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DENITRIFICATION IN ANAEROBIC DIGESTERS: POSSIBILITIES AND INFLUENCE OF WASTEWATER COD/N-NOx RATIO

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ABSTRACT

Laboratory-scale completely-stirred anaerobic digesters were fed with synthetic wastewaters containing nitrate and nitrite and with glucose as the only source of organic carbon in order to investigate and compare the denitrification potentials of anaerobic digesters in the presence of nitrate and nitrite. Varying the input nitrate and nitrite concentration at fixed COD and HRT, methane production without denitrification occurred at COD/N-NOx > 53 ; denitrification and methane production at 8.86 ≤ COD/N-NOx ≤ 53 and only denitrification at COD/N-NOx < 8.86. At COD/N-NOx > 53, ammonification appeared to be the main nitrate and nitrite reduction pathway. The successful competition of ammonia formers over the true denitrifiers at high ratios was attributed to the low initial nitrate and nitrite concentrations.

Keywords: Anaerobic digestion, denitrification, ammonification, methane, COD

INTRODUCTION

Anaerobic digestion of wastewaters to produce methane and especially to biodegrade the waste is widely employed in the treatment of agricultural wastewaters. In most cases, the methane is not always recovered and utilised. The by-products of this method of treatment are the biomass, methane, ammonia, carbon dioxide and hydrogen sulphide. Since ammonia is the result of protein digestion, high ammonia concentrations are often found in the treated effluents of food processing wastewaters. Beyond 1500-3000 mg $N\text{-}NH_3$ l^{-1} ammonia has been found to inhibit methanogenesis at high pH values (1,2).

The most widely used method for ammonia removal is the biological method which consists of nitrification and denitrification.

In nitrification, the nitrifying bacteria of types *Nitrosomonas* and *Nitrobactër* convert the ammonia to nitrate and nitrite under aerobic conditions.

The biological nitrate reduction can be either assimilatory or dissimilatory.

Assimilatory nitrate reduction involves the reduction of nitrate to nitrite and then to ammonia for cell synthesis. This reduction is controlled by the quantity of ammonia in the medium (3) and is common in cases where nitrate is the only nitrogen source. Plants, fungi, algae and some facultative bacteria are some organisms capable of carrying out this process.

Nitrate reduction under anaerobic conditions whereby some bacteria utilise nitrate instead of oxygen as a terminal electron acceptor is termed dissimilatory. Two types of reactions characterise this process: In the first reaction, nitrate is reduced to nitrite which is further reduced to gaseous products such as nitrogen or nitrous oxide, a process called nitrate respiration or denitrification. The second reaction involves the reduction of nitrate to ammonia via nitrite, a process known as ammonification.

Denitrification is a property of facultative or strict anaerobic bacteria. *Pseudomonas*, *Achromobacter*, *Bacillus* are some of the bacteria with high denitrifying capabilities (4).

