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Kinetic Study of Addition of Volatile Organic Compounds to a Nitrifying Sludge

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Abstract

The effects of different concentrations of several volatile organic compounds (VOC) such as ethanol, acetate, propionate, and butyrate ranging from 0 to 2000 mg/L as well as a mixture of volatile fatty acids (MVFA) at a 4:1:1 (acetate:propionate:butyrate) ratio on the nitrification rate in batch cultures were studied. The results showed that ethanol and acetate were consumed in a mixotrophic way by the nitrifying sludge. At a concentration of 500 mg/L, the nitrification rate of inhibition was different for each compound in which propionate and butyrate were the most inhibitory. At 2000 mg/L the inhibition was 80% with ethanol and 100% with acetate, propionate, and butyrate. With similar concentrations of MVFA, the inhibition was also similar to that in acetate. The effect of the addition of pulses of MVFA at a ratio of 4:1:1 during 14 h on the performance of the continuous nitrifying process with a hydraulic retention time of 3 d was also studied. No inhibition of the nitrification process was observed with pulses of 750–3000 mg of MVFA/L in the input of the reactor. The results in batch cultures suggest that the different degrees of inhibition of the nitrification process were related to the type of organic matter added. The noninhibitory effect of the organic matter in the continuous cultures on the nitrification efficiency of the nitrifying sludge might be related to the feeding pattern.

Index Entries: Nitrification; inhibition rate; ethanol; acetate; propionate; butyrate.

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Introduction

Removal of biological nitrogen from wastewater is carried out through nitrification and denitrification processes. Nitrification is an obligate aerobic process and is carried out sequentially by ammonia and nitrite oxidizing groups of bacteria, such as *Nitrosomonas* and *Nitrobacter* nitrifying microorganisms *(1)*. It is generally assumed that the variations in the respiration rates of a nitrifying sludge are owing to changes in the number of *Nitrosomonas* and *Nitrobacter*. The nitrifying bacteria are sensitive to changes in temperature, pH, substrate, and product concentrations affecting the rate of the process *(2,3)*. Anthonisen et al. *(4)* demonstrated that nitrite inhibited ammonia oxidation and ammonia, in turn, inhibited nitrite-oxidizing microorganisms. The effects of ammonia and nitrite on the nitrification process are related to its ionic forms, which depend on the environmental pH *(5,6)*. Inhibition of the nitrification at acid pH is well known.

Changes in the chemical composition of the influents may also modify the nitrification process. It necessary to keep the concentration of organic carbon at low levels in order to maintain the rate and efficiency of the nitrification because the nitrifying microorganisms of the sludge are chemolithoautotrophs. There is sufficient evidence suggesting that the ammonia-oxidizing flora might be a more obligate lithotroph than nitrite-oxidizing flora *(7,8)*. There are also many reports indicating that the effects of organic compounds on the growth of nitrifying microorganisms may vary from inhibition to stimulation *(9–11)*. Steinmüller and Böck *(12)* found that the organic matter was better assimilated by *Nitrobacter* if mixed culture filtrates were used as a source of growth factor. However, this would lead to a decrease in nitrite oxidation ability in the presence of a relatively high concentration of organic matter. It has been reported that there was a 40% inhibition in nitrite oxidation in the presence of 1.2 g/L of acetate with no significant effects on ammonia oxidation *(13)*. *Nitrosomonas*, the ammonia oxidaser, could grow in 36 g/L of acetate *(14)*, but other reports indicate that ammonia oxidation was partially inhibited at 10 g/L *(15)* or 6 g/L *(16)*. It is therefore reasonable to assume that at relatively high concentrations of organic matter, the process of nitrification for the wastewater treatment would be normally slow and that nitrite oxidation may be the limiting step for this slowness of the nitrite oxidation.

At present, discrepancies still exist about the effects of organic matter on the nitrifying microorganisms. The inconsistencies in the results reported so far in the literature might not be owing exclusively to environmental factors, but also to the specific physiological attributes and types of microorganisms in the sludge. It is assumed that the nitrifying sludge consists mainly of *Nitrosomonas* and *Nitrobacter*. However, other nitrifying microorganisms could also be present that may confer specific features to the sludge *(17)*.

In recent years, a number of anaerobic wastewater treatment plants have been installed for removing carbon, but the effluents may have a high

content of ammonia and residual volatile fatty acids (VFA) and ethanol. Therefore, the effluents will require an adjustment of the carbon content in order to maintain the nitrifying rate and efficiency.

