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COMBINED ORGANIC CARBON AND COMPLETE NITROGEN REMOVAL USING ANAEROBIC AND AEROBIC UPFLOW FILTERS

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ABSTRACT

Two laboratory upflow aerobic and anaerobic filters fed with synthetic wastewaters were used to study firstly the effects of aeration rate on the nitrification of anaerobically pre-treated effluents and secondly the effects of recycle-to-influent ratios on methane production rate, denitrification and nitrification performances of a combined aerobic and anaerobic wastewater treatment process. Nitrification of anaerobically pre-treated effluent was accompanied by aerobic post-treatment for residual COD removal. A comparison of nitrification performances using autotrophic medium and anaerobically pre-treated effluents (containing 1203 mg COD l^{-1}) with the same ammonia nitrogen concentration of about 300 mg NH_4-N l^{-1} showed that 3% of added ammonia nitrogen was assimilated by autotrophic nitrifiers during nitrification of the autotrophic medium while up to 30% was assimilated by both nitrifiers and heterotrophs during organic carbon removal and nitrification of anaerobically pre-treated effluent. Furthermore, it was suspected that significant nitrogen loss through denitrification occured in the aerobic filter especially at low aeration rates. In the study of the combined aerobic-anaerobic system, maximum ammonia nitrogen removal of 70% through denitrification was obtained at recycle-to-influent ratios of 4 and 5. COD removal efficiency in the anaerobic filter decreased from 77 to 60% for recycle-to-influent ratios of zero to 5. Overall COD removal efficiency of the entire system was constant at about 99% due to heterotrophic COD removal in the aerobic filter.

KEYWORDS

Anaerobic digestion; denitrification; nitrification; methane; nitrate; ammonia; COD; anaerobic filter; aerobic filter.

INTRODUCTION

The main advantages of anaerobic digestion compared to aerobic method are low sludge production and low energy needs. Furthermore, the methane produced could be recovered and utilized as an alternative energy source. These advantages make anaerobic digestion a relatively less expensive method of treating high COD containing wastewaters.

In food-processing and agricultural sectors, anaerobic treatment has been widely applied in both solid and wastewater treatment. For wastewaters containing nitrogenous compounds beyond the concentrations recommended by the legislation, a post-treatment must be carried out before their discharge into surface waters.

Post-treatment for nitrogen removal is usually a two-step process. The first step known as nitrification is the oxidation of ammonia to nitrite and to nitrate. It is an aerobic process carried out by autotrophic bacteria. Nitrification is inhibited if the wastewater contains organic matter. This is because the presence of organic matter provokes the growth of heterotrophs, which assimilate the ammonia, thus reducing the availablility of ammonia for nitrifying bacteria (Hanaki *et al*, 1990a, Figueora and Silverstein, 1992). This inhibition becomes more pronounced when the dissolved oxygen is very low (Hanaki *et al*, 1990b), because the heterotrophic bacteria compete successfully for the limited oxygen. It should be noted that the heterotrophic bacteria have a maximum growth rate five times higher and a yield (mg cells/mg nitrogen of carbon consumed) two to three times higher than that of nitrifiers (Grady and Lim, 1980). Nitrification of anaerobically pre-treated effluents can therefore be adversely affected by the residual organic matters, both soluble and particulate, normally associated with such effluents.

The second step in the nitrogen removal process involves the reduction of nitrate and nitrite to nitrogen gas. This process, called denitrification, is carried out by facultative or strict anaerobic microorganisms. In the absence of oxygen, the denitrifiers use nitrate and nitrite as terminal electron acceptors, oxidizing organic or inorganic matters which serve as electron donors. In heterotrophic denitrification, which is more frequently used in wastewater treatment, various electron donors can be obtained either by adding an external organic carbon source or by using the organic carbon already available in the wastewater to be treated. The process configuration discussed in this paper is shown in Fig. 1.