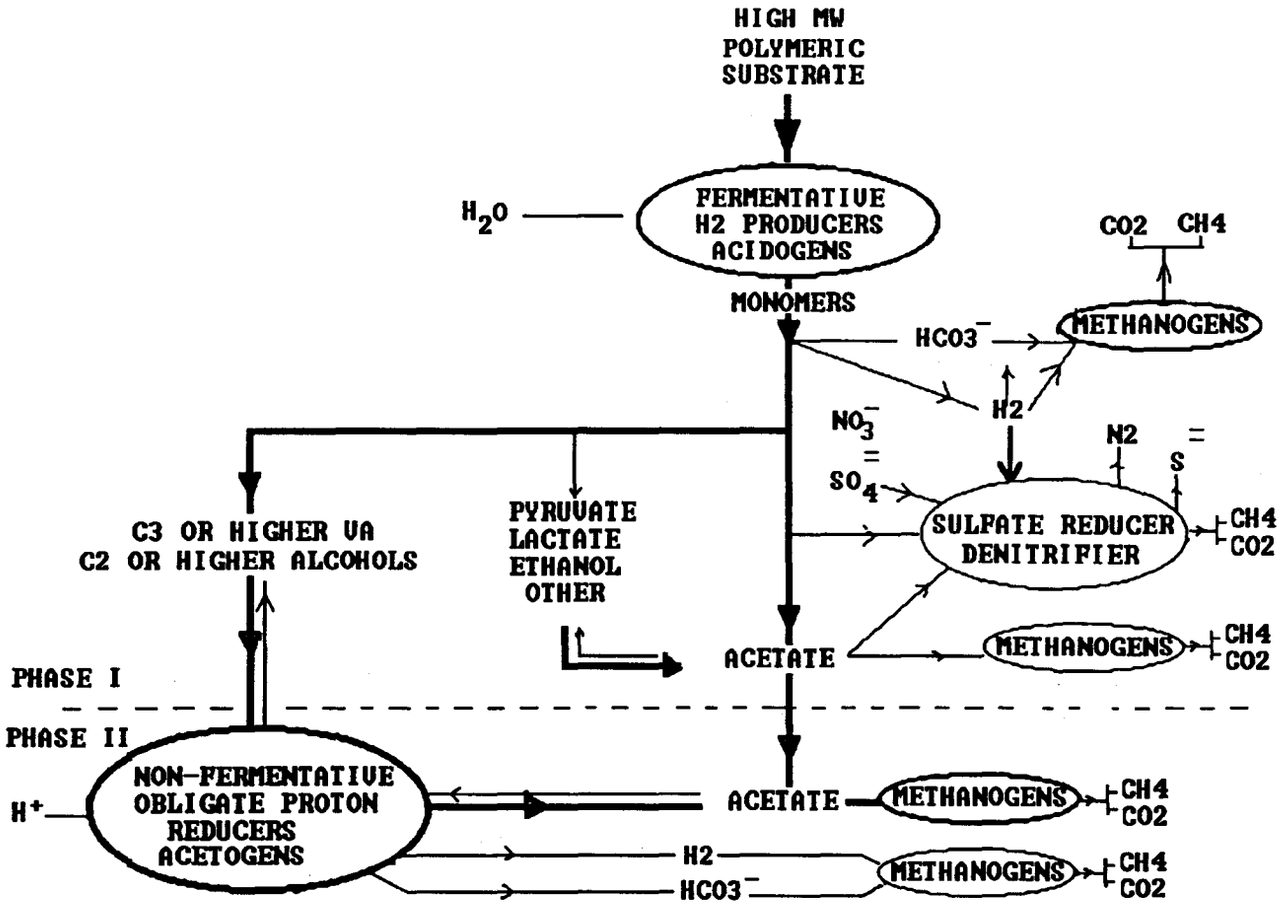

Figure 1. Microbial reactions in anaerobic digestion.

For denitrification to take place, there must be a source of organic carbon, which is the electron donor to be oxidised by nitrite or nitrate. The electron donor can be obtained either by adding an external carbon source or by using the carbon already available in the wastewater to be treated.

In the traditional anaerobic method of wastewater treatment, the effluent from an anaerobic digester is aerated to nitrify the ammonia present before denitrification. In most cases, carbon addition is necessary to ensure complete denitrification since most of the available carbon sources have been removed for methane and carbon dioxide production in the digester. This operation sometimes leads to either incomplete denitrification due to insufficient carbon matter or complete denitrification with high effluent COD due to excess carbon additions.

This problem can be solved if denitrifying bacteria are maintained in the anaerobic digester since it is generally believed that methanogenic activities begin only after denitrification (Figure 1) (5). Thus, the excess organic carbon remaining after denitrification can be converted to methane and carbon dioxide. The savings on costs, materials and space from this type of operation is far from being negligible.

Very few researchers have studied the feasibility of this combined system. Using an upflow filter fed with synthetic wastewater containing methanol as the only source of carbon, Hanaki and Polprasert (6) observed three reaction zones based on methanol/nitrate ratio which corresponded to processes of incomplete denitrification, complete denitrification and complete denitrification with methane production. A large scale reactor, equipped with a nitrification unit has been studied (7). With an anaerobic baffled reactor treating sewage wastewaters, these authors achieved more than 80% COD, suspended solids and nitrogen removal efficiency and produced 50% lesser sludge than the traditional nitrification/denitrification

process. Some other authors (8) obtained a slightly higher treatment efficiencies with fluidized bed reactors.

For this process to be applied in the treatment of agricultural and food processing wastewaters, it is necessary to understand the denitrification performances at very low nitrite or nitrate concentration (or very high COD/N-NOx ratio) since these wastewaters have a common characteristics of containing very high organic carbon with respect to nitrogen.

The experiments described in this article are the first part of a long-term research programme designed to study the feasibility and potential of an integrated denitrification/ methanogenesis system.

In this work nitrate and nitrite were separately fed to two methane-producing anaerobic digesters in order to evaluate and compare their relative performances. The influence of COD/nitrate and COD/nitrite ratios on methanogenic and denitrification activities were also studied. Microbiological studies were carried out at each ratio in order to evaluate the evolution of denitrifying bacteria with varying nitrate and nitrite loads. Synthetic wastewater containing glucose as the only source of carbon was used. Due to the microbiological studies involved, this work was carried out in continuous-flow stirred reactors.

Stoichiometric considerations for denitrification

Denitrification is a two-step reaction in which nitrate is biologically reduced to nitrogen gas.

Using glucose as the electron donor, the stoichiometric equations are as follows:

$$NO_3^- + 1/12\ C_6H_{12}O_6 \rightarrow NO_2^- + 1/2\ CO_2 + 1/2\ H_2O \quad (1)$$

$$NO_2^- + 1/8\ C_6H_{12}O_6 + H_2O \rightarrow 1/2\ N_2 + 3/4\ CO_2 + 5/4\ H_2O + OH^- \quad (2)$$

$$NO_3^- + 5/24\ C_6H_{12}O_6 + H_2O \rightarrow 1/2\ N_2 + 5/4\ CO_2 + 7/4\ H_2O + OH^- \quad (3)$$

Using equations (II) and (III), the glucose/N-NO_3 and glucose/N-NO_2 mass ratios for complete denitrification are 2.68 and 1.61 respectively. These ratios correspond to COD/N-NOx ratios of 2.86 and 1.72 for the nitrate and nitrite respectively, indicating that COD requirement is 40% lower for nitrite reduction than for nitrate reduction.

The practical ratios are more than the above because of the assimilation of glucose for biomass formation.

MATERIALS AND METHODS

Reactor performance studies

Experimental apparatus

Three bench-scale continuous-flow stirred reactors were used. Water (thermostatically regulated at 37°C) was circulated through a water jacket on each reactor. The working volume of each reactor was 3 litres. Apart from the influent and effluent inlet and outlet, there was also an outlet for the produced gas on top of each reactor. Feeding was done by pumping from the top of each reactor and the effluent was drawn from the bottom with also the aid of a pump.

Media

The composition of one litre of the synthetic wastewater was as follows: glucose - 5 g; peptone - 240 mg; yeast extract - 100 mg; $NaHCO_3$ - 400 mg; K_2HPO_4 - 3.5 g; KH_2PO_4 - 2.7 g; $MgSO_4\ 7H_2O$ - 5 mg. The trace elements were the following: $FeCl_2$ - 0.5 mg; $CaCl_2$ - 0.5 mg; KCl_2 - 0.5 mg; $CoCl_2$ - 0.1 mg.

