

EFFECT OF ALKALINITY TYPE AND CONCENTRATION ON NITRIFYING BIOFILM ACTIVITY

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ABSTRACT

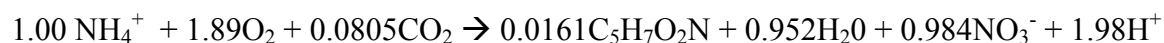
The effect of alkalinity on nitrifying biofilm activity was determined by collecting twenty-one day old biofilm samples from a full-scale nitrifying trickling filter and evaluating bench-scale nitrate plus nitrite generation rates at (1) various initial carbonate alkalinity concentrations and (2) with four types of available alkalinity; carbonate only, phosphate only, phosphate plus hydroxide, and phosphate plus carbonate alkalinity. Initial carbonate alkalinity concentrations were varied between 308 and 20 mg/L as CaCO_3 . Ammonia, nitrite, and nitrate concentrations were measured at time zero, 90 minutes, 180 minutes, and 270 minutes. Generation rates in $\text{g N/m}^2\cdot\text{day}$ were calculated for each time period and normalized against dry weight biomass. The effect of the initial carbonate alkalinity concentration on generation rate was evaluated by two-tailed ANOVA followed by LSD analysis at a 95% confidence interval. Generation rates were impaired at initial alkalinity concentrations at and below 40 mg/L as CaCO_3 and were unaffected at concentrations of 45 mg/L and above. This is consistent with Gujer and Boller's (1984) proposal and Szwerinski's *et al.* (1986) data that alkalinity is rate limiting below 1-2 millimoles/liter or 50-100 mg/L as CaCO_3 . For reactor runs with different alkalinity types, ammonia, nitrite, and nitrate concentrations were measured at time zero and at 375 minutes. Generation rates were calculated and normalized against dry weight biomass measurements. The effect of alkalinity type on generation rate was evaluated by two-tailed ANOVA followed by Tukey analysis. The type of alkalinity, carbonate versus phosphate, affected nitrification rates. If the carbonate alkalinity was below 45 mg/L, nitrification rates were impaired regardless of the total alkalinity. This effect seems to be independent of pH for the range of 6.92 to 7.99 evaluated here. This would suggest that in addition to neutralizing the acid generated by the nitrification process, a minimum level of carbonate alkalinity is necessary to meet the ammonia-oxidizer's inorganic carbon requirement for cellular synthesis and growth.

KEYWORDS

Alkalinity Limitation, Nitrification, Nitrifying Trickling Filter, Ammonia Removal, Fixed Film

INTRODUCTION

The nitrification process is carried out by two phylogenetically unrelated bacterial populations that sequentially oxidize ammonium to nitrate with the intermediate formation of nitrite. The stoichiometric equation for the complete oxidation of ammonium to nitrate, including cellular synthesis, is (USEPA 1993):



The stoichiometric coefficients show that per mole of ammonia-nitrogen removed a significant amount of oxygen is required and a substantial amount of alkalinity is destroyed by the production of hydrogen ions. Stoichiometric ratios for oxygen utilization and alkalinity destruction are 4.32 grams O₂ and 7.07 grams of alkalinity consumed per gram of ammonia-nitrogen oxidized to nitrate-nitrogen in a closed system (WEF MOP No. 8 1992, USEPA 1993). Reported ratios are between 6.0 and 7.4 mg of alkalinity consumed per mg of ammonia-nitrogen oxidized (UESPA 1993, Figueroa and Silverstein 1992, Wheeler 1998). Residual alkalinity may be needed to maintain a suitable pH range for nitrification.

Reported pH ranges for nitrification vary widely. The pH range for the growth of ammonia-oxidizing bacteria in pure culture has been reported to be between 5.8 and 8.5 (Princic *et al.* 1998). However, since nitrification rates are frequently not reported, or are qualitatively described as "good" or "poor", it is difficult to assess how well pure cultures of ammonia-oxidizing bacteria grow and/or nitrify at given pH within this range. Groeneweg *et al.* (1994) reported that the activity, i.e. the nitrification rate, of *Nitrosomonas* in pure culture was highest between pH 6.7 and 7.0 (0.4 g-N/g biomass*hour), was slightly lower between pH 7.0 and 9.0 (0.3 g-N/g biomass*hour), and was only 50% of the maximum observed rate between pH 6.3 and 6.7. Allison and Prosser (1993) saw a 39% decrease in the nitrite effluent concentration of their *Nitrosomonas* reactors when the pH was decreased from 8.0 to 7.0. Qualitative descriptors of nitrification rates in wastewater systems further complicate rate versus pH comparisons because rates of 0.185 g NH₃-N/g MLVSS*day (mixed liquor volatile suspended solids) and 0.140 g NH₃-N/g MLVSS*day would be considered "good" for activated sludge (Wild *et al.* 1971, Bratby *et al.* 1999), but would be considered "poor" for fixed film systems which are capable of achieving rates of 3.18 g-NH₃-N/m²*day (USEPA 1993, Logan 1990) and 4.00 g-NO₂+NO₃-N/gram of biofilm*day (Biesterfeld *et al.* unpublished data). Wild *et al.* (1971) observed a maximum nitrification rate of 0.185 g NH₃-N/g MLVSS*day in activated sludge between pH 7.8 and 9.0. When the pH was lower than 7.0 or higher than 9.8 for activated sludge samples, decreases in the nitrification rate by more than fifty percent of the maximum were observed (Wild *et al.* 1971, Princic *et al.* 1998). Shammas (1986) saw a reduction in nitrification rate of activated sludge when the pH was suddenly changed from 7.2 to 5.8, but not when changed from 7.2 to 6.4. In summary, ammonia-oxidizing bacteria have been reported to grow and nitrify over a wide range of pH values. However, since reported rates are often qualitative rather than quantitative, pH effects on nitrification rate cannot be predicted. Where quantitative rates are available, there is poor agreement on how much, and at what point, low pH begins to affect nitrification rates.