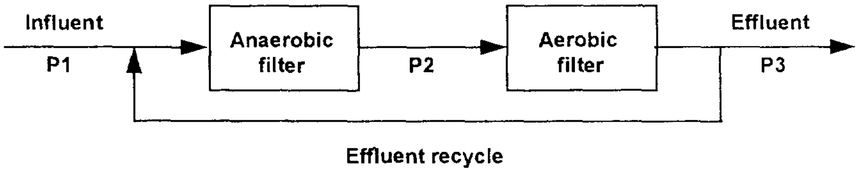

Fig. 1. Schematic diagram of the experimental process.

It consists of anaerobic and aerobic filters in series with effluent recycle. In the anaerobic unit, anaerobic digestion and denitrification take place simultaneously, producing methane and nitrogen gas. While in the aerobic unit, ammonia oxidation (nitrification) takes place. In this system, the raw wastewater serves as an organic carbon source for the denitrification and whatever remains is converted to methane. This system eliminates the use of a separate denitrification unit thus offering an enormous economic advantage. Denitrification and anaerobic digestion can successfully be carried out in the same system (Kuroda *et al.*, 1988; Hanaki and Polprasert, 1989; Akunna *et al.*, 1992). Anaerobic digested sludges have been found to have very high denitrification potential, especially in the presence of non-fermentative organic carbon susbstrates (Akunna *et al.*, 1993).

The main purpose of this work was to examine the feasibility and performance of the process configuration shown in Fig. 1. The effects of recyle-to-influent ratios on methane production, denitrification and nitrification performances, overall COD and nitrogen removal were investigated.

Before carrying out the study, nitrification of anaerobically pre-treated effluents was attempted in order to examine the interacting effect of aeration rates and organic loading on nitrification. Attached-growth reactors were used in this study. This was necessary in order to obtain a high solid retention time in both reactors and to avoid high solid load on the aerobic unit. Furthermore at high recycle-influent ratios, solid retention by the traditional sludge settling and recycling operations might be cumbersome. For the aerobic system, it was believed that earlier fixation of the nitrifiers on supports in the presence of autotrophic medium was necessary in order to assure their presence in satisfactory concentrations during heterotrophic growth brought about by the addition of anaerobically pre-treated effluents containing organic matters.

These studies were carried out using synthetic wastewaters. For the initial studies on nitrification, process performances using autotrophic wastewater (with no organic matter) containing inorganic carbon was compared with the performances using anaerobically pre-treated glucose-containing synthetic (or heterotrophic) wastewater. In the second study involving the whole units as shown in Fig. 1, the same synthetic wastewater containing glucose was used. The aeration rate employed in this second study was determined from the results of the preliminary studies on nitrification. The synthetic wastewater containing glucose (or heterotrophic wastewater) contained very low concentration of sulphate. This was to avoid hydrogen sulphide production during anaerobic digestion which might inhibit nitrification. Richardson (1985), found that 1 and 5 mg l^{-1} of sulphide could cause inhibition of up 28 and 67% respectively in the nitrification of activated sludge.

MATERIALS AND METHODS

Laboratory-scale reactors

The anaerobic upflow filter was a plastic cylindrical reactor, 15.3 cm inside diameter, 60 cm in height and 0.4 cm wall thickness. The empty-bed volume excluding headspace was approximately 10.5 litres. The filter media consisted of PVC rings average length, 1.5 cm and diameter of 1 cm. Media depth was 57 cm. Actual liquid volume was 8.3 l. To reduce the occurrence of dead zones and short-circuits due to uneven horizontal distribution of the influent in the bottom of the reactor, the influent entered vertically by a central channel from the top to the bottom of the column and mixing was done at the bottom of the column. The column body was covered with black plastic tubing through which water, thermostatically regulated at 37°C, was circulated to ensure a constant temperature. Six sampling ports at an average interval of 10 cm were made along the column.

