38.2	505	132.8
UREA–N (mg/l)								500	ND	500	ND	500	ND	500	ND	500	ND	500	ND	500	ND
NO_2^-–N (mg/l)								—	1.1	—	0.4	—	1.9	—	398.3	—	83.4	—	220.0	—	648.9
NO_3^-–N (mg/l)								—	898.7	—	889.4	—	1002	—	33.1	—	814.4	—	310.0	—	195.7
COD (mg/l)								1020	35	1020	47	1290	55	1010	62	1090	75	1010	86	1040	120
PO_4^{3-}–P (mg/l)								22.8	19.2	22.8	19.0	22.8	19.4	22.8	18.2	22.8	18.0	22.8	17.8	22.8	16.3
Alkalinity (mg/l as $CaCO_3$)								4800	180	4750	100	5450	336	4500	260	4410	160	4800	180	4400	140
SS (mg/l)								—	18.6	—	17.5	—	19.4	—	21.0	—	18.9	—	22.3	—	18.0

ND = not detectable.

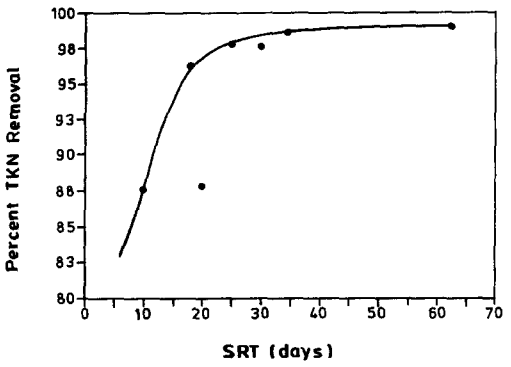

Fig. 3. Plot of percent TKN removal vs SRT.

Two 10 l CSTRs were initially operated at SRT of 62.5 and 34.5 d and HRT of 2 d. After these units reached pseudo-steady-state conditions, as determined by TKN concentrations in effluent, performance data were collected. After 42 d of operation the SRT were changed to 25 and 17.9 d and the HRT from 2 to 1.5 d respectively and operated for a period of 42 d. Subsequently three more CSTRs were operated at SRT of 30, 20 and 10 d and HRT of 2.1, 2 and 2 d respectively and pseudo-steady-state performance data were collected over a period of 75, 30 and 20 d.

RESULTS AND DISCUSSIONS

During the period of this study seven bench scale CSTRs were operated at solids retention time of 10, 17.9, 20, 25, 30, 34.5 and 62.5 d. Table 3 gives the average values for the analysis performed on influent, effluent and mixed liquors during this study. The Table shows that as the TKN loadings were increased from 0.135 to 0.300 kg N/kg MLVSS·d, the TKN removal efficiency decreased from 99 to 87.6%. The data on percent TKN removal were plotted against SRT as shown in Fig. 3. The figure shows the increase in TKN removal efficiency with increase in SRT up to 30 d after which the change in removal efficiency is not significant. A similar trend has been reported (Gupta, 1985). It has also been reported (Sutton *et al.*, 1981) that a minimum aerobic SRT of 25 d is required to obtain complete nitrification (Influent TKN = 300 mg N/l) of an industrial wastewater at approx. 22–24°C.

Nitrite build-up

Nitrite build-up was observed in CSTR units with SRT less than 30 d and TKN loadings more than 0.157 kg N/kg MLVSS·d. Nitrite build-up has been reported in the literature especially when influent TKN is high (Prakasam and Loehr, 1972; Taylor, 1956). The reason reported (Anthonison *et al.*, 1976) for this is inhibition of nitrifiers by free ammonia (FA) and free nitrous acid (FNA). The ranges of FA concentration that begin to inhibit *Nitrosomonas* are 10–150 mg N/l and that for inhibition of *nitrobacter* are 0.1–1.0 mg N/l. The FNA concentration that inhibits *Nitrobacter* activity ranges between 0.22 and 2.8 mg N/l.