Low pH is thought to adversely affect nitrification rates because of ammonia speciation and/or hydrogen ion toxicity. The relative proportions of NH₄⁺-N and NH₃-N change at different pH values, with ionization increasing as pH decreases (Allison and Prosser 1993). It is thought that the actual substrate of ammonia oxidation is not the ammonium-ion, NH₄⁺-N, but free ammonia, NH₃-N (Painter 1986). Reduced activity at lower pH may therefore result indirectly from ammonia limitation since the free ammonia concentration is much lower than the total ammonia concentration (Allison and Prosser 1993, Groeneweg *et al.* 1994). The variation in reported optimum pH ranges for nitrification may therefore be due in part to variations in the total

ammonia-nitrogen concentrations used. This is because when higher total ammonia concentrations are used, higher, non-limiting, concentrations of free ammonia may be maintained at lower pH values. Hydrogen ion toxicity at pH values of 5.7 and below has also been proposed as a possible mechanism for nitrification rate inhibition (Szweringi *et al.* 1986). Rate inhibition due to ammonia speciation and/or hydrogen ion toxicity is supported by numerous researcher's reports of complete nitrification inhibition near pH 5.7 (Shammas 1986, Allison and Prosser 1993, Painter 1986).

In addition to neutralizing the acid generated by the nitrification process, carbonate alkalinity satisfies the inorganic carbon requirement for cellular synthesis and growth of nitrifying bacteria (Watson *et al.* 1989). Consequently, it has been debated whether it is pH values near and below 7.0 or low alkalinity that ultimately causes rate limitation (Gujer and Boller 1984, Szweringi *et al.* 1986). While closely related, alkalinity and pH are not the same. Alkalinity is a measure of water's ability to neutralize acidity while pH is a measure of the hydrogen ion concentration. Water with high alkalinity will always have high pH, but water with high pH does not always have high alkalinity (Pankow 1991). Although low alkalinity has been suggested as a potentially rate limiting variable for nitrification, pH is more commonly reported. It is frequently reported without complementary alkalinity data; the implication being that at high pH values (greater than 7), high alkalinity also exists. To accurately describe the buffering capacity of an aqueous system, both pH and alkalinity values are needed.

Alkalinity limitations have been suggested as one reason for decreased ammonia removal rates in the lower portions of nitrifying trickling filters (NTFs) (Gujer and Boller 1986, Gujer and Boller 1984, Parker *et al.* 1989). This is thought to be due to decreased pH levels within the interior of the biofilm due to mass transfer limitations (Gujer and Boller 1986, Szweringi *et al.* 1986). Szweringi *et al.* (1986) calculated and experimentally verified a pH differential of up to one standard unit between the bulk aqueous phase and the back of a 400 μm thick nitrifying biofilm when the carbonate to dissolved oxygen mole ratio was less than or equal to three. Zhang and Bishop (1996) observed pH drops of up to 1.4 standard units in their 278 μm thick nitrifying biofilm under the same conditions. There is no consensus on exactly where alkalinity becomes rate limiting, however; literature values would indicate that it occurs somewhere below 100 mg/L as CaCO_3 (Gujer and Boller 1984, Szweringi *et al.* 1986, Seigrist and Gujer 1987). Few studies have been performed to determine the point at which alkalinity becomes rate limiting. This is due in part to the difficulty in separating pH effects from alkalinity effects and to the complexity of domestic wastewaters, which may contain alkalinity fractions in addition to carbonate. A better understanding of alkalinity and pH effects is needed. The effect of alkalinity on nitrifying biofilm activity was determined by collecting biofilm samples from a full-scale nitrifying trickling filter and evaluating bench-scale nitrate plus nitrite generation rates at (1) various initial carbonate alkalinity concentrations and (2) with four types of available alkalinity; carbonate only, phosphate only, phosphate plus hydroxide, and phosphate plus carbonate alkalinity. Different types of alkalinity were evaluated to determine where/if carbonate alkalinity, total alkalinity, and/or pH affected the nitrification rate.