TABLE 1. Wastewater Characteristics

Heterotrophic wastewater		**Autotrophic wastewater**	
Glucose (g l^{-1})	5.0	$(NH_4)_2SO_4$ (g l^{-1})	6.0
Yeast Extract (g l^{-1})	0.1	$NaHCO_3$ (g l^{-1})	3.75
$NaHCO_3$ (g l^{-1})	0.4	$MgSO_4$ (g l^{-1})	0.05
$MgSO_4$ (g l^{-1})	0.005	$CaCl_2$ (g l^{-1})	0.005
K_2HPO_4 (g l^{-1})	3.5	KH_2PO_4 (g l^{-1})	0.004
$NH_4H_2PO_4$ (g l^{-1})	2.875	Na_2HPO_4 (g l^{-1})	0.0171
		$FeCl_3$ (g l^{-1})	0.0015
Trace elements (mg l^{-1}):			
$FeCl_2$	0.5	Na_2MoO_4	3.0
$CaCl_2$	0.5	$ZnSO_4$	3.0
KCl	0.5	$CoCl_2$	0.06
$CoCl_2$	0.1	$MnCl_2$	6.0
		$CuSO_4$	6.0

The aerobic upflow filter was a transparent PVC cylindrical column, 11.2 cm inside diameter and 107.5 cm in height. Empty bed volume was about 10.5 l. Filter media was made up of grains of pozzolana of 3.55 mm average diameter, and 2 mm average length. Its density and porosity were respectively 2 kg l^{-1} and 0.52. Actual liquid volume was about 5.5 l. Aeration was from the bottom of the column, through 0.2 mm pore size glass air distributor. Aeration rate was controlled using an air flow meter. The column was covered with aluminium foil to keep the nitrifiers away from direct light. The reactor was operated at room temperature which varied from 20 to 22°C during the study period. Four sampling ports were made along the column at about 26 cm intervals.

Reactor start-up

The anaerobic filter was seeded with sludge obtained from a two-year old laboratory anaerobic digester enriched with glucose, peptone and yeast extracts. It was then fed with the heterotrophic wastewater at an initial hydraulic retention time (HRT) of 10 days. The HRT was gradually reduced to 23 hours when satisfactory biogas production and COD reduction were observed. Final COD and nitrogen loadings were respectively 4.4 g COD l^{-1} d^{-1} and 0.25 g NH_4-N l^{-1} d^{-1} .

The aerobic filter was inoculated with a flora obtained from seeding the autotrophic medium with a soil sample collected near a piggery farm. The filter was then fed with the autotrophic medium of pH 8.36 and HRT 14.72 hours. Nitrogen loading was about 0.25 g NH_4-N l^{-1} d^{-1} . Aeration rate was maintained constant at 120 l h^{-1}. After start-up, the influent pH was reduced to 7.5 1, which was closer to the pH value of the anaerobic filter effluent.

Experimental procedure

Case study 1: Nitrification of anaerobically pre-treated effluents

In this experiment, two synthetic wastewaters were used: the autotrophic medium and the anaerobically pre-treated effluent from the anaerobic filter. With the autotrophic wastewater, nitrogen load was 0.25 g NH_4-N l^{-1} d^{-1} while with the anaerobically pre-treated effluent, nitrogen and organic loads were respectively 0.24 g NH_4-N l^{-1} d^{-1} and 1 g COD l^{-1} d^{-1}. The characteristics of the anaerobically pre-treated effluent were constant during this study. For each wastewater, 4 aeration rates (10, 30, 60 and 120 l air h^{-1}) were applied.

Case study 2 : Study of the process configuration shown in Fig. 1

After case study 1, the nitrifying filter was washed and start-up was repeated using the autotrophic wastewater. After the start-up operations, the filter was connected to the anaerobic filter in series as shown in Fig. 1 but without effluent recycling. The anaerobic filter was maintained at the same loading characteristics as in case study 1. The aeration rate in the aerobic filter was maintained constant at 120 l air h^{-1} throughout the study period. Recycle-to-influent ratios were varied from zero to 5.