Thus, the object of the present study was to investigate the specific effect of intermediates from the anaerobic digestion such as VFA or ethanol, herein referred to as volatile organic compounds (VOC), at different concentrations on the kinetics of nitrification in stabilized nitrifying sludges in both batch and continuous cultures.

Materials and Methods

Batch Cultures

The effects of VOC on nitrification were studied in 250-mL aerated flasks each of which had a total working volume of 110 mL. Each flask was aerated at a constant airflow reaching a saturation of 65 ± 5% with oxygen. The air was passed through a flask containing distilled water to prevent the loss of water from the culture. The initial pH was adjusted to 7.7. The culture was placed on a shaker–incubator working at 200 rpm at 26°C.

Microbial growth was estimated by measuring protein in 1 mL of a well-homogenized sample that was digested at 90°C for 15 min with 0.1 mL of 10 *N* NaOH and then used for protein determination *(18)*. Ethanol and VFA were measured by the chromatographic method described by Soto et al. *(19)*. Nitrite and nitrate were measured in a microcapillary electrophoresis apparatus (Waters 4000). Nitrite and nitrate were quantified using a standard solution of 100 mg/L of each *(20)*. NH_4^+-N consumption was measured at the beginning and end of each of the batch experiments by a specific ammonia electrode *(21)*. The millivolt values measured in the sample were expressed as ammonia concentrations from a standard curve.

The chemical composition of the basal medium was 0.59 g/L of $(NH_4)_2SO_4$, 0.47 g/L of NH_4Cl, 0.1 g/L of NaCl, 0.15 g/L of $FeSO_4 \cdot H_2O$, 0.7 g/L of K_2HPO_4, 0.3 g/L of $MgSO_4$, 0.1 g/L of $CaCl_2 \cdot 2H_2O$, 0.15 g/L of $FeSO_4$, and 5 g/L of $NaHCO_3$ as carbon source and buffer. The total initial concentration of NH_4^+-N was 0.25 g/L. Ethanol, acetate, propionate, and butyrate were added individually to the inoculated basal medium at different concentrations (0–2000 mg/L). The effects of a mixture of volatile fatty acids (MVFA) at different concentrations (0–750 mg/L; acetate, propionate, and butyrate [4:1:1]) on the nitrification were also studied. The stabilized nitrifying sludge (inoculum) was taken from a feed-free VOC-aerated reactor operating under steady-state nitrification for 6 mo that was at the following operational conditions: temperature of 26°C; pH 7.7; stirring rate of 290 rpm; 70% air saturation; influent concentration of 0.5 g of NH_4^+-N/L; 4 d hydraulic retention time (HRT); microbial protein content of 0.38 ± 0.03 g/L; nitrate concentration of 1.8 ± 0.1 g/L; and a 95% efficiency of NH_4^+-N nitrification. Samples of stabilized nitrifying sludge were taken and inoculated to each flask culture. The size of the inoculum in all batch cultures represented 20% of the total working volume (110 mL).

The specific rate of microbial growth (μ) was calculated by the following equation:

$$\mu = (1/\Delta t)\ln(x/x_0)$$

in which μ (h^{-1}) is the specific rate for microbial growth, Δt is the interval of time (h), x_0 is the initial protein concentration (g/L), and x is the microbial protein concentration (g/L) at any given time. Nitrification was calculated as follows:

$$q = \Delta NO_3^-/X \cdot \Delta t$$

in which q (g of NO_3^-/[g of protein·h]) is the specific nitrification rate, and ΔNO_3^- is the change in concentration at the interval of time Δt. X represents the microbial protein value (g/L) estimated as $x - x_0/\ln(x/x_0)$ in the Δt interval. The procedure for calculating μ and q values always used the same criterion.

Continuous Cultures

The continuous culture was done in an instrumented 1.2-L fermentor with a working volume of 1 L. An external settler (0.2-L working volume) was also incorporated to recycle the biomass at the rate of 9 L/d (Fig. 1). The settled flocs at the bottom were pumped into the fermentor using a peristaltic pump connected to a timer that controlled the cycle of pause and operation (15 and 5 min, respectively).