METHODOLOGY

Sampling Location

The Littleton/Englewood Wastewater Treatment Plant, Englewood, Colorado, has three NTFs that are operated in parallel. These covered filters were designed with 7.3 meters of media depth, are 32 meters in diameter, have an average hydraulic loading rate of 88 cubic meters per square meter per day, and cross-flow media with a specific surface area of 138 square meters per cubic meter. They are equipped with rotational, four arm distributors that complete one rotation every four minutes. To maintain a relatively constant hydraulic loading rate, flow is recirculated from the composited NTF effluents. Approximately 40,000 liters of 1,800 mg/L $\text{NH}_3\text{-N}$ centrate solution is returned nightly from the solids dewatering process to the plant headworks between 11:00 p.m. and 1:30 a.m. Returning the centrate solution during periods of low flow minimizes diurnal ammonia loading variations to the NTFs, which could result in reduced performance. Average secondary clarifier effluent values, plus or minus one standard deviation, for ammonia-nitrogen, alkalinity, and pH are 25.7 ± 5.7 mg/L, 246 ± 20 mg/L as CaCO_3 , and 7.96 ± 1.01 , respectively. Average NTF influent values, which include recycle flow, for ammonia-nitrogen and pH are 14.5 ± 3.1 mg/L and 7.59 ± 1.5 , respectively.

Biofilm Samples

Biofilm samples were collected from the top of the NTF media on standard glass microscope slides. The slides were held in place for twenty-one days with a wire basket fitted with two layers of eight microscope slide holders each (Figure 1). Each slide holder held five microscope slides for a total of eighty slides. Each slide provides 38.4 cm^2 of biofilm growth area. All slides were held perpendicular to flow to minimize disruptions of the forming biofilm and to promote uniform growth.

At the end of the twenty-one day sampling period, the entire biofilm sampler was removed from the NTF, submerged in process water, and transported to the laboratory. Approximately fifteen minutes elapsed between the removal of the sampler from the NTF and bench-scale reactor start up.

Bench-Scale Reactors

Slides were removed from the sampler in groups of five and suspended in miniature batch reactors filled with 600 mL of ammonia-oxidizer growth media (Figure 2). The base growth media contained 106.8 mg/L NH_4Cl , 584 mg/L NaCl , 49.3 mg/L MgSO_4 , 147 mg/L CaCl_2 , and 54.4 mg/L K_2HPO_4 . Initial ammonia concentrations were 27.4 ± 2.3 mg/L as N for all reactors. Final ammonia-nitrogen concentrations for all reactors were greater than 20 mg/L. Initial alkalinity concentrations were varied between 308 and 20 mg/L as CaCO_3 by adjusting the volume of sodium bicarbonate solution added to each reactor. The media was added to the reactors one day prior to biofilm sample collection where it was aerated continuously and allowed to equilibrate overnight. Standard five-gallon aquarium pumps equipped with air stones were used to aerate the reactors at a rate of 0.38 liters per minute per reactor. Dissolved oxygen concentrations were maintained at greater than 6.0 mg/L. Ammonia, nitrite, nitrate, alkalinity,

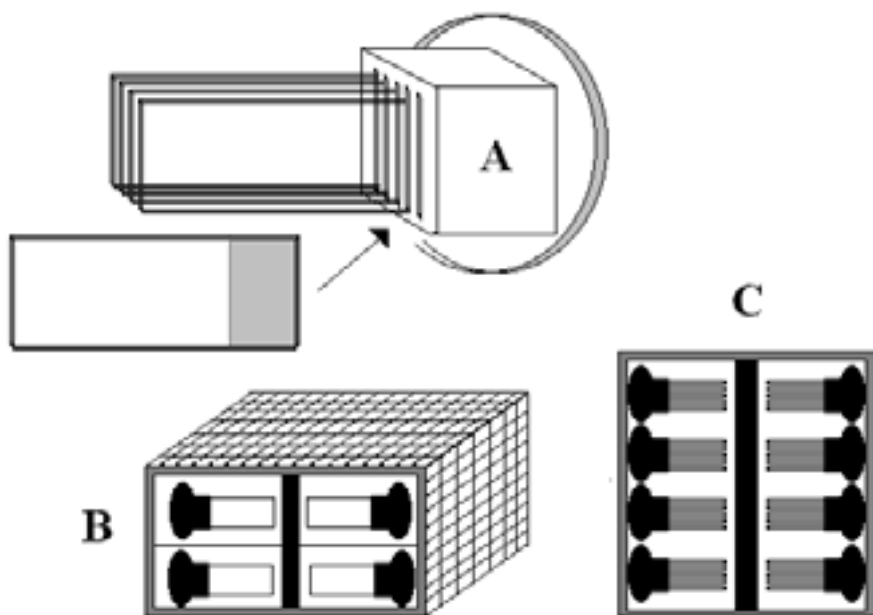


Figure 1 Biofilm Sampling Device. (A) individual slide holder, (B) side view of sampling basket, (C) aerial view of sampling basket