Calculation methods (Case study 2)

The percentage of influent ammonia nitrogen eliminated through denitrification in the anaerobic filter was estimated using equation (1):

$$\textbf{Ammonia nitrogen eliminated through denitrification} \quad (\%) = \frac{28 \times N(g)}{100 \times (NH_4\text{-}N)_{P1} \times V_T} \times 100 \qquad (1)$$

where:

N (g) = Rate of production of nitrogen gas at 20°C (l d^{-1})
$(NH_4\text{-}N)_{P1}$ = Quantity of ammonia nitrogen fed daily to the anaerobic filter (mg d^{-1})
V_T = Volume of 1 mole of gas at standard gas at 20°C (24.04 l)

The COD removal efficiency of the anaerobic filter and the combination of the anaerobic and aerobic filters were calculated using the following equations :

$$COD_{AN}(\%) = \left[\frac{1-(1+R)(COD_{P2})}{COD_{P1}+RCOD_{P3}}\right] \times 100 \tag{2}$$

$$COD_{AA}\ (\%) = \left[\frac{COD_{P1}-COD_{P3}}{COD_{P1}}\right] \times 100 \tag{3}$$

where

COD_{AN} = COD removal by anaerobic filter (%)
COD_{AA} = Overall COD removal by both anaerobic and aerobic filters (%)
COD_{P1} = Influent wastewater COD at P_1 (Fig. 1) (mg l^{-1})
COD_{P2} = COD of effluent from anaerobic filter at P_2 (Fig. 1)(mg l^{-1})
COD_{P3} = COD of effluent from aerobic filter at P_3 (Fig. 1)(mg l^{-1})
R = Recycle-to-influent ratio = Recycling rate (l h^{-1})/Influent feeding rate (l h^{-1})

Analytical methods

Chemical oxygen demand (COD) concentration determination on unfiltered samples was done using the potassium dichromate-ferrous ammonium sulphate method. Nitrate and nitrite were analysed on centrifuged supernatant filtered through 0.2 µm nylon membrane syringe filters (NALGENE), by ion chromatography system using conductivity detection (DIONEX-100). Separation and elution of the anions were carried out on IonPac AS4A Analytical Column utilizing a carbonate/bicarbonate eluant and sulphuric acid regenerant. Integration was done using Chromjet integrator (SPECTRA-PHYSICS). Volatile fatty acids' (VFA) analysis was done by gas chromatography fitted with an ionization detector (CHROMPAC CP 9000) and coupled with an integrator (CHROMATOPAC CR 3A). The gas produced from the anaerobic filter was passed through a SCHLUMBERGER gas meter and its composition determined by gas chromatography using thermal conductivity detector. Total Kjedahl Nitrogen (TKN) and ammonium nitrogen analysis were done according the method described by Rodier (1975). Unfiltered effluent samples were analysed for Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). Total Carbon (TC), Inorganic Carbon (IC) and Total Organic Carbon (TOC) were measured on filtered samples using a carbon analyser DOHRMANN TOC DC 90. Dissolved oxygen concentration was measured using an oxygen sensor (INGOLD SENSOR TYPE 19/50) coupled to an oxygen amplifier (INGOLD TYPE 170 % AIR).

RESULTS AND DISCUSSION

Case study 1 : Nitrification of anaerobically pre-treated effluents

Autotrophic synthetic wastewater

The influent ammonia nitrogen concentration was 320 mg NH_4-N l^{-1}. For all the aeration rates studied, complete nitrification was observed. Nitrate was the final product of ammonia oxidation in all cases. Nitrogen balance showed that the amount of ammonia assimilated for biomass production was constant and it represented 3% of the total ammonia in the influent, corresponding to about 10 mg NH_4-N l^{-1}.

Anaerobically pre-treated wastewater

The characteristics of the anaerobically pre-treated effluent together with the characteristics of the effluents from the nitrifying filter for the four aeration rates studied are presented in Table 2.

