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Respirometric evaluation of side-stream treatment of reject water as a source of nitrifying bacteria for main-stream activated sludge bioreactors

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Abstract

A laboratory-scale bioreactor study was conducted to characterize differences in nitrification function in main-stream reactors due to bioaugmentation from side-stream reactors treating reject water. The objective was to evaluate how configuration of a suspended growth side-stream bioreactor impacts nitrification function in the main-stream bioreactor. A bioaugmentation effect was not observed in main-stream reactors operated at warm temperatures. Complete oxidation of ammonia to nitrate was observed in the bioaugmented and control main-stream reactors although nitrite accumulation was observed in each case. Furthermore, respirometry did not reveal superior kinetics in bioaugmented reactors operated at warm temperatures. At cold temperatures bioaugmentation may have stabilized ammonia oxidation in main-stream reactor B2 bioaugmented from a PFR side-stream. Complete ammonia oxidation was observed for most of cold period of operation in the main-stream bioreactor B2. Furthermore, respirometry revealed a higher rate of ammonia oxidation and more stable nitrite oxidation compared with the control bioreactor.

Keywords

ammonia-oxidizing bacteria; bioaugmentation; nitrite-oxidizing bacteria; reject water; respirometry

INTRODUCTION

Anaerobic digester reject water may comprise up to 20% of the total reactive nitrogen load influent to a municipal wastewater treatment plant-even greater proportions in centralized solids handling facilities-but is generally less than 1% of wastewater flow (Mossakowska *et al.* 1997). Thus treatment of digester reject waters can benefit operation by eliminating a troublesome recycle stream. Furthermore, bioaugmentation of main-stream bioreactors with nitrifying biomass generated in a side-stream treatment system can increase main-stream solids retention time (SRT) and release reactor volume for other purposes thereby also

providing a significant economic benefit to treatment plants needing to add treatment capacity or nutrient removal capability. The development in recent years of multiple side-stream treatment configurations means that the engineer has many choices when it comes to designing a side-stream treatment system. One of the most fundamental decisions engineers make is the choice between a completely stirred tank reactor (CSTR) configuration and a plug flow reactor (PFR) configuration. The potential for inhibition due to toxicity from high concentrations of ammonia nitrogen in reject water would be the classic case for a CSTR configuration. On the other hand, past research (Chudoba *et al.* 1985) has shown that a plug-flow configuration provides higher rates of nitrification and is less susceptible to substrate inhibition. The objective of this study was to evaluate how configuration of a suspended growth side-stream bioreactor impacts nitrification function in the main-stream bioreactor.

METHODS

Bench-scale reactor operation

Two sets of laboratory-scale bioreactors were operated. Set one was composed of two aerated, stirred, chemostats (A1 and B1) treating synthetic anaerobic digester reject water. A CSTR or PFR configuration was simulated by feeding hourly or in one batch each day, respectively. Set two was composed of three aerated, 6L sequencing batch reactors (A2, B2, and C2) treating a synthetic domestic wastewater and operated in a PFR configuration by feeding once per cycle. A portion of the waste biomass (60 mL) from the side-stream reactors was transferred from A1 to A2 and from B1 to B2 in a single dose each day. The experimental reactor configuration is described in Figure 1 and Table 1.

Reactor operation was divided into two periods: warm and cold. During the warm period the reactors were operated at 20°C for 8 SRT's following start-up. A transition period followed during which the main-stream reactors were moved into a temperature-controlled room and temperature was gradually reduced down to 10°C for the cold period of operation. The reactors remained at 10°C for 32 SRTs. Reactor nitrification performance was evaluated during both warm and cold periods by routine analysis of ammonia-nitrogen (NH₃-N), nitrite-nitrogen (NO₂-N), and nitrate-nitrogen (NO₃-N).

Respirometry

Biomass wasted from the main-stream reactors was recovered for use in batch respirometry. Two types of respirometric assays were conducted. Oxygen uptake assays using a commercial respirometer were conducted for the purpose of estimating kinetic coefficients. A second type of assay was conducted by measuring inorganic nitrogen concentrations for the purpose of characterizing ammonia and nitrite oxidation to improve estimation of kinetic coefficients from the oxygen uptake assays.