Analytical methods employed were similar to those used in batch cultures. Nitrite, nitrate, and ammonia contained in the effluents of the reactor were measured every day. Protein in the nitrifying sludge was measured by withdrawing samples directly from the fermentor. Microbial protein content in the fermentor was maintained at 200 ± 10 mg/L by periodic biomass purges (twice per week, 20 mL after homogenization of the culture system).

A main medium (MM) containing 5.9 g/L of $(NH_4)_4SO_4$, 4.8 g/L of NH_4Cl, 7 g/L of NaH_2PO_4, 3 g/L of $MgSO_4$, and 5 g/L of NaCl was employed. A 1:5 dilution of MM was prepared to hold an inlet NH_4^+-N concentration of 500 mg/L. $FeSO_4$ at 0.075 g/L was added to diluted MM and kept in the dark before being pumped into the fermentor. A secondary medium (SM) consisting of 40 g/L of $NaHCO_3$ and 0.4 g/L of $CaCl_2 \cdot 3.5H_2O$ was also pumped into the fermentor. MM and SM were fed separately to decrease the precipitation of salts, and no evident nutrient limitation was noticed. The MM and SM solutions were fed into the reactor in such a way that the C:N ratio was maintained at 1.8 ± 0.1.

The fermentor was inoculated with 20% (v/v) of stabilized nitrifying sludge from a reactor in steady state. NH_4^+-N consumption, microbial growth, and nitrite and nitrate produced were measured as indicated earlier. Once ammonia in the batch culture was exhausted, continuous addition of MM and SM was initiated. The flow rate was increased until an HRT of 3.3 d was reached through the addition of 0.26 L/d of MM and 0.040 L/d of SM.

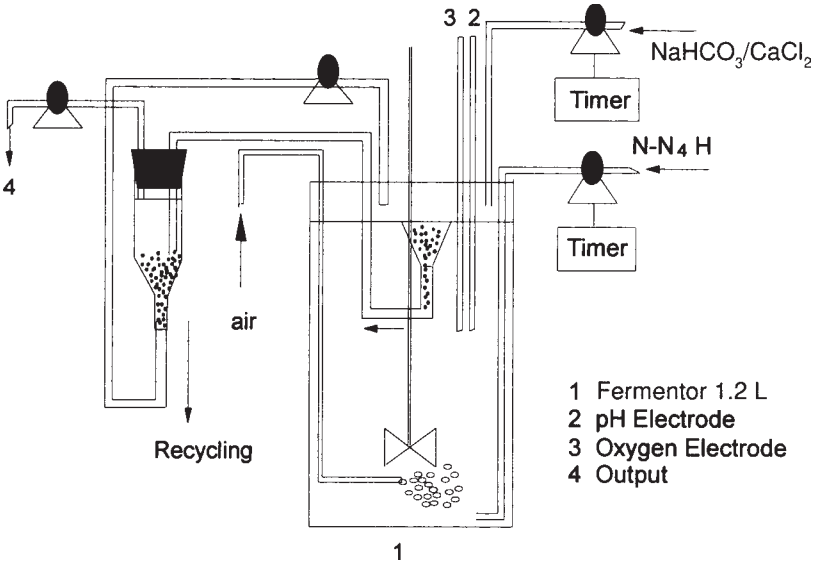

Fig. 1. Diagram of fermentor adapted for continuous culture with biomass recycling. Each peristaltic pump was connected to the timer.

The same conditions were maintained for 2 mo before starting the experimental assays. The C:N ratio of 1.8 ± 0.1 was maintained except when VOC was added. Temperature, pH, stirring rate, and oxygen were maintained at 26°C, 7.7 ± 0.4, 300 rpm, and 70%, respectively.

To evaluate the effect of VFA on a steady-state nitrifying reactor, a mixture of acetate, propionate, and butyrate at a 4:1:1 ratio was added to the MM and different organic loading rates (OLRs) were applied. Before testing each OLR, the nitrifying reactor was fed with lithotrophic medium for at least four times the HRT.

Results and Discussion

Batch Cultures

During the initial hours of culturing, the pH increased to 8.7 in all batch cultures. In a preliminary work with nitrifying continuous cultures, changes in pH (from 7.0 to 8.5) were made, but no significant changes in the nitrification rate and efficiency were observed. However, to decrease ammonia stripping, concentrated HCl was added to the culture flask to adjust the pH to 7.7. VOC and ammonia were measured in all the cultures at the beginning and end (80 h) of culture. At the end of each experiment, neither ethanol nor VFA were detected, and no more than 5% of the initial concentration of NH_4^+-N was detected.