The COD of the influent was made up of principally acetic acid (308 mg l^{-1}) and propionic acid (529 mg l^{-1}). Table 2 shows that almost all the influent COD was eliminated in the nitrifying filter. The final effluent total COD and TOC content were low, about 70 and 20 mg l^{-1} respectively in all cases. VFA analysis did not show significant values.

Ammonia oxidation to nitrate occured in all aeration conditions (Table 2). Effluent dissolved oxygen concentration was satisfactory, greater than 5 mg l^{-1} in all cases. Nitrogen balance showed that more nitrogen was lost in the nitrification of anaerobically pre-treated wastewater than in the nitrification of autotrophic synthetic wastewater (which was about 10 mg NH_4-N l^{-1} in the latter). This was because the anaerobically pre-treated wastewater contained organic matter. Thus, some of the influent ammonia nitrogen was assimilated by the heterotrophic bacteria during aerobic removal of contained COD.

But the quantity of ammonia nitrogen lost in all the aeration conditions was not constant, it was found to decrease with increasing aeration rate. The ratio of ammonia nitrogen lost to removed COD at different aeration rates is also shown in Table 2. It decreased with increase in aeration rates. This ratio should be constant because nitrogen assimilation is proportional to COD removal. Since the ratio was high at low aeration conditions, it was assumed that denitrification occurred and the quantity of ammonia nitrogen lost by denitrification decreased with increasing aeration rates.

TABLE 2. Performance of the Nitrification of Anaerobically Pre-treated Wastewater

Parameter	Influent (anaerobic effluent)	Effluent from aerobic filter			
		10 l air h^{-1}	30 l air h^{-1}	60 l air h^{-1}	120 l air h^{-1}
pH	6.8	6.6	6.4	6.4	6.4
TC (mg l^{-1})	765	41	25	29	20
TOC (mg l^{-1})	452	18	14	14	12
IC (mg l^{-1})	313	23	11	15	8
Total COD (mg l^{-1})	1203	70	70	70	70
Dissolved O_2 (mg l^{-1})	0	5.3	7.7	8.0	8.3
MLSS (mg l^{-1})	146	0	0	0	0
MLVSS (mg l^{-1})	107	0	0	0	0
NH_4-N (mg l^{-1})	287	8	3	6	10
NO_2-N (mg l^{-1})	0	0	0	0	0
NO_3-N (mg l^{-1})	0	121	136	151	180
Lost NH_4-N (mg l^{-1})	-	158	148	130	97
Lost NH_4-N (%)	-	55	55	55	55
Lost NH_4-N (mg) per $COD_{removed}$	-	0.14	0.13	0.12	0.09

This experiment shows that the quantity of ammonia to be nitrified depends on the COD content of the anaerobically pre-treated wastewater. In the presence of degradable COD, the heterotrophic bacteria assimilate a part of the influent ammonia for the COD elimination. COD : NH_4-N ratio of the wastewater is therefore an important factor to be considered in the nitrification process.

Case study 2 : Study of the process configuration shown in Fig. 1

Process performance

Performance of the anaerobic filter

The characteristics of the effluents from the anaerobic filter are shown in the column P_2 of Table 3. P_1, P_2 and P_3 are respectively the characteristics of influent wastewater, wastewater from the anaerobic filter and final effluent from the aerobic filter.

It could be seen that an increase in the recycle-to-influent ratio brought about a decrease in the concentration of the following effluent parameters : TC, IC, TOC, COD, TKN and NH_4-N.