In this study, the concentration of FA and FNA were calculated for all the reactors. FA was calculated by substituting the value of total ammonia nitrogen, pH, the ionization constant of ammonia equilibrium equation, K_b, and the ionization constant of water, K_w, in the following equation (Anthonisen *et al.*, 1976)

$$\text{FA as } NH_3 \text{ (mg/l)} = \frac{17}{14} \times \frac{\text{total ammonia as N (mg/l)} \times 10^{pH}}{\frac{K_b}{K_w} + 10^{pH}} \quad (1)$$

$$\frac{K_b}{K_w} = \exp(6{,}344/273 + {}^\circ\text{C}). \quad (2)$$

FNA was calculated by substituting the values of nitrite nitrogen, pH and the ionization constant of nitrous acid equilibrium equation, K_a, in the following equation (Anthonisen *et al.*, 1976)

$$\text{FNA as } HNO_2 \text{ (mg/l)} = \frac{46}{14} \times \frac{NO_2^- \text{–N (mg/l)}}{K_a \times 10^{pH}} \quad (3)$$

$$K_a = \exp(-2{,}300/273 + {}^\circ\text{C}). \quad (4)$$

Table 4 gives the maximum FA and FNA concentration in the CSTRs. Maximum FA concentration was in the range of 0.742–12.28 mg/l and maximum FNA concentration was found to be in the range of 1.33×10^{-4}–0.46 mg/l. Figure 4 shows boundary conditions of FNA inhibition to *Nitrobacter* (zone [A]), FA inhibition to *Nitrobacter* (zone [B]) and FA inhibition to *Nitrobacter* and *Nitrosomonas* (zone [C]). During this study maximum FA concentration in all the CSTRs was in zone [B] and hence were inhibitory to *Nitrobacter*. It has also been reported that nitrifiers can acclimate to higher concentrations of FA depending upon the length of operation (acclimation) period and SRT (Turk and Mavinic, 1989). This could probably explain no NO_2^-–N accumulation observed in CSTR I, II and III. However, FA concentration in CSTR IV, V, VI and VII was relatively high and may have caused *Nitrobacter* inhibition leading to accumulation of NO_2^-–N.

FNA in CSTR IV and VII was found to be in the inhibitory zone [A] to *Nitrobacter* as shown in Fig. 4. The higher FNA could have caused further inhibition of *Nitrobacter* in CSTR IV, VI and VII resulting in

Table 4. Maximum free ammonia (FA) and free nitrous acid (FNA) obtained in CSTRs at 27°C from this study

Reactor number	FA (mg/l)	FNA (mg/l)
I	0.742	7.72×10^{-4}
II	1.030	2.81×10^{-4}
III	1.134	1.33×10^{-4}
IV	4.916	0.28
V	11.661	0.06
VI	7.957	0.15
VII	12.281	0.46

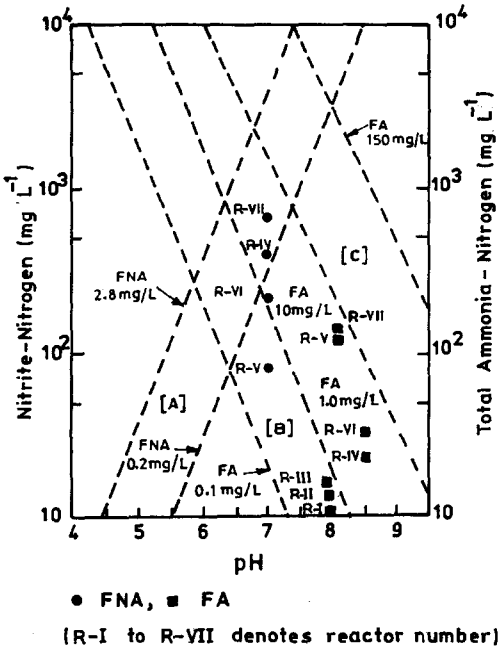

Fig. 4. Maximum free ammonia (FA) and free nitrous acid (FNA) concentrations, obtained in different CSTRs, showing boundary conditions of zone of nitrification inhibition.

accumulation of higher concentration of NO_2^-–N as given in Table 3.