Figure 2 shows the effect of different ethanol concentrations on the microbial growth of the nitrifying sludge. It was observed that increasing ethanol concentrations resulted in increased microbial protein, so that at

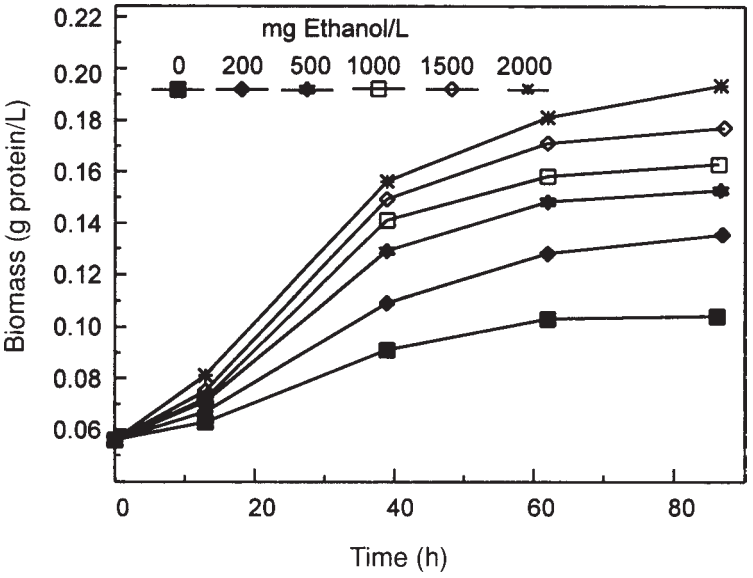

Fig. 2. Microbial growth of the sludge at different concentrations of ethanol.

2000 mg/L the nitrifying biomass concentration was doubled compared to control, in which no VOC was added.

Figure 3 shows nitrification profiles at different concentrations of ethanol. Nitrite was not detected in the cultures at any ethanol concentration. It can also be observed that the nitrification ability of the sludge decreased as ethanol concentration increased. In general, in the presence of ethanol, nitrate was always the main product of the mixotrophic microbial respiration, and, consequently, biomass formation was limited. It has been previously reported that this pattern was normal when organic compounds are present in the nitrifying culture *(5,12)*.

The NH_4^+-N consumption of the nitrifying sludge in the presence of acetate was mixotrophically assimilated since nitrate was produced in concentrations lower than 1500 mg/L. At higher concentrations of acetate, nitrate formation was no longer detected. The nitrification was completely inhibited (Table 1), and the initial nitrate (from inoculum), NH_4^+-N, and acetate were possibly consumed for microbial protein formation through the heterotrophic pathway by the heterotrophic microorganisms present in the nitrifying sludge.

The microbial growth and nitrate formation profiles at different concentrations of propionate are shown in Figs. 4 and 5, respectively. It can be observed (Fig. 4) that at concentrations close to 500 mg/L of propionate, NH_4^+-N was depleted mixotrophically as nitrate was still being produced. Profiles of nitrification and microbial growth at the same concentrations of butyrate were similar to propionate (data not shown). In both types of VOC, at concentrations higher than 500 mg/L, nitrate production was no longer detected. On the contrary, at this concentration of VOC the initial

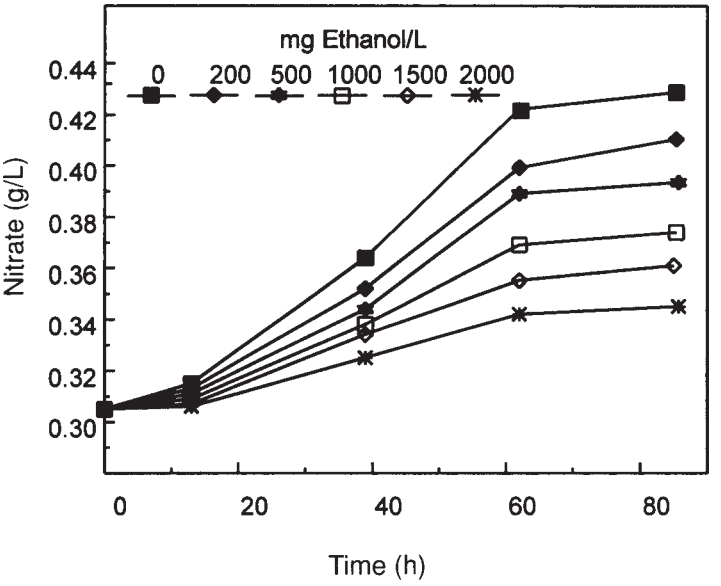

Fig. 3. Nitrate production with a nitrifying sludge at different concentrations of ethanol.