The pH of the medium was adjusted to about 7.2 by the addition of sodium hydroxide. A 20 fold concentrated stock solution was prepared for each run and stored in a refrigerator at 4°C. Various concentrations of nitrate and nitrite were respectively prepared from potassium nitrate and sodium nitrite.

Experimental design and start-up

One of the three reactors served as a single stage anaerobic digester and received the control feed which did not contain nitrate or nitrite. It was the blank. The second reactor received feed containing added nitrate and the third received feed containing added nitrite.

The original seed sludge was obtained from an anaerobic digester in the Narbonne Municipal Sewage Treatment Plant. The nitrate-fed and nitrite-fed reactors were started with very low concentrations of nitrate and nitrite respectively. All the reactors were run with an initial hydraulic residence time (HRT) of 30 days which was shortened to 10 days after stable activity was confirmed.

Throughout the experimental period, the HRT and influent wastewater COD were kept constant for all the reactors but the nitrate and nitrite concentrations in the feeds to the latter two reactors were increased in steps to bring about large variations of COD/N-NOx ratios. For each ratio samples were collected from each reactor for bacterial population studies which

was essentially the numeration and characterisation of denitrifying bacteria population. The Table 1 shows the experimental conditions used in the study.

Analytical methods

The pH of the influent and mixed liquor of each reactor was measured daily with a glass-electrode pH meter.

The influent COD, nitrate, nitrite and total kjeldahl nitrogen (TKN) were measured twice for each experimental condition. Filtered effluents (supernatants of samples centrifuged at 20000 rpm for 15 minutes with Beckman J2-21 M/E Centrifuge) were analysed for COD, nitrate, nitrite, volatile fatty acids (VFA) and ammonia nitrogen. Unfiltered effluent samples were analysed for TKN, and Mixed Liquor Volatile Suspended Solids (MLVSS).

The COD was measured by 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 utilising a carbonate/ bicarbonate eluant and sulphuric acid regenerant. Integration was done using Chromjet integrator (Spectra-Physics). VFA analysis was done by gas chromatograph fitted with a flame ionisation detector (Chrompac CP 9000) and coupled with an integrator (Chromatopac CR 3A). The gas produced was passed through a Schlumberger gas meter and its composition determined by gas chromatography using a thermal conductivity detector. Other parameters were determined according to Standard Methods (9).

Bacterial population studies

Media

The culture media used were the following: Endo Agar (Biomerieux 51321); Cetrimid Agar (Merck 5203); MacConkey Agar (Biomerieux 71071) ; Nitrate Agar (Nitrate broth Merck added 1.5% Agar).

Culture conditions

Samples collected for each run were diluted and spread evenly on petri dishes. The petri dishes were then incubated at 28°C in the dark. Developed colonies were counted after 48 hours in aerobic conditions or after 96 hours in anaerobic conditions. Denitrifiers were isolated on Nitrate Broth Agar medium in anaerobic jars (BBL). Developed colonies were counted and about 30 to 50 of them were then purified and characterised. Characterisation was carried out with the help of oxydase tests, selective media and API 20 E/API 20 NE systems (Biomerieux 20050-20100).

RESULTS AND DISCUSSION

The results presented were those obtained at steady-state conditions. Since nitrate reduction is always accompanied by nitrite production, nitrate removal efficiency is given in terms of N-NOx ($N\text{-}NO_3$ + $N\text{-}NO_2$), thus disregarding any conversion of nitrate to nitrite.

Methane Fermentation Performances in the Blank Reactor

The blank reactor was run for about 120 days. About 25 days after start-up, most of the parameters studied became stable. Daily gas

Table 1. Influent characteristics and experimental design.

	Influent variations to N-NOx fed reactors						
	1	2	3	4	5	6	Blank
Influent $N\text{-}NO_3/N\text{-}NO_2$	50	100	300	600	800	2500	-
Influent COD (mgL^{-1})	5318	5318	5318	5318	5318	5318	5318
COD/N-NOx Ratio	106	53	17.73	8.86	6.65	2.13	-
Influent TKN (mgL^{-1})	47	47	47	47	47	47	47
Influent NH_4 (mgL^{-1})	-	-	-	-	-	-	-
HRT (days)	10	10	10	10	10	10	10

production varied from 800 to 1000 ml and the average composition was 58% methane and 39% carbon dioxide (Figure 2). The effluent pH was unstable, varying from 5.9 to 6.5 because of the accumulation of volatile fatty acids (1.2 g l^{-1} on average). MLVSS remained constant at 1.7 g l^{-1} throughout the period of the study. The effluent COD varied from 0.17 to 0.22 g l^{-1} d^{-1} after the start-up period (Figure 3), thus representing between 60% and 70% COD reduction. This rather low treatment efficiency could be attributed to the low pH values caused by low degradation rate of the produced volatile fatty acids.

Figure 3 also shows that after the start-up period, the ammonia nitrogen in the effluent was not detectable. This was expected since the influent wastewater did not contain ammonia nitrogen but only 46-47 mg l^{-1} of organic nitrogen in the forms of yeast extracts and peptone. The initial ammonia nitrogen observed during start-up must therefore be from sludge residuals used as inoculum. TKN analysis showed that about 7% of the added organic nitrogen was used for biomass production.