Figure 2 Bench-Scale Reactors.

pH, and dissolved oxygen concentrations were measured at the time of slide group placement, 90 minutes, 180 minutes, and 270 minutes. The nitrate plus nitrite generation rates in grams of $\text{N/m}^2 \cdot \text{day}$ were calculated per reactor for each time segment. This was repeated on three different dates for a total of 128 rate calculations.

Slide groups were then transferred to reactors containing 600 mL of one of four media types; only carbonate alkalinity, only phosphate alkalinity, phosphate plus hydroxide alkalinity, or phosphate plus a small amount of carbonate alkalinity. The carbonate alkalinity media was made by adding NaHCO_3 to the base media to obtain a final concentration of 217.6 mg/L. The phosphate alkalinity media contained 218.0 mg/L NaH_2PO_4 and 965.0 mg/L Na_2HPO_4 in addition to the base media ingredients. The phosphate plus hydroxide alkalinity media was made by adding 10 N Sodium hydroxide solution to reactors already containing phosphate alkalinity media to obtain a final pH of 7.37 ± 0.07 . The phosphate plus carbonate alkalinity media was made by adding 0.25 mls of a 54.4 g/L sodium bicarbonate solution to reactors already containing phosphate alkalinity media. The final carbonate alkalinity concentration was 25 mg/L. Initial ammonia concentrations were 25.1 ± 3.7 mg/L as N for all reactors. Final ammonia-nitrogen concentrations for all reactors were greater than 20 mg/L. The media were added to the reactors one day prior to biofilm sample collection where it was aerated continuously and allowed to equilibrate overnight. Standard five-gallon aquarium pumps equipped with air stones were used to aerate the reactors at a rate of 0.38 liters per minute per reactor. Dissolved oxygen concentrations were maintained at greater than 6.0 mg/L. Ammonia, nitrite, nitrate, alkalinity, pH, and dissolved oxygen concentrations were measured at the time of slide group transfer and at 375 minutes. The nitrate plus nitrite generation rates ($\text{g N/m}^2 \cdot \text{day}$) were calculated per reactor for each time period. This was repeated on three different dates for a total of 48 rate calculations.

Chemical Analyses

Thirty milliliter samples were collected from each batch reactor at the specified time intervals and were transferred to polystyrene snap-cap vials. All samples were analyzed by accepted USEPA methods and/or in accordance with *Standard Methods for the Examination of Water and Wastewater* (1995). Dissolved oxygen and pH measurements were taken at the time of sampling with calibrated meters (two-point calibration). The ammonia-nitrogen concentration of each reactor was determined by the Nesslerization method (APHA 1995) without distillation. Nitrate- and nitrite-nitrogen concentrations were determined by Hach (Loveland, Colorado) method numbers 10020, chromotropic acid method for nitrate, and 8507, diazotization method for nitrite. All samples were analyzed in replicate and the results averaged. Samples that could not be analyzed immediately were preserved with the granular nitrification inhibitor 2-Chloro-6-(Trichloromethyl)pyridine (HACH Company, Loveland, CO), stored at 4°C , and analyzed within 24 hours of collection.

Dry Weight Biomass Measurements

Accumulated biomass was quantified as dry weight biomass in milligrams per microscope slide. Prior to placing the microscope slides into the NTF, each slide was engraved with a serial number, rinsed with de-ionized water to remove residual glass dust, and allow to air dry. Each

slide was weighed on an analytical balance. The recorded weights were confirmed by re-weighing the slides in groups of five. If the added individual slide weights did not equal the collective slide weight plus or minus 0.5 mg, all of the slides in the group were re-weighed. When reactor runs were complete, the slides were removed from the reactors and dried at 103°C for at least two hours before reweighing to obtain the dry weight biomass per slide. The dry weight biomass per microscope slide was calculated for each reactor. Slides with obvious nicks and/or chips were not included in these calculations.