Table 1
Inhibition Rate (%) at Different Concentrations of VOC on Nitrification

Concentration (mg/L)	Inhibition rate (%)			
	Ethanol	Acetate	Propionate	Butyrate
0	0	0	0	0
200	30	32	38	40
500	48	53	66	70
1000	64	70	100	100
1500	75	96	100	100
2000	82	100	100	100

nitrate and the NH_4^+-N (Fig. 5) were possibly used up for microbial protein formation, indicative of a change in the respiratory pattern of the sludge from lithotrophic to heterotrophic. The VOC was mainly depleted as the carbon and energy source and the NH_4^+-N as the nitrogen source.

When MVFA at different concentrations (0–750 mg/L at a ratio of 4:1:1) were assayed, profiles similar to that of acetate were observed. At an MVFA concentration of 750 mg/L, nitrate formation decreased to 40%, as compared to control. Regardless of this reduction, nitrate was always present at a lesser level, indicating that the respiration profile in this range of concentrations of VFA was mixotrophic.

Böck et al. *(22)* observed that the growth rate of the isolated nitrifying microorganisms was slower when the culture conditions changed from

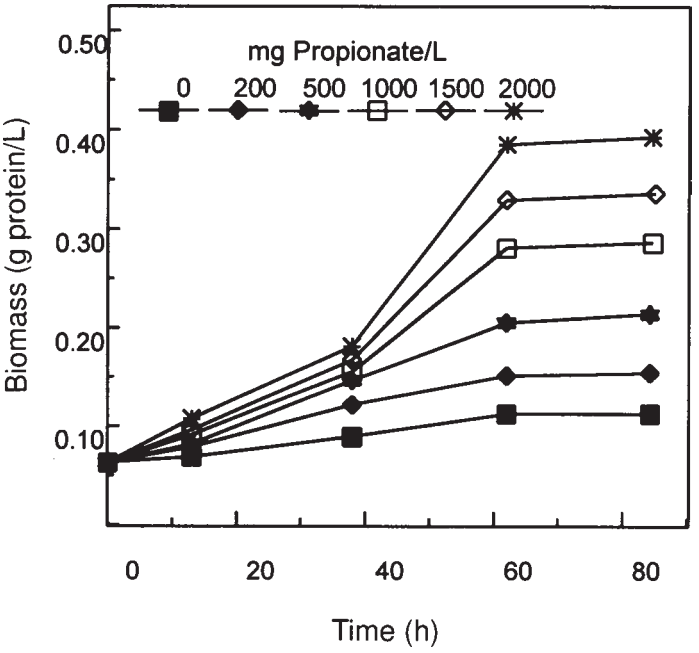

Fig. 4. Microbial growth of nitrifying sludge at different concentrations of propionate.

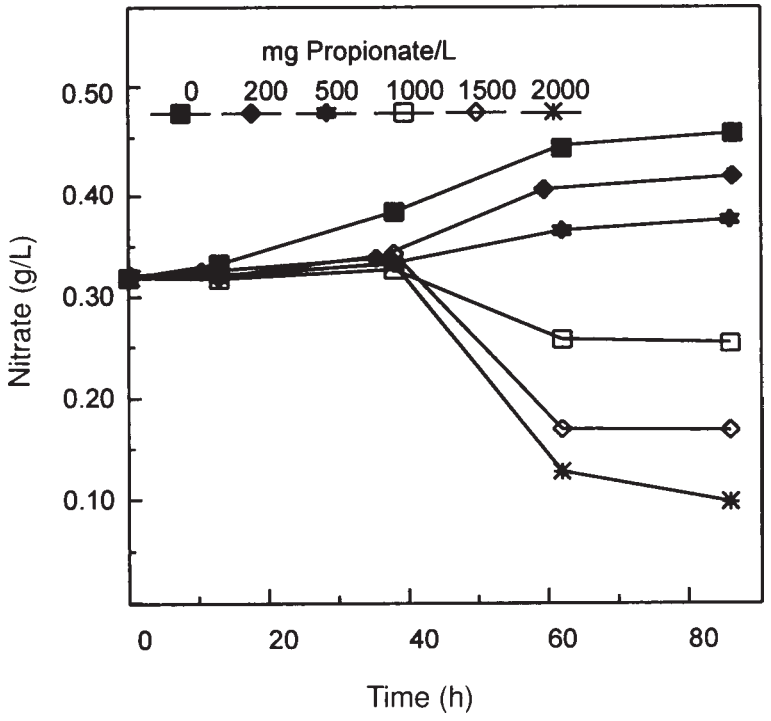

Fig. 5. Nitrate formation of nitrifying sludge in the presence of different concentrations of propionate.