Oxygen uptake assays

After washing residual oxygen demand from the biomass using a phosphate buffered saline (PBS) solution biomass was diluted with PBS, combined with nutrients and substrate in 250-mL media bottles, and placed into a commercial oxygen replenishment type respirometer (Challenge Environmental Systems) set up in a room with a controlled temperature of 26°C.

A concentrated solution of ammonium chloride was injected into each assay to provide an initial substrate concentration equal to 46 mg/L nitrogenous oxygen demand (NOD). The endpoint of the assay was the point at which cumulative oxygen uptake reached the NOD of the input substrate or leveled off without increasing for an extended period. Assays were conducted daily or semi-daily for one SRT each during the warm and cool period of operation. One or more assays with biomass but not exogenous substrate (seed) were conducted with each batch of assays.

Equations representing biomass growth, substrate consumption, and oxygen uptake for a two-step nitrification model developed by others (Chandran & Smets 2000) are summarized in Table 2. Differential equations for each species can be obtained by multiplying the kinetic term in the last column of Table 2 with the appropriate stoichiometric term in columns 2 through 5. S is the reduced nitrogen concentration (mg NOD L⁻¹), f_s is the biomass yield coefficient, OU is the cumulative oxygen uptake, $\mu_{\max}$ is the maximum specific growth rate (h⁻¹), and K_s is the half-saturation coefficient. The subscript ns is used to denote ammonia oxidizing bacteria (AOB) activity and the subscript nb is used to denote nitrite oxidizing bacteria (NOB) activity.

The kinetics of NH₄⁺-N to NO₂⁻-N oxidation and NO₂⁻-N to NO₃⁻-N oxidation were estimated by fitting the numerical solutions of differential equations described in Table 2 to oxygen uptake profiles obtained in response to NH₄⁺-N pulses. The differential equations were solved simultaneously using a fourth-order Runge-Kutta method. Biokinetic parameters for each oxidation step were mathematically estimated by minimizing the sum of squared errors (SSE) between the solution of the appropriate differential equation and the experimental oxygen-uptake data using the SOLVER utility in MS Excel. Substrate and biomass concentrations were expressed in terms of oxygen demand such that each mg of ammonia nitrogen is equal to 3.5 mg NOD, each mg of nitrite nitrogen is equal to 1.1 mg NOD, and each mg biomass volatile suspended solids is equal to 1.42 mg chemical oxygen demand (COD). The values for yield coefficients were assumed as reported in the literature (Chandran & Smets 2000).

On account of correlation between $\mu_{\max}$, X , and f_s , the maximum specific NH₄⁺-N and NO₂⁻-N oxidation activities were expressed as pseudo first order rate coefficients, k as follows:

$$k = \frac{\mu_{\max}}{f_s} \times \frac{X}{K_s}$$

Nitrogen assays

500 mL of waste biomass from each of the main-stream reactors was directly placed into a 1 L open-top beaker. Each beaker was mixed mechanically using a magnetic stir bar, flushed with air supplied through an aquarium diffuser stone, and buffered with bicarbonate. Assay duration was chosen to match the length of the aeration cycle in the laboratory-scale reactors. A concentrated solution of ammonium chloride was injected into each assay to

provide an initial substrate concentration equal to 46 mg/L nitrogenous oxygen demand (NOD). A small sample was taken from each beaker at routine intervals to measure pH, $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$.

RESULTS AND DISCUSSION

Bench-scale bioreactor operation

Nitrification performance for the laboratory-scale bioreactors is shown in Figure 2. Nitrification was complete and stable in all five reactors during the entire period of main-stream reactor operation near 25°C. Periods of instability were experienced in all reactors thereafter. Two brief periods of persistent elevated effluent nitrite occurred in the CSTR side-stream reactor (A1). Complete nitrite oxidation resumed after each period. On the other hand, nitrite oxidation was very unstable in the PFR side-stream reactor (B1) about two-thirds of the final 350 days of the experiment. The instability of the PFR side-stream may have been due to inhibition from free ammonia which would have been at a concentration of around 5 mg/L as N at the beginning of each cycle. Inhibition to NOB is thought to occur at a free ammonia concentration of 0.1 to 1.0 mg/L as N (Anthonisen *et al.* 1976). Although, complete and stable nitrite oxidation did occur at the beginning of the experiment, the reaction could be prone to upset from the slightest perturbation. On the other hand, ammonia oxidation may be all that is desired if denitrification is practiced.