RESULTS

Results from the variable initial carbonate alkalinity concentration experiments and the variable alkalinity type experiments, plus or minus one standard deviation, are presented in [Table 1](#). Eleven reactor runs were eliminated from the data set because the differences between initial and final alkalinity concentrations were greater than 35 mg/L or because alkalinity had been completely depleted. One hundred and seventeen reactor runs remained. Reactor results were grouped by initial alkalinity concentration with no more than 10 mg/L as CaCO₃ difference between individual data points within a group. This resulted in 23 groups with a minimum of 2 reactor runs per group. All groups with average initial alkalinity concentrations of 150 mg/L as CaCO₃ or less contained at least four reactor runs per group. Generation rates in g N/m²*day were calculated for each time period and normalized against dry weight biomass to obtain grams of NO₂+NO₃-N generated per gram of dry weight biomass per day. The effect of the initial alkalinity concentration on generation rate was evaluated by two-tailed ANOVA followed by a least squares difference (LSD) analysis at a 95% confidence interval. Generation rates were impaired at initial alkalinity concentrations of 20, 24, 30, 35, and 40 mg/L as CaCO₃ and were unaffected at concentrations of 45 mg/L and above. This data is presented graphically in [Figure 3](#).

Results from exposing nitrifying biofilm samples to alternate alkalinity types are presented at the bottom of Table 1. The effect of alkalinity type on generation rate was evaluated by two-tailed ANOVA followed by Tukey analysis at a 95% confidence interval. Nitrate plus nitrite generation rates were significantly impaired in the absence of adequate carbonate alkalinity i.e. with phosphate and/or phosphate plus either hydroxide alkalinity or 25 mg/L of carbonate alkalinity as CaCO₃.

DISCUSSION

Reactor results for the variable alkalinity experiment were grouped to minimize the change in alkalinity concentration from beginning to end of a time segment in an effort to pinpoint the rate limiting concentration. The nitrate plus nitrite generation rates were used for all reactor runs instead of the ammonia oxidation/removal rates because of concern that the pH range of the different reactors, 6.92 - 8.62, may have resulted in differential volatilization of ammonia-nitrogen. This could have resulted in the calculation of erroneously high removal rates at higher pH values as ammonia is readily stripped from solution near pH 8.5. Ninety percent ammonia removal can be achieved at pH 9.0 with an aeration rate of 0.78 liters per minute (Aragaman *et al.