Figure 2 shows that methane gas production rate decreased from 1.04 to 0.32 l CH_4 l^{-1} d^{-1} while nitrogen gas production rate increased from zero to 0.15 l N_2 l^{-1} d^{-1} on increasing recycle-to-influent ratio from zero to 5. At the first recycle-to-influent ratio, recycled nitrate was not reduced within the first 24 hours causing a sharp fall in methane gas production rate. This initial period was considered as the period of development of nitrate-reducers present in the methanogenic sludge. The increase in the nitrogen gas production rate was due to denitrification activities because during recycling operation, oxidized ammonia was introduced into the anaerobic filter and the amount introduced increased with increase in recycle-to-influent ratio. The decrease in carbon dioxide production rate with increasing recycle-to-influent ratio could probably be due to its dissolution, brought about by the deficiency of inorganic carbon (IC) in the recycled aerobic effluent (column P_3). It should be noted that IC is the carbon substrate of ammonia oxidizers. Table 3 shows that the amount of IC in the recycled effluent (P_3) was very low, less than 40 mg l^{-1} in all cases.

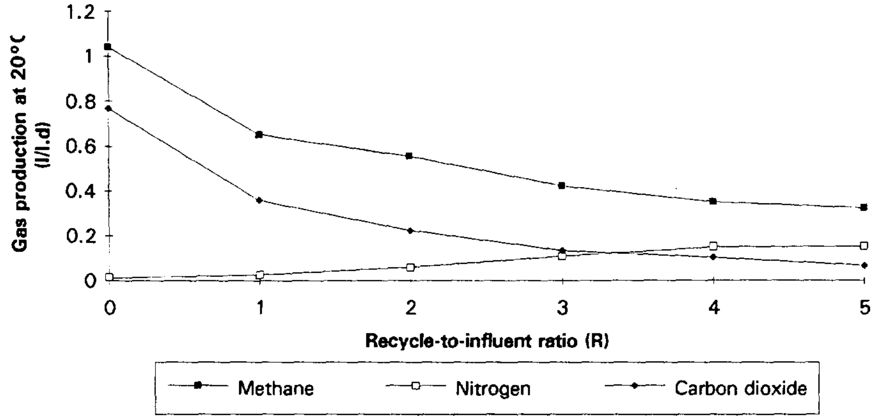

Fig. 2. Influence of recycle-to-influent ratios on the anaerobic gas production rates and composition.

Performance of the aerobic filter

The characteristics of the effluents from the aerobic filter are shown in column P_3 of Table 3. Increasing recycle-to-influent ratios brought about both aerobic elimination of added COD and nitrification. From Fig. 3, it will be seen that for the recycle-to-influent ratios of zero and 1, nitrification activities were concentrated in the first 26 cm of the filter column. Beyond these ratios, a decrease of nitrification activities in this part of the column and its increase in the upper parts was observed. At a ratio of 5, nitrification practically stopped in this lower zone but continued in the upper parts. This behaviour was probably due to the competition for the ammonia between the autotrophic (ammonia oxidizers) and heterotrophic bacteria which eliminated influent COD. At the beginning of the experiment, all the support media were well colonized by the autotrophs. But as the experiment progressed, the heterotrophs dominated gradually the lower part of the filter. It seemed that these bacteria fixed on top of the autotrophic biofilm thereby preventing the latter from having access to the available oxygen and ammonia. Thus, as the recycle-to-influent ratio was increased, nitrifiers at the lower part became redundant while those at the upper part became more active. It should be noted that almost all the influent COD was eliminated in the lower part of the filter. As a result, there were relatively lower concentrations of heterotrophs at the upper parts of the filter (beyond a height of 26 cm). The effluent was almost saturated with dissolved oxygen (above 7 mg l^{-1}) in all the recycle-to-influent ratios studied, indicating that there was enough oxygen for both the heterotrophs and the autotrophs.