Nitrification became unstable in all three main-stream reactors after the operating temperature reached the minimum of 10°C on Day 128. Both ammonia oxidation and nitrite oxidation were unstable in reactor A2, which receives biomass input from the CSTR side-stream. Effluent nitrite peaked at 23.8 on Day 167; effluent ammonia peaked at 16.5 mg/L on Day 273. Complete nitrification did not resume until after Day 388, following 260 days of operation at 10°C. Although ammonia oxidation remained strong throughout the experiment, nitrite accumulation did occur in reactor B2, input from the plug flow side-stream. Effluent nitrite from reactor B2 peaked at 19.5 mg/L on Day 273. Nitrate became the dominant inorganic nitrogen species again on Day 358. A period of partial ammonia oxidation occurred in reactor C2 from Day 240 through Day 290, otherwise ammonia oxidation was strong. However, nitrite oxidation was very weak during the initial 300 days of cold-temperature operation in reactor C2 with no biomass input. Nitrate became the dominant effluent inorganic nitrogen species after Day 388.

Performance results from bench-scale reactor operation do not indicate a benefit from bioaugmentation for main-stream reactor operation at warm temperatures. At cold temperatures bioaugmentation may have stabilized ammonia oxidation during the transition from warm to cold temperatures and shortened the recovery period for nitrite oxidation in main-stream reactor B2. A similar bioaugmentation effect was not observed in main-stream reactor A2. One possible explanation is that AOB from the PFR side-stream biomass was acclimated to a high substrate gradient and, hence could have been expected to more successfully incorporate into a PFR main-stream. Conversely, AOB from the CSTR side-stream would have acclimated to a low gradient and may have been sensitive to relatively high concentrations of ammonia.

Respirometry nitrogen assays

A set of nitrogen assays was conducted on day 18 during the warm period of operation at 25°C (Figure 3). Nitrification was complete and stable in the main-stream reactors at the time the assays were conducted as indicated in Figure 2. Despite the sampling interval, characteristics of nitrification function were evident. All three assays exhibited substantial nitrite accumulation indicating that both ammonia oxidation and nitrite oxidation were rate-limiting. Nitrite accumulation appeared to be most severe for bioaugmented biomass from reactor B2 and least severe for control reactor biomass. Nitrite accumulation in all three main-stream reactors is probably attributable to a relatively higher growth rate for AOB compared to NOB at temperatures in excess of 20°C (Hellinga *et al.* 1998). Aside from that, control reactor biomass (C2) appeared to exhibit a higher initial rate of ammonia oxidation and a lower half-saturation coefficient for nitrite oxidation than bioaugmented biomass (A2 and B2). Thus, carryover of factors from the side-stream reactors may have been detrimental to main-stream nitrification.

A second set of nitrogen assays was conducted on day 223 during the cold period of operation at 10°C. The data presented in Figure 4 reflect the observations in Figure 2. Ammonia oxidation was incomplete in reactor A2 resulting in nearly equal concentrations of NH₃-N and NO₂-N in the effluent. Ammonia oxidation was strong in reactor C2 but it appears that NOB were nearly completely washed out resulting in an effluent NO₂-N concentration equal to the input NH₃-N. In general, persistence of reduced forms of nitrogen in the effluent from reactors A2 and C2 was believed to be attributable to low concentrations of AOB and NOB. On the other hand, nitrification was complete and stable in reactor B2 resulting in nearly complete oxidation of ammonia to nitrate.

Respirometry oxygen uptake assays

Typical experimental seed-corrected cumulative oxygen uptake (markers) and modeled total cumulative oxygen uptake (dashed lines) for assays conducted during the warm period of reactor operation between Day 20 and Day 27 are shown in Figure 5. Modeled oxygen uptake associated with ammonia and nitrite oxidation is also shown in Figure 5. A two-step model was utilized on account of the significant nitrite accumulation observed from each of the nitrogen assays presented in Figure 3. The initial slope of each respirogram represents the maximum oxygen uptake rate (OUR) which is directly proportional to the maximum growth rate and the initial active biomass concentration. The model indicates that this portion of each assay was dominated by ammonia oxidation for each assay. Thus it appears that AOB biomass from reactors A2 and C2 had superior growth properties. On the other hand, a more abrupt transition from the maximum rate of ammonia oxidation to completion of the reaction indicates higher substrate affinity in the biomass from reactor B2 compared with the biomass from reactors A2 and C2. Comparison of the slope for nitrite oxidation according to the model indicates that NOB exhibited the highest growth rate in reactor C2. The model indicates that nitrite oxidation did not complete in reactor A2 during the course of the assay. Variation in the tail end of each assay was attributed to heterotrophic endogenous activity and not to nitrifying bacteria.