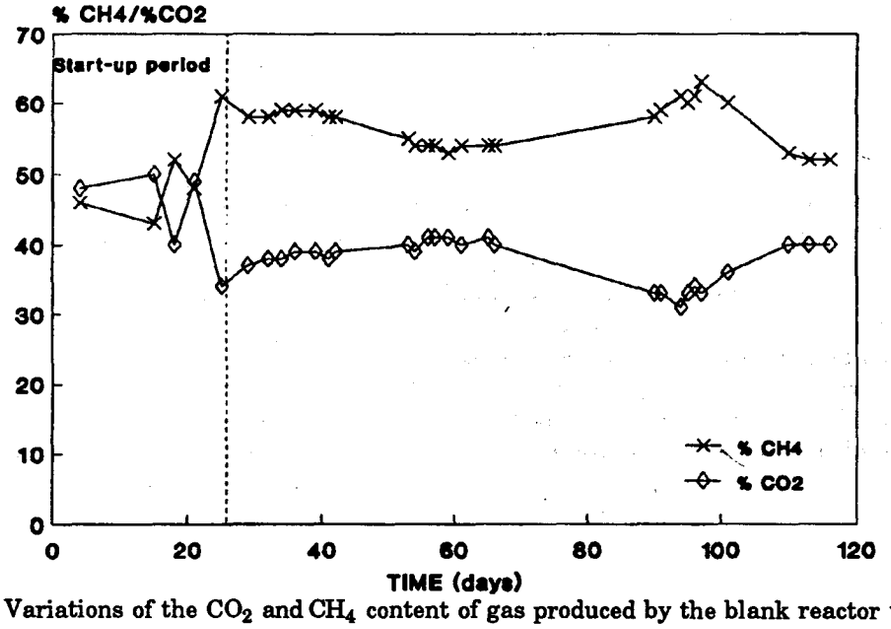

Figure 2. Variations of the CO_2 and CH_4 content of gas produced by the blank reactor with time.

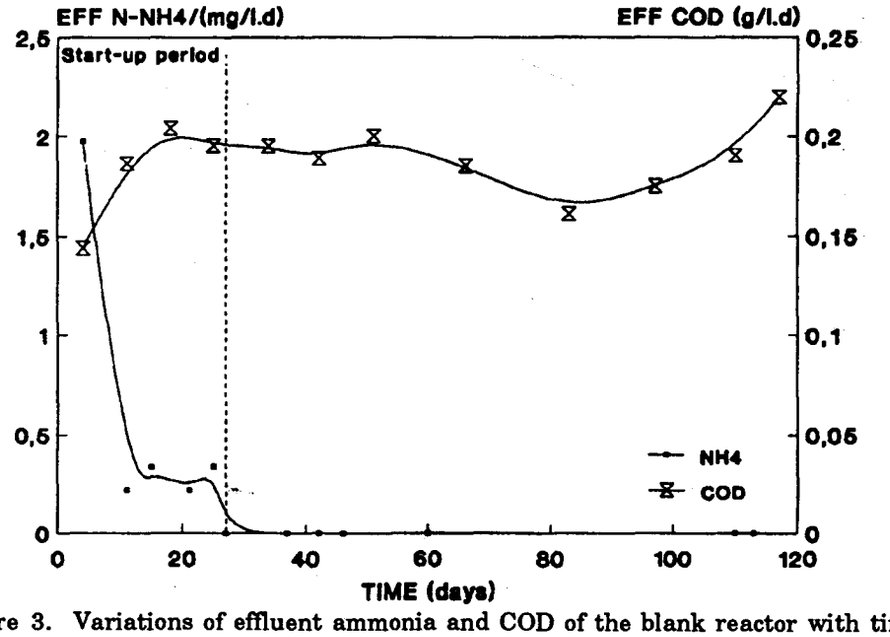

Figure 3. Variations of effluent ammonia and COD of the blank reactor with time.

Performances of the Nitrate-fed and the Nitrite-fed Reactors

Denitrification:

The denitrification performances of both reactors are shown in figure 4. The percentage of added N-NOx (nitrate nitrogen and/or nitrite nitrogen) denitrified in both systems increased with decreasing COD/N-NOx ratio. The nitrate-fed reactor exhibited a better denitrification performance. At a ratio of 8.86, 64% of added nitrate was denitrified as compared with 32% observed in the nitrite-fed reactor. At lower ratios, the percentage of nitrate denitrified diminished while that of nitrite increased to about 50% at a ratio of 6.65. Decreasing the ratio further to 2.13 brought about a remarkable decrease in denitrification efficiencies to 16 and 10% in the nitrate-fed and nitrite-fed reactors respectively.

From the ratios of 106 to 6.65, neither nitrite nor nitrate was significantly detected in the effluent indicating a total reduction of all the added nitrate and nitrite to ammonium and nitrogen gas in both reactors. But at a ratio of 2.13, the effluents contained high concentrations of N-NOx (figures 5 and 6), much more than expected signifying probably an activity inhibition caused by high influent N-NOx (2500 mg l^{-1}).

Ammonification:

At low nitrate and nitrite loads (or high COD/N-NOx ratios), the reduction of nitrate and nitrite to ammonia was dominant (Figures 5 and 6). A 100% reduction of nitrate and nitrite to ammonia was observed at COD/N-NOx of 106 (or at a load of 5 mg N-NOx l^{-1} d^{-1}).in both reactors. This proportion decreased while denitrified proportion increased as the nitrate and nitrite loads were respectively increased. No significant ammonia was observed at a load of 250 mg N-NOx l^{-1} d^{-1}. Nitrite accumulation at this load in the nitrate-fed reactor was low (about 130 mg l^{-1}). Effluent analysis showed that more than 80% of added nitrate and nitrite were not reduced indicating that the high N-NOx load was toxic to the entire microbial ecosystem.

The figures also show that ammonia production was higher in the nitrite-fed reactor at all ratios, suggesting that ammonia-formers have a greater affinity for nitrite than nitrate. Furthermore, the competition between denitrifiers and ammonia-formers appears to be more favourable to the latter under nitrate and/or nitrite-limiting conditions.