TABLE 3. Steady State Performance of the Treatment Process

		Recycle-to-Influent ratio (R)											
	Influent	0		1		2		3		4		5	
Para-meter	P_1	P_2	P_3	P_2	P_3	P_2	P_3	P_2	P_3	P_2	P_3	P_2	P_3
pH	7.7	6.8	6.3	6.8	6.5	6.6	6.6	6.7	6.6	6.7	6.7	6.7	6.7
TC (mg l^{-1})	2282	765	27	570	30	402	43	389	63	296	44	280	38
TOC (mg l^{-1})	2282	452	12	352	12	246	28	208	28	176	28	154	26
IC (mg l^{-1})	0	313	15	218	18	156	15	181	35	120	16	126	12
Total COD (mg l^{-1})	5318	1213	70	896	70	645	130	544	130	450	130	400	130
Dis. O_2 (mg l^{-1})	8.8	0	8.4	0	8.1	0	7.8	0	7.6	0	7.7	0	7.9
TKN (mg l^{-1})	333	323	24	249	24	196	33	150	33	113	28	90	39
NH_4-N (mg l^{-1})	300	287	22	199	22	159	28	117	22	86	8	61	11
NO_3-N (mg l^{-1})	0	0	185	0	130	0	110	0	92	0	69	0	49
NO_2-N (mg l^{-1})	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 3 suggests that nitrification activities in a biofilm reactor can be completely inhibited by heterotrophic growth, even in the presence of sufficient dissolved oxygen and abundant ammonia nitrogen.

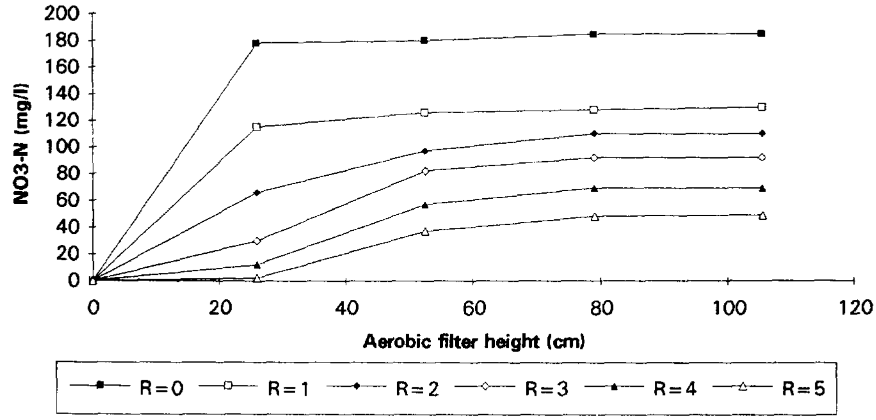

Fig. 3. Profile of nitrate nitrogen concentrations in the aerobic filter for all recycle-to-influent ratios.

Overall performance

Using equation (1), the percentage of added ammonia nitrogen denitrified in the anaerobic filter was estimated for each recyle-to-influent ratio studied. This parameter increased with increase in the ratio as shown in Fig. 4, with maximum value of 72 at a ratio of 5. Increasing the ratio beyond 4 did not result in a significant increase in nitrogen elimination through denitrification.

Figure 4 also shows the COD removal efficiency obtained from the anaerobic filter and from the two filters. The values used in these graphs were obtained for each ratio by using respectively equations (2) and (3). Before effluent recycling, COD removal efficiencies were 77% for only the anaerobic filter and 99% for the entire system. At the recycling ratio of 1, a sharp fall in anaerobic COD removal was observed. This was assumed to have been caused by nitrate toxicity on methanogenic bacteria. There was, as a consequence, a sharp fall in methane production rate (Fig. 2). This toxicity was pronounced because during the first 24 hours, added nitrate was found in the effluent. There was therefore a latent period for nitrate reduction corresponding probably to the period of development and multiplication of the nitrate reducers (essentially denitrifiers). Beyond the recycle-to-influent ratio of one, the percentage COD removal was more or less constant, with an average value of about 63%. The slight variation in COD removal efficiency, despite significant diminution in methane gas production rate (Fig. 2) observed in the anaerobic filter, was due to the occurence of denitrification. It should be noted that from stoichiometric equations, 2.86 g of COD are oxidized per 1 g nitrate nitrogen denitrified.