From the above discussion it is inferred that an SRT of 30 d and an HRT of 2 d may be considered optimum to achieve maximum nitrification with minimum accumulation of NO_2^-–N. Figure 5 shows the performance of the unit at an SRT of 30 d during the operation period. The alkalinity consumption for every mg of nitrogen oxidized ranged between 4.25 and 4.97 mg $CaCO_3$/l which is lower than the theoretical value of 7.1. Similar results have been reported (5.8 mg alkalinity consumed/mg of ammonia nitrogen removed) in the literature (Benninger and Sherrard, 1978). The phosphate phosphorus removal ranged from 14.9 to 28.7%.

Nitrogen balance

In order to determine the fate of influent nitrogen in the CSTRs, the nitrogen balance was calculated as shown in Table 5. The percent nitrogen which could be accounted for ranged from 45 to 92%. The unaccounted portions may be attributed to air stripping, biomass assimilation and possible denitrification. The maximum loss of nitrogen due to air stripping from CSTR I, II, III, IV, V, VI and VII was calculated according to the procedure given in literature (Tchobanoglous and Burton, 1990) and was found to be 0.06, 0.08, 0.09, 0.40, 0.93, 0.64 and 0.95% respectively of total influent TKN. The above values were calculated by assuming complete

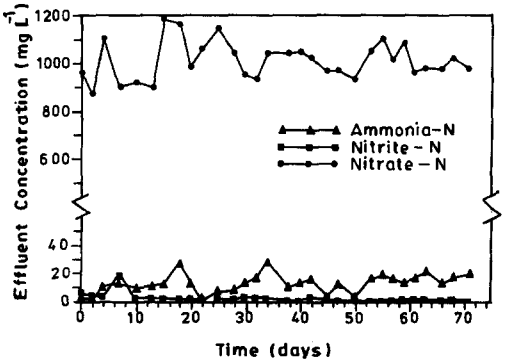

Fig. 5. Variation of NH_4^+–N, NO_2^-–N and NO_3^-–N (mg/l) in the effluent from CSTR operated at SRT of 30 d and HRT of 2 d with time.

Table 5. Nitrogen balance data

Reactor number	Influent TKN (mg/l)	Nitrogen balance as a percent				
		As NH_4^+–N	As NO_2^-–N + NO_3^-–N	In biomass	Air stripping	Unaccounted
I	1016	0.97	88.56	0.07	1.07	9.33
II	1016	1.36	87.54	0.10	1.70	9.30
III	1055	1.44	95.16	0.11	2.01	1.28
IV	1020	2.27	42.29	0.48	2.02	52.94
V	1034	12.20	86.83	1.13	2.21	—
VI	1020	3.75	51.96	0.78	2.45	41.06
VII	1062	12.62	79.53	1.16	3.00	3.81

stripping of free ammonia present as the compressed air flow rate to each reactor was 3–4 l/min or 864–1155 $l_{air}/l_{mixed\ liquor}$ over a period of 48 h (HRT).

Nitrogen content incorporated into cell synthesis was calculated on the basis that uptake of nitrogen by each bacterial cell is 9%. Assimilation of nitrogen into biomass was found to be 1.07–3.00% of the influent TKN in all the CSTRs as given in Table 5.

Denitrification in the aeration unit of the CSTRs operated at SRT of 25 and 17.9 d seemed to be the dominant mechanism. The contamination of *Thiosphaera pantotropha* in these units was suspected because the work was carried out in the same laboratory where aerobic denitrification using *T. pantotropha* dominated activated sludge was being studied. This was confirmed by isolating this bacterium using a specific medium (Robertson *et al.*, 1988). Examination under the microscope showed that the type of colonies obtained had a similar appearance to that of *T. pantotropha*. Moreover the bacterium was able to perform denitrification under fully aerobic conditions in a shake flask experiment.

The data obtained for different level of unaccounted nitrogen was plotted against various COD/TKN ratios as shown in Fig. 6. The figure does not show any connection between COD/TKN ratio and level of unaccounted nitrogen.