Methane production:

The composition of the produced gas as a function of the COD/N-NOx ratio is shown in Figures 7 and 8 for the nitrate-fed and nitrite-fed

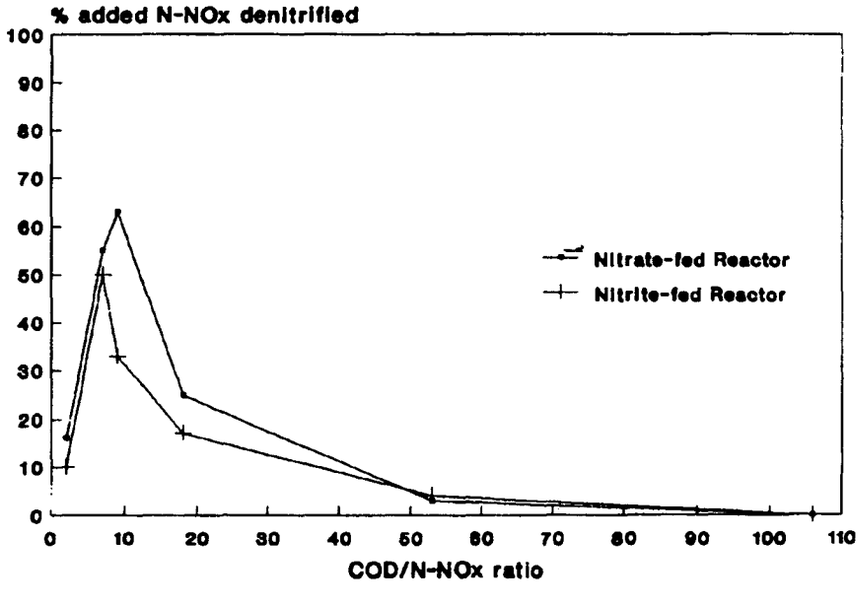

Figure 4. Percentage of added nitrate and nitrite denitrified as a function of COD/N-NOx ratio.

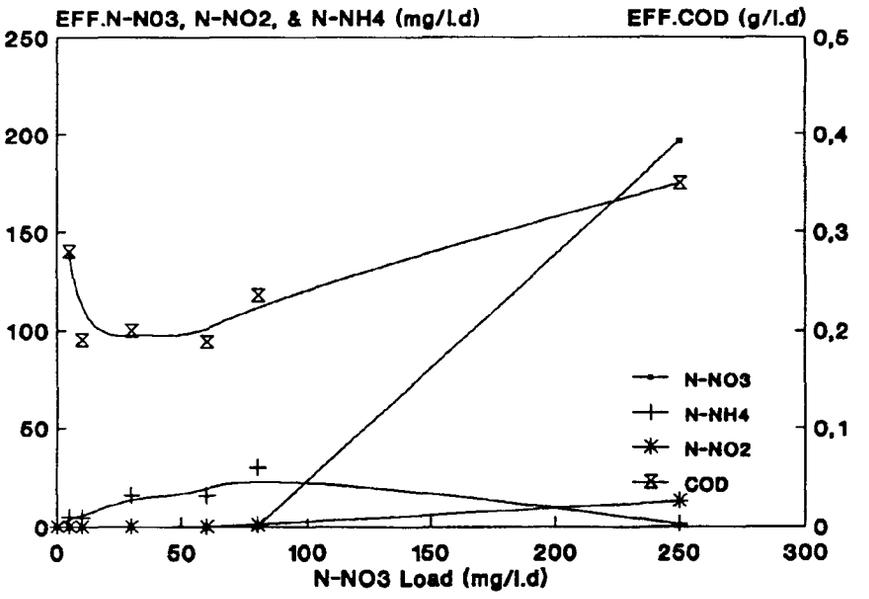

Figure 5. Relationship between nitrate load and effluent nitrate, nitrite, ammonia and COD.

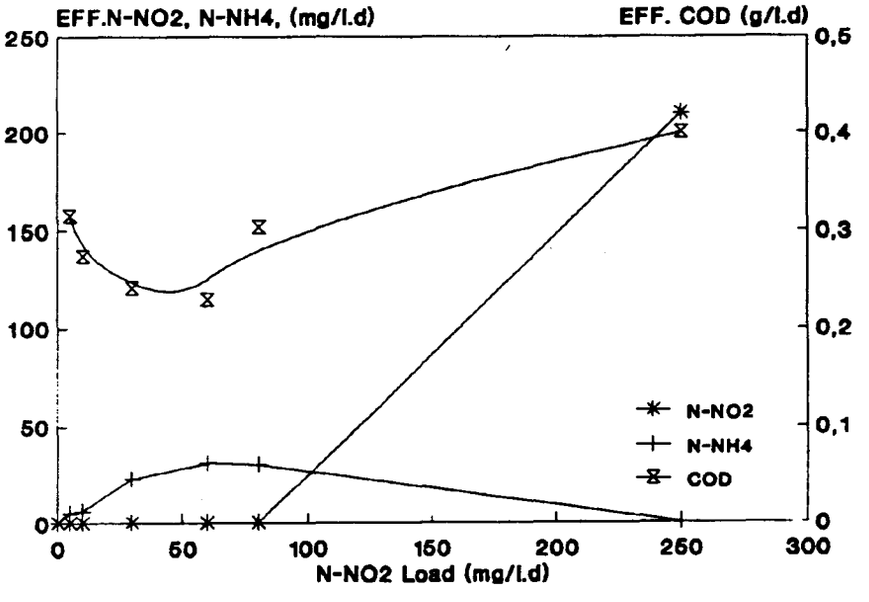

Figure 6. Relationship between nitrite load and effluent nitrite, ammonia and COD.

reactors respectively. The methane content of the gas decreased as the ratio was decreased while the nitrogen content increased. The maximum methane percentage (55% in the nitrate-fed reactor and 57% in the nitrite-fed reactor) was recorded at a ratio of 53. At a ratio of 2.13, methane production ceased, while nitrogen gas content increased to 62% and 68% for the nitrate and nitrite-fed reactors respectively.