* 1991). For reactor runs conducted at pH 8.46, the ammonia-nitrogen oxidation rate was 2.55 g NH₃-N/m²*day while the nitrate plus nitrite generation rate was 2.15 g NO₂+NO₃-N/m²*day..

Table 1 Reactor Data

Media Type	# Reactors	Initial Alkalinity ¹	Final Alkalinity ¹	pH	Biomass mg/slide	NO ₂ +NO ₃ Generation Rate ²	Rate per mg of Biomass ³
<div style="text-align: center;"> CaCO₃ ↓ CaCO₃ </div>	4	308 ± 3	294 ± 12	8.49 ± 0.04	2.55 ± 0.34	1.39 ± 0.87	2.09 ± 0.66
	2	270 ± 5	311 ± 0	8.62 ± 0.01	2.55 ± 0.34	2.18 ± 0.11	3.28 ± 0.08
	3	251 ± 6	238 ± 15	8.46 ± 0.25	3.92 ± 0.28	2.15 ± 0.50	2.11 ± 0.25
	4	208 ± 6	201 ± 7	8.29 ± 0.05	2.37 ± 0.19	1.65 ± 0.33	2.68 ± 0.22
	2	181 ± 2	211 ± 5	8.41 ± 0.00	2.37 ± 0.19	1.42 ± 0.27	2.30 ± 0.21
	2	168 ± 0	148 ± 5	8.13 ± 0.02	4.12 ± 0.19	2.24 ± 0.68	2.09 ± 0.31
	6	150 ± 4	140 ± 10	8.11 ± 0.16	3.96 ± 0.31	2.65 ± 0.33	2.57 ± 0.14
	6	139 ± 1	135 ± 12	8.04 ± 0.19	3.94 ± 0.36	2.81 ± 0.50	2.74 ± 0.20
	4	126 ± 5	117 ± 5	8.02 ± 0.09	3.09 ± 0.47	2.69 ± 0.53	3.35 ± 0.24
	4	119 ± 1	117 ± 6	8.06 ± 0.10	3.15 ± 0.28	2.62 ± 0.63	3.19 ± 0.27
	5	110 ± 4	99 ± 10	7.89 ± 0.04	3.46 ± 0.53	1.90 ± 0.80	2.11 ± 0.46
	6	96 ± 3	85 ± 16	7.90 ± 0.10	3.70 ± 0.43	2.50 ± 0.43	2.59 ± 0.20
	4	85 ± 3	77 ± 6	7.78 ± 0.11	3.49 ± 0.36	2.68 ± 0.28	2.95 ± 0.13
	6	72 ± 2	60 ± 10	7.80 ± 0.15	3.32 ± 0.33	2.14 ± 0.50	2.47 ± 0.26
	7	63 ± 1	51 ± 6	7.66 ± 0.14	3.74 ± 0.37	2.62 ± 0.41	2.70 ± 0.18
	6	54 ± 2	39 ± 15	7.59 ± 0.10	4.31 ± 0.61	2.72 ± 0.42	2.42 ± 0.19
	7	50 ± 1	34 ± 12	7.50 ± 0.16	3.27 ± 0.47	2.10 ± 0.47	2.46 ± 0.26
	7	45 ± 3	32 ± 11	7.46 ± 0.08	2.83 ± 0.35	1.67 ± 0.45	2.27 ± 0.30
	6	40 ± 1	27 ± 4	7.34 ± 0.12	3.43 ± 0.49	1.63 ± 0.31	1.83 ± 0.23
	5	35 ± 1	32 ± 9	7.28 ± 0.09	2.88 ± 0.71	1.38 ± 0.44	1.84 ± 0.39
	11	30 ± 2	24 ± 6	7.16 ± 0.14	3.41 ± 0.62	1.28 ± 0.45	1.44 ± 0.40
	4	24 ± 1	24 ± 4	7.05 ± 0.19	3.09 ± 0.60	1.16 ± 0.37	1.44 ± 0.37
	6	20 ± 2	18 ± 9	7.09 ± 0.13	3.27 ± 0.52	1.31 ± 0.25	1.53 ± 0.23
CaCO ₃	15	119 ± 40	ND	7.99 ± 0.22	3.28 ± 0.85	1.87 ± 0.24	2.37 ± 1.01
PO ₄ ⁼	15	170 ± 14	ND	6.92 ± 0.05	3.18 ± 0.85	1.11 ± 0.55	1.39 ± 0.75
NaOH + PO ₄ ⁼	6	126 ± 12	ND	7.37 ± 0.07	2.62 ± 0.26	0.95 ± 0.35	1.39 ± 0.68
CO ₃ +PO ₄ ⁼	12	180 ± 18	ND	6.99 ± 0.09	3.05 ± 0.64	1.41 ± 0.57	1.76 ± 0.60