Sludge settling

The sludge volume index of the sludge obtained in the bench scale reactors was found to be in the range of 56–78 ml/g MLSS. The suspended solids in the effluent from a secondary clarifier ranged from 18 to 23 mg/l. These parameters indicate good settling of the sludge. The good settling of sludge could have resulted from proper maintenance of COD/TKN ratio (about 1) and volumetric COD loading (0.51–0.67 kg $COD/m^3 \cdot d$) in the feed to the reactors. Rashed (1987) also reported C/N ratio and volumetric BOD_5 loading as the major factors affecting sludge structure while investigating nitrification process of ammonia rich wastewaters by using activated sludge systems. He observed that at BOD_5/NH_4^+–N ratio $\geqslant 1$ and volumetric BOD_5 loading $\geqslant 0.4$ kg $BOD_5/m^3 \cdot d$ the SVI of activated sludge decreases below 100 ml/g MLSS.

Lower DO levels in full scale plants have been reported to cause filamentous growth in activated sludge systems and hence increase in SVI. This may result in loss of sludge and nitrification capacity of the system. In a full scale treatment plant a tower reactor with jet aerator at its bottom may be used to achieve high oxygen transfer efficiency (Eckenfelder, 1980).

Determination of kinetic parameters

Values for biological coefficients for heterotrophs and nitrifiers were estimated using the pseudo-steady-state data for biological oxidation of organic carbon and TKN respectively. The values of specific substrate utilization rate, U, were plotted against the reciprocal of the SRT according to equations (5)

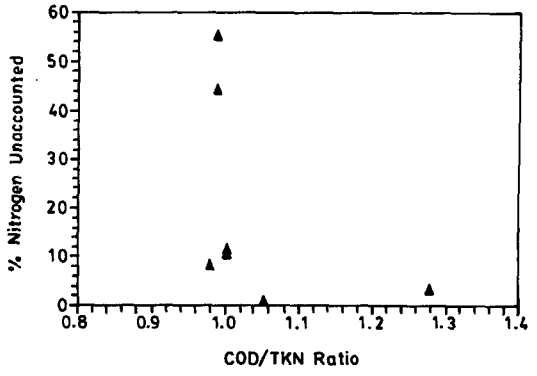

Fig. 6. Plot of different level of unaccounted nitrogen against various COD/TKN ratios.

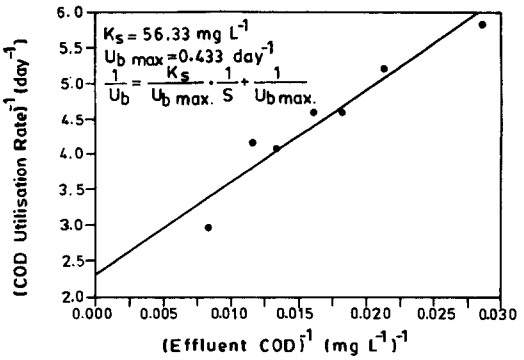

Fig. 7. Relationship between (COD utilization rate)$^{-1}$ and (effluent COD)$^{-1}$.

and (6). Kinetic coefficients for heterotrophs and nitrifiers were determined from these plots

$$\frac{1}{\Theta_c} = Y_b U_b - k_{db} \tag{5}$$

$$\frac{1}{\Theta_c} = Y_n U_n - k_{dn} \tag{6}$$

where Θ_c = SRT (days), Y_b = yield coefficient for heterotrophs (mg X_b/mg COD), X_b = heterotrophs concentration (mg/l), Y_n = yield coefficient for nitrifiers (mg X_n/mg TKN), X_n = nitrifiers concentration (mg/l), U_b = COD utilization rate (d^{-1}), U_n = TKN utilization rate (d^{-1}), k_{db} = decay coefficient of heterotrophs (d^{-1}), k_{dn} = decay coefficient of nitrifiers (d^{-1}).

For heterotrophs

$$U_b = \frac{S_0 - S}{\Theta_H X_b} \tag{7}$$

where S_0 = influent COD (mg/l), S = effluent COD (mg/l), and Θ_H = hydraulic retention time (day).