It should be noticed that for a given COD/N-NOx ratio, the gas produced in the nitrite-fed reactor was richer in methane than that produced in nitrate-fed reactor. Since the influent COD was the same, this phenomenon was expected because at the same N-NOx load more COD would be available after nitrite reduction for methane production than that remaining after nitrate reduction knowing that the COD requirement for nitrite reduction is about 40% lower than that required for nitrate reduction (equations I and II).

Furthermore, the fact that methane

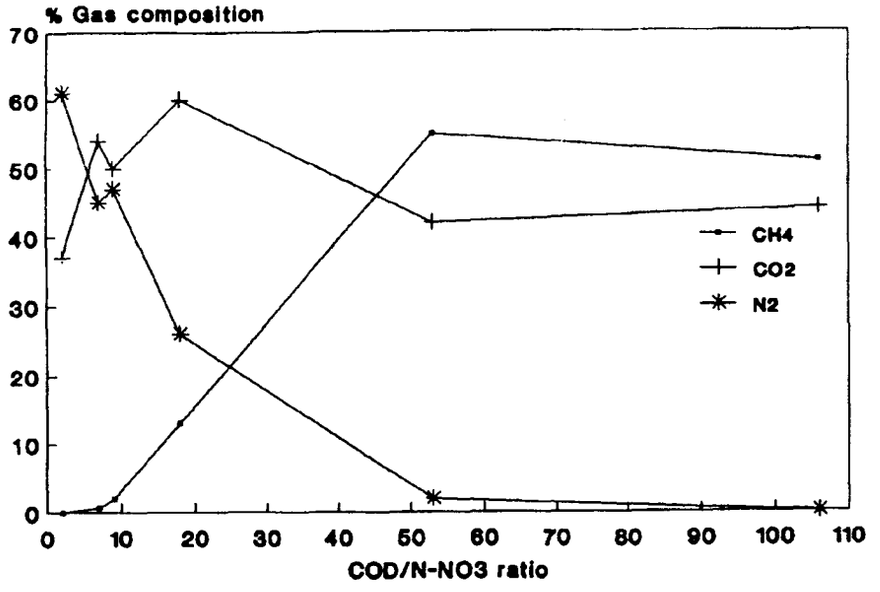

Figure 7. Gas composition as a function of COD/N-NO_3 ratio.

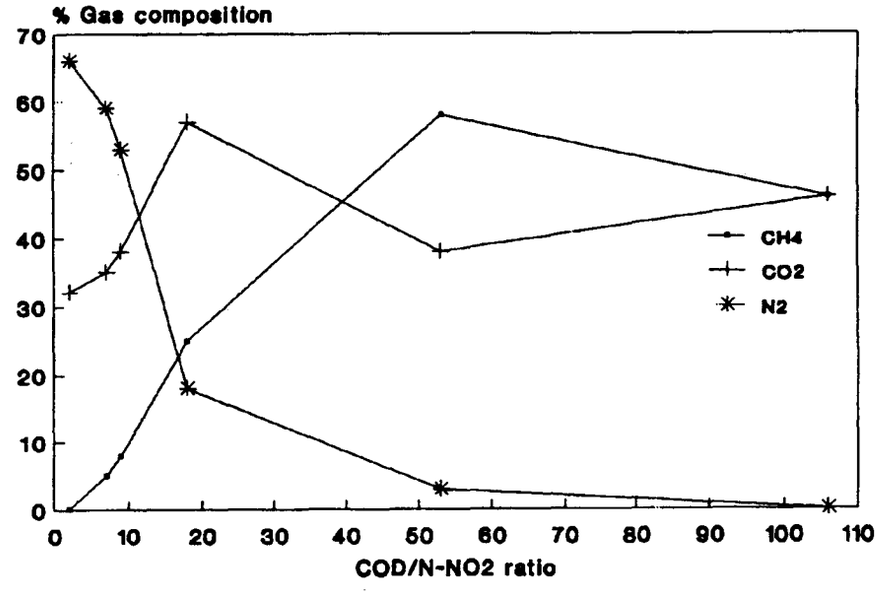

Figure 8. Gas composition as a function of COD/N-NO_2 ratio.

production was possible in both reactors at an influent concentration of 800 mg N-NOx l^{-1} suggests that nitrite did not cause greater inhibition to methanogenesis than nitrate up to this concentration. It means then that the system can withstand high nitrite accumulation which frequently occurs in the traditional denitrification process from nitrates. The system can also be used for nitrite elimination in cases where selective inhibition is used to enhance nitrite build-up, a method that can bring about significant cost savings in aeration requirements in the treatment of highly nitrogenous wastes (10).

COD Removal:

The variations of effluent COD of both reactors are shown in Figures 5 and 6. The lowest effluent COD was observed at nitrate nitrogen loads from 10 to 80 mg l^{-1} d^{-1} and at nitrite

nitrogen loads of 60 and 80 mg l^{-1} which corresponded to the COD/N-NOx ratios when significant denitrification occurred (Figure 4). At these loads about 61 and 59% COD reduction were respectively achieved. These values compare favourably with the maximum COD reduction observed in the blank reactor which varied from 60 to 70% (Figure 3). The lower COD eliminated via methanogenic pathways in the nitrate-fed and nitrite-fed reactors was compensated by a higher COD removal by the denitrification process thereby maintaining the overall COD removal efficiency similar to that of the blank reactor. Thus, a system of combined denitrification/ methanogenesis processes is likely to achieve a greater COD removal efficiency than a single system of anaerobic digestion or denitrification if the system is such that biomass loss is minimal and the existence in zones of high concentrations of each of the bacterial populations involved is possible. MLVSS varied slightly with average values of 2.9 and 2.5 g l^{-1} in the nitrate-fed and nitrite-fed reactors respectively, more than 30% greater than the MLVSS in the blank reactor.