¹ Alkalinity results are reported as Total Alkalinity in mg/L as CaCO₃.² Generation rates are given as g-NO₂+NO₃-N/m²*day.³ Normalized generation rates are given as g-NO₂+NO₃-N/g biomass*day.⁴ ND = Not Determined

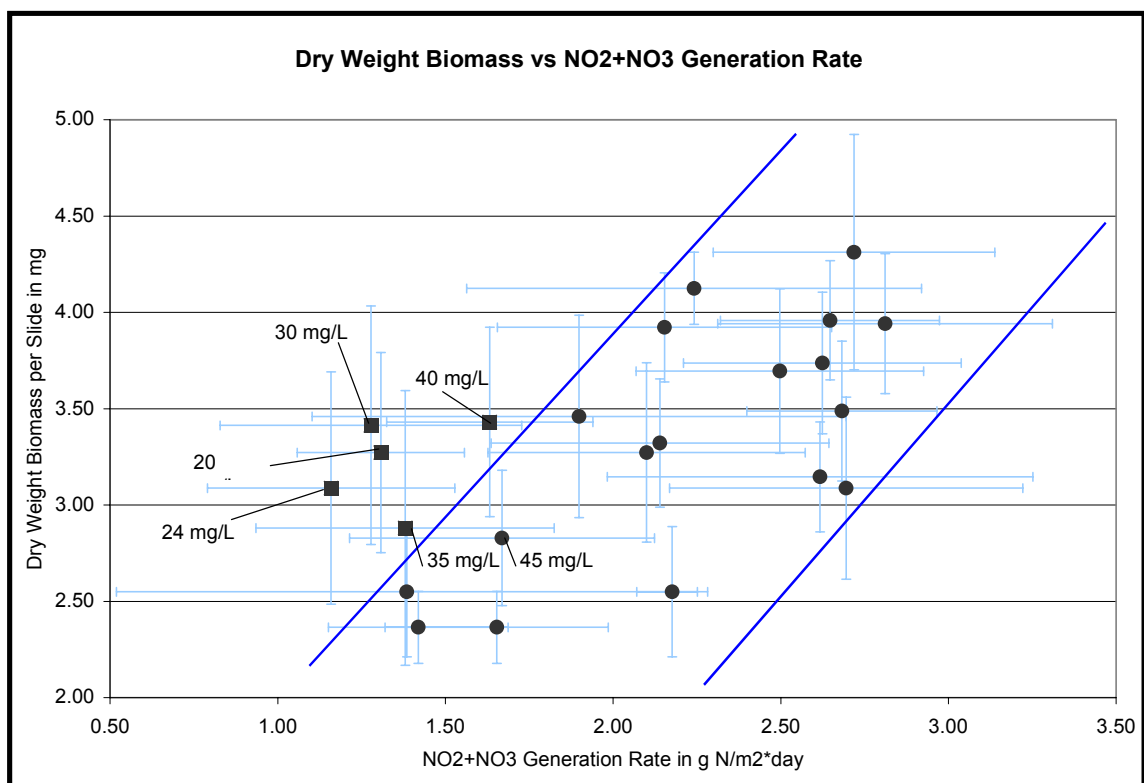


Figure 3 - Scatterplot showing effect of carbonate alkalinity concentrations in mg/L on the nitrate plus nitrite generation rate per mg of biomass. Data point labels indicate initial carbonate alkalinity concentration in mg/L as CaCO₃. Squares denote data points where the initial alkalinity concentration was rate limiting. Circles denote data points where the initial alkalinity concentration was greater than or equal to 50 mg/L as CaCO₃. The heavy lines bracket the range where alkalinity was not rate limiting.

Observed nitrate plus nitrite generation rates of 1.16 to 2.69 g N/m²*day are consistent For reactor runs conducted with pH 7.80, the ammonia-nitrogen oxidation rate was 2.19 g NH₃-N/m²*day while the nitrate plus nitrite generation rate was 2.14 g NO₂+NO₃-N/m²*day. The NO₂+NO₃ generation rates were normalized against the dry weight biomass measurements to account for rate variances due to differences in total biomass accumulation per slide with apparent zero-order nitrification rates of 0.98 to 2.77 g N/m²*day reported by Parker *et al.* (1995) and others (Gullicks and Cleasby 1986, Logan *et al.* 1990). They are also consistent with the theoretical maximum nitrification rates

predicted by Logan's model for cross flow plastic media (Logan *et al.* 1990). The rate limiting carbonate alkalinity concentrations of approximately 40 mg/L and below found here are consistent with Gujer and Boller's (1984) proposal and Szwerinski's *et al.* (1986) and Zhang and Bishop's (1996) data that alkalinity is rate limiting below 1-2 millimoles/liter or 50-100 mg/L as CaCO_3 and is severely limiting at 30 mg/L as CaCO_3 .

Szwerinski *et al.* (1986) and Zhang and Bishop (1996) proposed ratios of one reactant to another to predict when alkalinity would become rate limiting due to mass transfer. Szwerinski proposed a predictive ratio of 3.8 grams of alkalinity as CaCO_3 per gram of dissolved oxygen and/or a ratio of 12.1 grams of alkalinity as CaCO_3 per gram of ammonia-nitrogen. Zhang and Bishop (1996) suggested that when the ratio of carbonate to dissolved oxygen concentration was greater than five, nitrification rates would be unaffected, and that rate limiting conditions occurred only when the ratio was less than three. These predictions are based on theoretical mass transfer limitations of HCO_3^- and CO_2 through a thick, flat, homogenous biofilm resulting in substantial pH drops with biofilm depth (Zhang and Bishop 1996, Gujer and Boller 1986, Gullicks and Cleasby 1986, Szwerinski *et al.* 1986). However, nitrifying biofilms are relatively thin, 20-280 μm thick (Gullicks and Cleasby 1986, Gujer and Boller 1984, Seigrist and Gujer 1987, Boller *et al.* 1994, Zhang and Bishop 1996). Additionally, biofilms are known to have complex tertiary structures such as cell clusters, discrete aggregates of cells, interstitial voids, and open channels connected to the bulk liquid that further decrease the maximum thickness that alkalinity (HCO_3^- and CO_2) or other component would be diffusing through (Thorn *et al.* 1996, DeBeer *et al.* 1996). There is also evidence that ammonia-oxidizing bacteria primarily occupy the upper 20-50 μm of the biofilm, regardless of its total thickness (Schramm *et al.* 1996, Liu and Capdeville 1994). This is consistent with microelectrode measurements by Zhang and Bishop (1996) and Schramm *et al.* (1996) that show greater than ninety percent of ammonia oxidation occurring in the top 100 μm of biofilm. Zhang and Bishop (1996) saw smaller experimental pH drops across their biofilm than what was predicted by their models which assumed all 278 μm of biofilm was actively nitrifying. Consequently, mass transfer limitations of the inorganic carbon species may be much less than that assumed by diffusion based models. This is supported by the lower limiting alkalinity concentration of 40 mg/L as CaCO_3 found here as compared to the accepted theoretical limit of 50-100 mg/L as CaCO_3 .