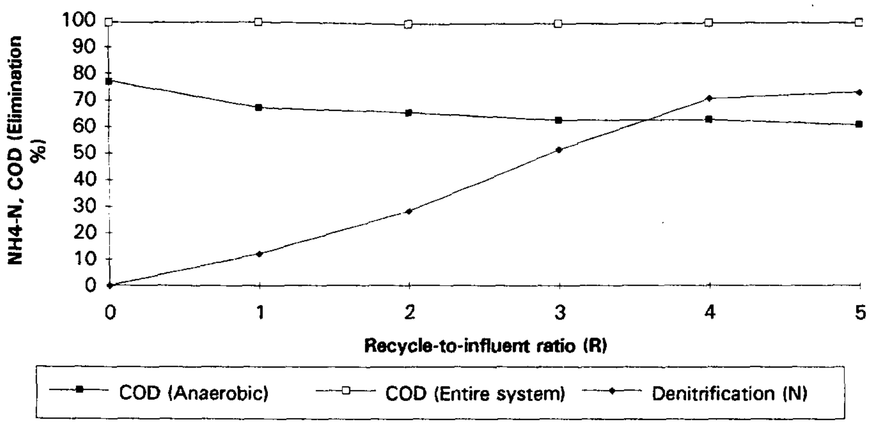

Fig. 4. Denitrification and COD removal performances as a function of recycle-to-effluent ratio.

The data in Fig. 4 further indicate that the entire system achieved final COD removal efficiency of about 99%, implying that aerobic elimination of residual COD from the anaerobic filter was significant, almost 40% of influent COD. Table 3 shows that final effluent TOC was generally less than 30 mg l^{-1} , while effluent total COD varied from 70 to 130 mg l^{-1}.

CONCLUSION

In this study, it was observed that nitrification of anaerobically pre-treated effluents was accompanied by aerobic post-treatment for residual COD removal. It involved therefore a cohabitation of two principal groups of bacteria: the autotrophic ammonia oxidizers and the heterotrophic COD removers. The results showed that the amount of ammonia nitrogen to be nitrified depends on the amount of organic matter remaining in the digested effluent. The comparative study of nitrification performances using autotrophic medium (without organic carbon compounds) and anaerobically pre-treated effluents (containing 1203 mg COD l^{-1}) with the same nitrogen concentration of about 300 mg NH_4-N l^{-1} showed that 3% of added ammonia nitrogen was used by autotrophic nitrifiers for cell synthesis during nitrification of the autotrophic medium, while up to 30% was used for both autotrophic and heterotrophic cell synthesis during aerobic organic carbon removal and nitrification of the anaerobically pre-treated effluent. It was believed that significant nitrogen loss through denitrification occurred in the aerobic filter especially at low aeration rates. Finally, it was observed that heterotrophic growth could completely inhibit nitrification in an aerobic filter even in the presence of sufficient dissolved oxygen and abundant ammonia nitrogen.

For the study of the combined anaerobic and aerobic upflow filters, it was found that methane gas production rate decreased from 1.04 to 0.32 m^3 CH_4 m^{-3} d^{-1} while nitrogen gas production rate increased from zero to 0.15 m^3 N_2 m^{-3} d^{-1} on increasing recycle-to-influent ratios from zero to 5.

Denitrification in the anaerobic filter accounted for up to 70% of added ammonia nitrogen. Highest complete added nitrogen removal was obtained at recycle-to-influent ratios of 4 and 5.

COD reduction in the anaerobic filter decreased slightly from 77 to 60% at recycle-to-influent ratios of zero to 5. The small variations in percentage COD reduction despite significant reduction in methane production rates were associated to increasing denitrification performances whereby organic carbon compounds serve as electron donor. Overall COD reduction was constant at 99%, due to heterotrophic COD removal in the aerobic filter. The study also shows that organic carbon removal by anaerobic digestion associated with organic carbon and nitrate removal by denitrification is feasible in one system.

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