Effluent VFA values were high for both reactors (about 2.7 and 3.0 g l^{-1} in nitrate-fed and nitrite-fed reactors respectively) at N-NOx load of 5 mg l^{-1} d^{-1}, thus producing high effluent COD as shown in Figures 5 and 6. But at a N-NOx load of 10 mg l^{-1} d^{-1} these values decreased respectively to about 1 and 1.8 g l^{-1}. With higher N-NOx loads they increased gradually and reached maximum values of about 2.2 and 2.4 g l^{-1} respectively at N-NOx of 60 mg l^{-1} d^{-1}. This later increase in effluent VFA values was attributed to low methane producing performances of both reactors at higher N-NOx loads.

At nitrate and nitrite loads of 250 mg N-NOx l^{-1} d^{-1}, methane production and ammonification stopped while denitrification decreased considerably with high effluent N-NOx (Figures 4, 5, 6, 7 and 8),thus indicating a global inhibition induced by the high influent nitrate and nitrite concentrations in both reactors. This is thus the reason for the high effluent COD recorded in both reactors. Furthermore, the effluent VFA values were low, about 0.4 and 0.2 g l^{-1} respectively in

Table 2. Evolution of nitrate/nitrite-reducers in all the reactors.

Sludge		A (%) oxydase (-) Red on endo agar	B (%) oxydase (-) White on endo agar	C (CFU*) oxydase (+)	Total CFU* (anaerobic nitrate reducers)
Start-up sludge		90	<10	3.10^3	4.10^6
	Time				
Blank reactor	50 days	89	<11	4.10^3	12.10^6
	100 days	62	<38	5.10^2	8.10^6
	$COD/N\text{-}NO_3$				
	106	90	10	*	5.10^6
	53	96	4	*	14.10^6
nitrate-fed reactor	17.73	96	<4	2.10^3	72.10^6
	8.86	94	6	12.10^4	201.10^6
	6.65	94	*	3.10^6	550.10^6
	2.13	11	0	$1.8.10^4$	2.10^4
	$COD/N\text{-}NO_2$				
	106	40	60	*	5.10^6
	53	23	70	*	7.10^6
nitrite reactor	17.73	66	34	4.10^3	6.10^6
	8.86	80	20	10.10^3	150.10^6
	6.65	90	*	1.10^5	239.10^6
	2.13	*	*	*	*

CFU : colony forming unit per ml

*: populations not detected in the diluted samples examined

Table 3. List of principal species isolated in groups A, B and C (table 2) in all the reactors.

Sludge	A	B	C
start-up sludge	*Klebsiella oxytoca*	*Providentia sp*	*Pseudomonas fluorescens*
blank reactor	*Enterobacter cloacae, Echerichia coli, Klebsiella oxytoca*	*Chryseomonas luteola*	*Ps. fluorescens Pseudomonas sp*
nitrate-fed reactor	*Klebsiella oxytoca*	*Citrobacter diversus*	*Ps. fluorescens Ps. mendocina Alcaligenes denitrificans*
nitrite-fed reactor	*Echerichia coli, Klebsiella oxytoca*	*Citrobacter diversus*	*Ps . fluorescens Alcaligenes denitrificans Aeromonas sp*

the nitrate-fed and nitrite-fed reactors suggesting that glucose conversion to VFA was also inhibited.

Evolution of Nitrate/Nitrite-reducers in the reactors

Table 2 gives the evolution of the bacterial population capable of reducing nitrate and nitrite in the three reactors throughout the experimental period. In Table 3 the principal species isolated and characterised with the aid of the API methods are presented. All the isolates in the C column are true denitrifiers. Others (in columns A and B), except *Chryseomonas luteola* (also a denitrifier), can also denitrify but ammonification is their major activity.

From the tables it is seen that the start-up sludge contained some nitrate reducers in which the true denitrifiers represented but a minute proportion, with *Pseudomonas fluorescens* as the dominant species. The blank reactor maintained similar population characteristics as the start-up inoculum throughout the experimental period. In the nitrate-fed and nitrite-fed reactors the true denitrifiers were not detectable at COD/N-NOx ratios of 106 and 53 probably because of their relatively lower quantities with respect to the ammonia-formers. But as the ratio was reduced their presence became evident with a maximum colony forming units (CFU) per mL of 3.10^6 and 1.10^5 in the nitrate-fed and nitrite-fed reactors respectively at a ratio of 6.65. *Pseudomonas fluorescens* was also the most common species. The *Enterobacter* was the most dominating group even when significant denitrification was taking place. At a ratio of 2.13 the nitrate/nitrite-reducers in the nitrite-fed reactor were not detectable in the examined diluted sample confirming our earlier suspicion of strong inhibition. The inhibition was not as strong in the nitrate-fed reactor where some nitrate/nitrite-reducers were found (about 2.10^4 $CFU(mL)^{-1}$). True denitrifiers dominated by *Alcaligenes denitrificans* represented about 89% of the population.

Facultative anaerobes like *Escherichia coli*, *Citrobacter sp.* and *Klebsiella sp.* abundant in soils and wastewaters and which represented more than 90% of the nitrate/nitrite-reducers in these reactors at high COD/N-NOx ratios have been found to be the major agents of ammonification. These bacteria (also known as non-respiratory denitrifiers) could also produce N_2O from nitrate or nitrite but ammonification is their major activity. Their successful competition over the true denitrifiers could probably be because of the initial low N-NOx loading.

The choice of glucose as a carbon source, a substrate found inefficient for denitrification by some workers (13,14) might have contributed to the abundance of the ammonia-formers.