For the second part of this research, different types of alkalinity were evaluated to determine whether or not carbonate alkalinity, total alkalinity, and/or pH were affecting the nitrification rates. Although alkalinity concentrations were comparable between the carbonate buffered and phosphate buffered medias, nitrification rates and media pH differed significantly. Biofilms submerged in complete media, which contained excess carbonate alkalinity, nitrified at a significantly higher rate than the biofilms in either the phosphate only, phosphate plus hydroxide, or the phosphate plus carbonate alkalinity

media. Moreover, the nitrification rates between the later three media types did not differ significantly from each other. This would suggest that either the 45 mg/L of alkalinity needed to avoid reductions in the ammonia oxidation rate must be carbonate alkalinity or that the decreased rates are a result of pH effects. Nitrifying bacteria require an inorganic carbon source for growth and synthesis of organic molecules (USEPA 1993). That the nitrification rate may be dependent on the inorganic carbon concentration, i.e. carbonate alkalinity, suggests that the minimum carbonate alkalinity requirement is a function of the inorganic carbon needed for cellular synthesis and does not necessarily reflect the neutralization requirement of the acid generated by the nitrification process.

For the four alkalinity types evaluated, the average final pH values were 8.14 (carbonate alkalinity), 6.92 (phosphate alkalinity), 7.37 (phosphate plus hydroxide alkalinity) and 6.94 (phosphate plus carbonate alkalinity). It is possible that pH, and not the lack of carbonate alkalinity, is the cause of the decreased $\text{NO}_2+\text{NO}_3\text{-N}$ generation rates. Zhang and Bishop (1996) saw nearly identical ammonia removal rates in their biofilms when alkalinity was 30 mg/L as CaCO_3 and below, despite a pH range of 6.56 to 7.16. This would suggest that alkalinity, and not pH, was affecting the nitrification rate. Biofilm nitrification rates in the full-scale NTF were unaffected between pH 7.0 and 7.5 (Table 2). This implies that the differences in $\text{NO}_2+\text{NO}_3\text{-N}$ generation rates between the different media types are not due to pH effects; at least not at pH 7.0 and above. The reactor rate data presented here suggests that it is the concentration of carbonate alkalinity (mg/L), and not pH, is responsible for the rate reduction.

Table 2 Removal Rates versus pH for Full-Scale NTF

Sample Date	pH	Alkalinity as CaCO_3	Ammonia Removal Rate $\text{g-NH}_3\text{-N/m}^2\text{*day}$
10/23/1996	7.06	151	2.01
01/15/1997	7.04	160	2.87
03/19/1997	7.10	164	2.58
08/20/1997	7.56	168	2.06
09/10/1997	7.45	119	2.58
12/10/1997	7.56	210	1.62

The results presented here have implications for wastewater treatment plant operation. Plants that currently add alkalinity to achieve a final residual alkalinity concentration of 100 mg/L may be able to decrease their chemical addition to achieve a final residual alkalinity concentration of only 50 mg/L, a significant cost savings. The Utoy Creek Wastewater Plant (Atlanta, Georgia) and the Blue Plains Wastewater Plant (Washington D.C.) are currently performing chemical addition for alkalinity to enhance nitrification (Parker 2001). New York City is considering the addition of chemical dosing facilities to

their existing wastewater plants (NYCDEPa 1999). Average source water alkalinity concentrations of 11.8 mg/L as CaCO_3 (Catskill watershed), 44.7 mg/L as CaCO_3 (Croton watershed) and 57.7 mg/L as CaCO_3 (groundwater sources) are not thought to be sufficient to support nitrification (NYCDEPb 1999). This plant may be able to nitrify without added alkalinity.

Knowing that the required alkalinity must be carbonate alkalinity affects the chemical choice for addition. Wastewater plants may add lime, sodium hydroxide, sodium carbonate, or sodium bicarbonate to increase pH and alkalinity (Lind and Ruehl 1998). For plants that are adding alkalinity to enhance nitrification rates, it may be preferable and more effective to add sodium carbonate or bicarbonate instead of lime or sodium hydroxide, despite their relatively higher cost (Lind and Ruehl 1998). The need for sufficient inorganic carbon also explains unusual instances where there appears to be adequate alkalinity, but maximum nitrification rates are not achieved. It is possible that while the total alkalinity is high, the available carbonate alkalinity is low enough to be rate limiting. By testing for cations and anions, such as phosphate and borate, in addition to alkalinity, it may be possible to estimate the fraction of carbonate alkalinity. This approach may be used to troubleshoot nitrification processes.

CONCLUSIONS

Nitrate plus nitrite generation rates were impaired in nitrifying biofilms at initial alkalinity concentrations at and below 40 mg/L as CaCO_3 and were unaffected at concentrations of 45 mg/L and above. The type of alkalinity, carbonate versus phosphate, does affect nitrification rates. If the carbonate alkalinity is below 45 mg/L, nitrification rates are impaired regardless of the total alkalinity. This effect appears to be independent of pH for the range of 6.92 to 7.99 evaluated here. This would suggest that in addition to neutralizing the acid generated by the nitrification process, a minimum level of carbonate alkalinity is necessary to meet the ammonia-oxidizer's inorganic carbon requirement for cellular synthesis and growth.

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