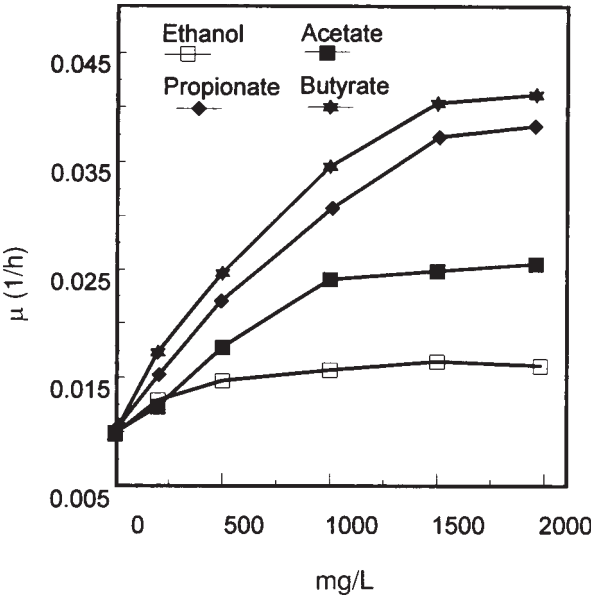

Fig. 6. Specific microbial growth rate at different concentrations of VOC: ethanol, acetate, propionate, and butyrate.

lithoautotrophic to heterotrophic, diminishing the nitrate formation. The rate of microbial growth of the sludge studied here was faster with VOC than in control, but the nitrification rate was slower because the NH_4^+-N was assimilated in a heterotrophic manner. The specific microbial growth (μ, h^{-1}) and nitrification (q, g of NO_3^-/[g of protein·h]) rates at different concentrations of ethanol, acetate, propionate, and butyrate are shown in Figs. 6 and 7, respectively. It was observed that the microbial growth rate was faster with propionate and butyrate than with either ethanol or acetate in the same range of concentrations, but the ammonia nitrification rate was lower with propionate and butyrate. Table 1 shows that the nitrification rate was completely inhibited in the presence of 1000 mg/L of either propionate or butyrate. These results indicate that the nitrifying sludge was more sensitive to the presence of VOC, contrary to previous reports *(13,15,16)*.

It is well known that for microbial protein synthesis, a chemical reducer power is needed for nitrate or ammonia uptake *(23)*. Therefore, the increase in microbial growth and in the specific microbial rate, as a function of VOC, might be understood in terms of its reducer power or oxidation number of carbon (0, –0.66, and –1 for acetate, propionate, and butyrate, respectively). This criterion suggests that ethanol as VOC (with –2 as oxidation number) the values for microbial growth and specific microbial growth rate should have been higher. On the contrary higher values for microbial growth and specific microbial growth rate were obtained with propionate and butyrate as explained earlier. Thus, it is not easy to explain the differences in the physiological behavior of the nitrifying sludge only through the intrinsic reducer power of VOC.

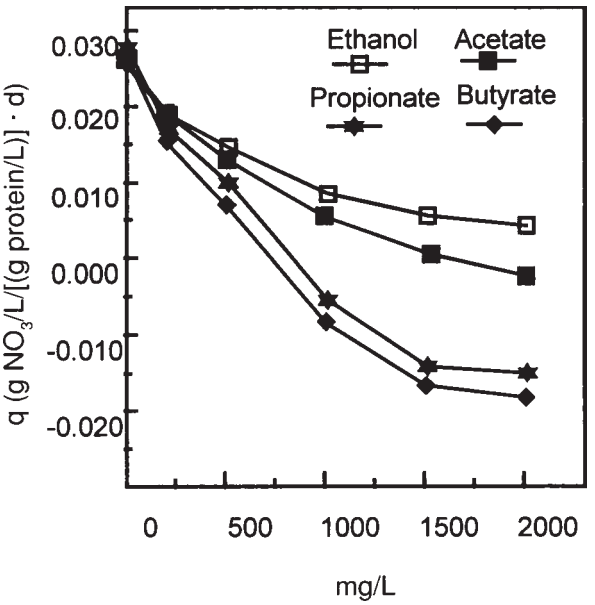

Fig. 7. Nitrification rate at different concentrations of VOC: ethanol, acetate, propionate, and butyrate.