For nitrifiers

$$U_n = \frac{N_0 - N}{\Theta_H X_n} \tag{8}$$

where, N_0 = influent TKN (mg/l) and N = effluent TKN (mg/l).

X_n and X_b were determined using equations (9) and (10) respectively

$$X_n = \frac{\text{MLVSS}}{\frac{(S_0 - S)(Y_b)}{(N_0 - N)(Y_n)} + 1} \tag{9}$$

$$X_b = \text{MLVSS} - X_n \tag{10}$$

where, MLVSS = Mixed liquor volatile suspended solids.

The values of yield coefficient for nitrifiers, Y_n, and for heterotrophs, Y_b used to calculate X_n and X_b were taken from another study (Gupta and Sharma, 1991). X_n and X_b were then used to calculate U_n and U_b which were further used to obtain a new set of Y_n and Y_b from equations (5) and (6). The new values of Y_n and Y_b were used to calculate another set of Y_n and Y_b and so on till a constant value of Y_n and Y_b was obtained. The half saturation constant was determined from the plot of the inverse of the substrate utilization rate vs the inverse of the substrate in the effluent for heterotrophs (Fig. 7) and nitrifiers (Fig. 8). Table 6 shows the kinetic coefficient for nitrifiers and heterotrophs obtained from this study and those reported in the literature (Sharma and Alhert, 1977; Wong-Chong and Loehr, 1975).

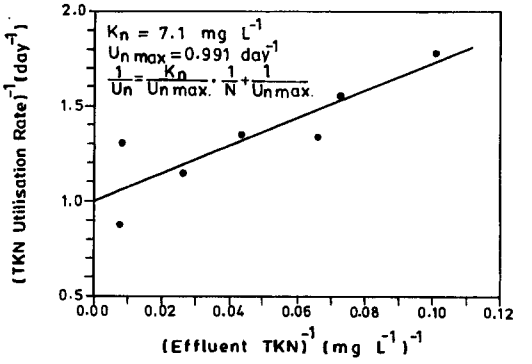

Fig. 8. Relationship between (TKN utilization rate)$^{-1}$ and (effluent TKN)$^{-1}$.

Table 6. Kinetic coefficients for heterotrophs and nitrifiers

Type of organism kinetic coefficient	This study		Other studies	
	Nitrifiers	Heterotrophs	Nitrifiers	Heterotrophs
$Y \frac{\text{mg biomass}}{\text{mg substrate} \cdot \text{d}}$	0.149	0.498	0.05–0.21[a] 0.03–0.09[b]	0.37–0.79[a]
k_d (d^{-1})	0.065	0.068		
K_s (mg/l)	7.1	56.330	0.06–8.40[a] 1.00–10.0[b]	<1–181[a]
U (d^{-1})	0.991	0.433		
μ_m (d^{-1})	0.148	0.216	0.74–3.64[a] 0.21–0.62[b]	7.2–17[a]

[a]Sharma and Alhert (1977).
[b]Wong-Chong and Loehr (1975).

The yield coefficients and half saturation coefficients for nitrifiers and heterotrophs are in agreement with the values reported for activated sludge by other investigators.

CONCLUSIONS

The following conclusions can be drawn from the present study:

1. The biological oxidation of high strength nitrogenous wastewater could be carried out successfully after supplementing it with pharmaceutical wastewater as an external source of organic carbon.
2. The percent nitrification up to 96.6 and TKN removal up to 99.0% could be achieved for nitrogenous wastewater containing TKN between 1016 and 1062 mg N/l.
3. SRT of 30 d and HRT of 2 d was found to be optimum to nitrify high strength nitrogenous wastewater having a TKN of 1016–1062 mg N/l.
4. The nitrifiers and heterotrophs yield coefficients and cell decay coefficients were 0.149 (TKN basis) and 0.065 d^{-1} and 0.498 (COD basis) and 0.068 d^{-1} respectively for the mixed population.
5. A COD/TKN ratio of about 1 was found to give good settling of sludge (SVI = 55–78 ml/g MLSS) and nitrifier fraction (0.196–0.238) in the total biomass.

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