Another possible cause of high ammonification especially in the nitrite-fed reactor could be the presence of sulphate reducing bacteria (not studied in this work) which might have been present in significant quantities in the original seed sludge. It is believed that one of the enzymes involved in the nitrite reduction to ammonia is sulphide reductase (3). Since the wastewater used in this work contained very low sulphate concentration (5 mg l^{-1} of $MgSO_4.7H_2O$), nitrite reduction to ammonia under this sulphate-limiting condition may be enhanced.

SUMMARY AND CONCLUSION

The results of this laboratory scale study has led to the following conclusions:

1. Anaerobic digesters treating liquid wastes have denitrification potentials. Varying influent nitrate or nitrite concentrations with respect to the influent COD brings about any of the following three processes: methane production only, methane production/denitrification, and denitrification only. With glucose as the only carbon source, these three reactions can be respectively carried out in the following ratios: COD/N-NOx > 53; 8.86 ≤ COD/N-NOx ≤ 53; and COD/N-NOx < 8.86. Denitrification percentage decreases as the ratio decreases from 8.86 if nitrate is the principal source of nitrogen and from 6.65 if nitrite is used. In the zone of combined methane production/denitrification processes, the produced gas from a nitrite-fed reactor is richer in methane than that from a nitrate-fed reactor at the same ratio.
2. In the zone of only methane production, ammonification appears to be the main pathway of nitrate and nitrite reduction in anaerobic digesters. As the ratio decreases denitrification increases while ammonification decreases. Ammonification is higher with nitrite than with nitrate as the nitrogen source.
3. The competition between the true denitrifiers and the ammonia formers has been identified as the major factor that determines nitrogen removal efficiency in the combined methane production/denitrification system. To enhance denitrification the initial influent COD/N-NOx must not be very high.
4. The nitrite does not appear to cause relatively greater toxicity than nitrate up to the influent concentration of 800 mg N-NOx l^{-1}. Strong activity inhibition occurs with either of them at an influent concentration of 2500 mg N-NOx l^{-1}.
5. True denitrifiers dominated by *Pseudomonas fluorescens* were not detected at high COD/N-NOx ratios because of their relatively lower quantities with respect to the ammonia-formers. Their population increased as the ratio was decreased. *Alcaligenes denitrificans* showed a high tolerance to very high N-NOx concentrations.
6. The COD reduction obtained in the zone of methane production/denitrification processes in the nitrate-fed and nitrite-fed reactors compared favourably with that obtained from the blank reactor where only methane fermentation was carried out (60 to 70%). A combined system can therefore achieve a greater COD removal efficiency than either of the single systems (anaerobic digestion or denitrification) if the system is such that solid loss is low and the existence in zones of the different bacterial populations involved is possible.

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REFERENCES

1. McCarty P.L., Anaerobic waste treatment fundamentals III: toxic materials and their control. *Public Works*, 91-94 (1964).
2. Demeyer A., Jacob F., Jay M., Menguy G. and Perrier J., La Conversion Bioénergétique du Rayonnement Solaire et les Biotechnologies, (ed) Technique et Documentation, Paris, 213-249 (1981).
3. Tiedge J.N., Ecology of Denitrification and Dissimilatory Nitrate Reduction to Ammonium. In: *Biology of Anaerobic Microorganisms*, Zehnder A.J.B. (ed), John Wiley, New York, 179-244 (1988).
4. Payne W.J., *Denitrification*. John Wiley, New York, (1981).
5. Ghosh S., Microbial Production of Energy, Plenary Lecture 7th International Biotechnology Symposium, New Delhi, India, (1984).
6. Hanaki K. and Polprasert C., Contribution of Methanogenesis to Denitrification with an Upflow Filter. *J. Water Poll. Control Fed.*, **61**, 1604-1611 (1989).
7. Garuti G., Dohanyos M., and Tilch A , Anaerobic-Aerobic Combined Process for the Treatment of Sewage with Nutrient Removal: The ANANOX Process, Proceedings 6th International Symposium on Anaerobic Digestion, Sao Paulo, Brazil, 371-379 (1991).

8. Acïtores M., Garcia Encina P.A. and Fdz-Polanco F., Simultaneous Organic Carbon and Nitrogen Removal in Domestic Sewage, Posters-Abstracts 6th International Symposium on Anaerobic Digestion, Sao Paulo, Brazil, 54-55 (1991).
9. APHA.-AWWA.-WPCF., *Standard Methods for the Examination of Water and Wastewater*. 16th edition, Washington D.C. (1985).
10. Turk O. and Mavinic D.S., Benefits of using Selective Inhibition to Remove Nitrogen from highly Nitrogenous Wastes. *Environmental Technology Letters*, **8**, 419-426 (1987).
11. Blaszczyk M., Effects of various sources of organic carbon and high nitrite and nitrate concentrations on the selection of denitrifying bacteria : continous cultures in packed bed reactors. *Acta Microbiologica Polonica*, **1**(1), 65-71 (1983).
12. Carley B.N. and Mavinic D.S., The effects of External Carbon Loading on Nitrification and Denitrification of a High Ammoniun Landfill Leachate. *J. Water Poll. Control Fed.*, **69**, 51-59 (1991).
13. Stouthamer A.H., Bioenergetics and yields with electron acceptors other than oxygen. In: *Handbook on Anaerobic Fermentation*, Erickson L.E. and Yee-Chak Fung D. (eds), Marcel Dekker, New York, 345-437 (1988).
14. Kuroda M., Shima H., Sakakibara Y., A study on simultaneous treatment of organic matter and nitrate with a biofilm consisting of methane fermentative bacteria and denitrifying bacteria. *Proc. Environmental and Sanitary Engineering Research*, **24**, 231-239 (1988).