The physiology of respiration of the microorganisms may also be influenced by the C:N ratio. In lithotrophic and anoxic respiratory processes, the range of action of the C:N ratio is rather narrow *(24,25)*, in contrast to methanogenic, fermentation, or aerobic processes, in which the C:N ratio is wide. We found that when there were increases in the VOC concentration there were also increases in the C:N ratio. The C:N ratio in the control was initially 2.86, but with the addition of VOC (from 200 to 2000 mg/L) the ratio ranged from 3.3 ± 0.06 to 6.8 ± 0.5. The levels of inhibition in the nitrification rate were determined during this interval and were found to be different for each substrate tested, particularly with ethanol as compared to propionate or butyrate (Fig. 7 and Table 1). Thus, the C:N ratio alone does not explain the physiological differences in the nitrifying sludge. Our results suggest that even at the same concentration of VOC, the microbial growth and respiration profiles could be influenced by the type of substrate in the nitrifying culture. Many investigators have reported that the effect of VOC is only related to CO_2 fixation rather than to the enzymes of ammonia or nitrite oxidation. In fact, there is evidence indicating that the ribulose biphosphate carboxylase enzyme of the Benson-Calvin involved in the CO_2 fixation could be the site of inhibition by organic carbon in nonphotosynthetic bacteria *(5,7,22,26)*. The inhibition of CO_2 fixation by the type of VOC in the nitrifying sludge might be a plausible explanation for the reduction in nitrification by microorganisms such as *Nitrosomonas* and *Nitrobacter* and the increased growth of the heterotrophic microflora as the NH_4^+-N is used heterotrophically.

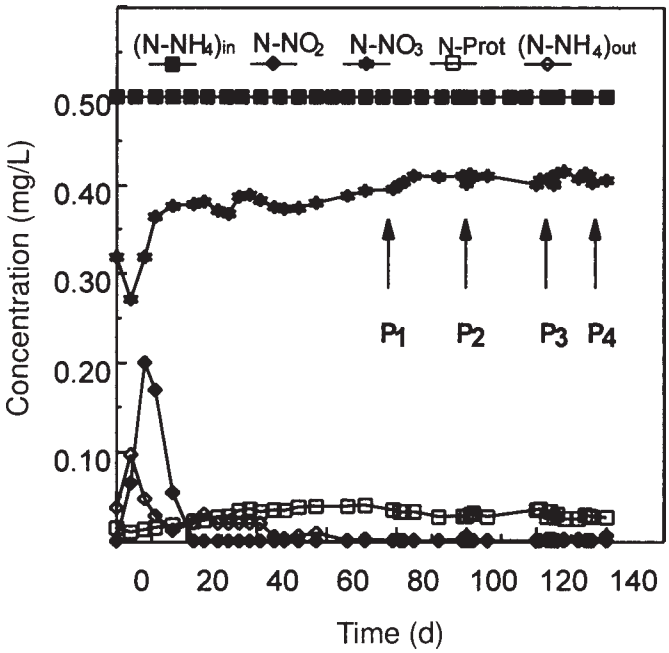

Fig. 8. Nitrification in steady-state culture. Pulses of VFA (P_1, 750 mg/L; P_2, 1500 mg/L; P_3, 2250 mg/L; P_4, 3000 mg/L) dissolved in lithotrophic medium were added, as shown by arrows.

Continuous Culture

Nitrification units are frequently connected on line with anaerobic digesters. In disturbed conditions (usually of short duration), some of the VOC from the anaerobic reactor may reach the nitrifying unit, decreasing the nitrification rate and efficiency. The VOC concentration in the effluent of the anaerobic digesters depends on many factors such as type of influent, HRT, and type of reactor, which may reach levels higher than normal. That is why an evaluation was made to study the influence of several pulses (P_1–P_4) of VOC on the performance of a continuous nitrifying reactor using a synthetic influent.