No attempt was made to characterize nitrification kinetics during the cold period of operation because of the instability of the reaction in the bench-scale reactors experienced during that phase of the experiment.

The pseudo first order rate coefficient, k , and half-saturation coefficient, K_s , for ammonia oxidation and nitrite oxidation for the biomass from each main-stream reactor are shown in Table 3. The estimated biokinetic coefficients for ammonia oxidation for control reactor biomass were superior to the coefficients for bioaugmented biomass with a higher k and a lower K_s . As for nitrite oxidation, the estimated kinetics of nitrite oxidation were least favorable for biomass bioaugmented from a CSTR side-stream and most favorable for biomass bioaugmented from a PFR side-stream. Thus, the respirograms and the estimates of biokinetic properties made from the respirogram data for nitrification at warm temperatures confirm the findings of bench-scale reactor operation, specifically that there is no clear benefit to nitrification from bioaugmentation.

CONCLUSIONS

Ammonia oxidation and nitrite oxidation are both rate-limiting in bioreactors with a plug flow configuration and operated at temperatures of 25°C and higher.

A CSTR side-stream reactor configuration provided more stable nitrite oxidation in side-stream reactors treating reject water.

There was no apparent benefit to bioaugmentation for main-stream bioreactors operated at warm temperatures. In fact, carryover of factors from the side-stream reactors may have been detrimental to main-stream nitrification.

Bioaugmentation may have prevented washout of critical populations in the main-stream bioreactor coupled to a PFR side-stream bioreactor thereby stabilizing nitrification. The potential benefit to municipal wastewater treatment is faster recovery of nitrification following a seasonal deterioration due to reduced wastewater temperature.

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Figure 1 |.
Laboratory-scale bioreactor configuration.

Figure 2 |.
Influent and effluent nitrogen for bench-scale bioreactors: (a) A1, (b) B1, (c) A2, (d) B2, (e) C2.

Figure 3 |.
Ammonia and nitrite profiles for main-stream reactors acclimated to $T = 25^{\circ}\text{C}$: (a) A2, (b) B2, (c) C2.

Figure 4 |.
Ammonia and nitrite profiles for main-stream reactors acclimated to $T = 10^{\circ}\text{C}$: (a) A2, (b) B2, (c) C2.

Figure 5 |.
Respirograms from main-stream reactors showing modeled and experimental cumulative oxygen uptake for assays conducted during warm period of operation: (a) A2 (b) B2 (c) C2.

Table 1 |

Laboratory-scale bioreactor system characteristics

Characteristic	Side-stream reactors	Main-stream reactors
Volume (L)	1	6
Configuration	CSTR (A1) PFR (B1)	PFR
Temperature (°C)	35	Variable
Hydraulic residence time (days)	5	0.5
Solids residence time (days)	5	12

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Summary of equations for substrate consumption, oxygen consumption, and biomass synthesis for two-step nitrification model

Table 2 |

Oxidation by	S_{nh}	S_{no2}	OU_{ns}	OU_{nb}	X_{ns}	X_{nb}	Rate expression
X_{ns}	$-\frac{1 + (0.3 \times fs, ns)}{fs, ns}$	$+\frac{1}{3 \times fs, ns}$	$\frac{1 - fs, ns}{fs, ns}$		+1		$\mu_{max}^{ns} \times \frac{X_{ns} \times S_{nh}}{K_{s, ns} + S_{nh}}$
X_{nb}		$-\frac{1}{fs, nb}$		$\frac{1 - fs, nb}{fs, nb}$		+1	$\mu_{max}^{nb} \times \frac{X_{nb} \times S_{no2}}{K_{s, nb} + S_{no2}}$

Table 3 |

Kinetic parameter estimates for main-stream reactor biomass acclimated to 25°C

	Ammonia oxidation		Nitrite oxidation	
	<i>k</i> (1/hr.)	K _s (mgNOD/L)	<i>k</i> (1/hr.)	K _s (mgNOD/L)
A2	11.9	1.56	1.35	1.98
B2	5.50	2.71	7.12	0.387
C2	44.7	0.339	1.61	1.62