Figure 8 shows the input and output concentration of nitrogen-compounds and the general pattern of the global process operating at a constant HRT of 3 d. The arrows labeled as P_1–P_4 indicate the moment when the feeding was changed from autotrophic to heterotrophic. Each pulse was applied for 14 h. Each arrow points to a different concentration of the VOC ranging from 750 (P_1) to 3000 (P_4) mg/L of MVFA at an acetate, propionate, and butyrate ratio of 4:1:1. It can be seen that the steady-state nitrification was not disturbed because the rate of nitrification remained constant. No VFA in the output of the reactor were detected and the increase in microbial protein was negligible. The nitrification efficiencies for the four OLR were similar (Table 2). Nevertheless, increases in OLR had a remarkable effect on the adhesion of cells to the glass wall of the fermentor.

Table 2
Loading Rates and Efficiency of Nitrifying Reactor Operating at HRT of 3.3 D, at Different Concentrations of a Mixture of Acetate, Propionate, and Butyrate (ratio 4:1:1)[a]

MVFA (g/L)	Q_{VFA}	$\%E_{NO3}$
0	0.000	87
750	0.195	86
1500	0.390	87
2250	0.585	86
3000	0.780	87

[a]Q_{VFA}, loading rate of VFA (g/[L·d]); $\%E_{NO3}$, nitrification efficiency calculated as nitrate-nitrogen output/ammonium-nitrogen input.

This behavior may be associated with the change in extracellular substances in activated sludges *(27)*.

The kinetic behavior of the nitrifying sludge in the presence of MVFA was significantly different in batch than in continuous cultures. In the former, the nitrification rate of the sludge was affected by the MVFA, whereas in the latter nitrate formation by the MVFA was not at all disturbed. In batch trials the rates for microbial protein growth at higher VOC concentrations were close to five, four, and three times greater for butyrate, propionate, and acetate, respectively, than the control (Fig. 6). On the contrary, in continuous cultures with heterotrophic pulses of 14 h, the protein increase was not significant even at 3000 mg/L of MVFA in the input (OLR of 0.780 g/[L·d]). Concentrations lower than 500 mg/L of VOC in batch cultures showed similar (mixotrophic) tendencies in nitrification patterns as compared to control. Thus, inhibition of the nitrifying process was not predictable in continuous nitrification because the concentration of the VOC was not detectable in the steady state. Furthermore, the C:N ratio for nitrification was kept low in order to limit nitrogen uptake as well as to reduce the microbial growth, particularly of the heterotrophic microflora. With the nutritional limitation (or unbalance of the culture medium), the energy coefficient for the maintenance of the heterotrophic microflora of the sludge was too high. There was a significant depletion of substrate that was not owing to microbial growth, as evidenced by the high nitrification efficiency and low rate of microbial formation.

More work is need in this direction. There are reports suggesting *(28,29)* that the heterotrophic microflora associated with the nitrifying sludge decreased the negative effect of organic matter on nitrification efficiency rate by up to 20% if the OLR was equivalent to 0.28 g/(L·d). OLR values obtained in our experiments were three times higher than 0.28 g/(L·d), and still there was no evidence of inhibition of nitrification.

The differential behavior of the nitrifying sludges could be explained in terms of the type of associated heterotrophic microflora and its specific rate of low consumption relating to the C:N ratio. Our results reveal that the heterotrophic microorganisms were faster consumers of VOC than reported previously, but because the C:N ratio was low no microbial growth was possible. Work is being done in our laboratory to understand better the role of the associated microflora in the nitrifying sludge.

Conclusion

It is reasonable to assert that in batch assays at different concentrations of VOC (ethanol, acetate, propionate, and butyrate), the microbial growth rate increased when the VOC concentration also increased, causing a concomitant decrease in nitrification. The effect of each one of the components of the VOC showed different degrees of inhibition in the nitrification process, suggesting that the type of VOC played an important physiological role in the kinetics of nitrification. Ethanol and acetate had a lower inhibitory effect than propionate and butyrate. In steady-state continuous culture conditions, VOC at a loading rate of 0.780 g/(L·d) did not inhibit the nitrification. The presence of VOC was also not detected in the continuous nitrifying reactor owing to its own complete depletion. Under these conditions, the microbial protein formation rate was not significant owing to the nutritional limitations and the presence of the heterotrophic microorganisms.

Acknowledgments

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