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Bioaugmentation for nitrification at cold temperatures

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Abstract

Bioaugmentation of nitrifying bacteria for short solids retention time (short-SRT) nitrification is an attractive alternative for wastewater treatment plants in cold climates or for those in the process of upgrading to include nitrification. One possible source of ammonia for the production of nitrifying bacteria is the liquor generated during the dewatering of anaerobically digested sludges. The objectives of this study was to determine the impact of sudden decrease in temperature on nitrification rates and to determine if nitrification could be accomplished in sequencing batch reactors (SBRs) at 10°C by seeding nitrifying bacteria acclimated to 20°C. In this research, biomass produced during warm nitrification of dewatering liquor was seeded into cold SBRs at various hydraulic retention times from 43.3 to 96 h. The average decreases in nitrification rates were 58%, 71% and 82% for biomass cooled to 10°C when the biomass was acclimated to 20°C, 25°C and 30°C, respectively. The seeded SRTs of the cold SBRs were raised above the minimum solids retention time (SRT_{min}) required for nitrification. Full ammonia nitrogen removal was achieved in cold SBRs that were operated at an apparent SRT less than SRT_{min}.

Keywords: Dewatering liquors; Bioaugmentation; Nitrification; Cold-shock; Biological nutrient removal; Hydraulic retention time; Solids retention time

1. Introduction

Treatment of sludge dewatering liquors (centrate) by nitrification in a dedicated side-stream nitrification tank is an effective means of removing up to 25% of the ammonia nitrogen (NH_3-N) entering a wastewater treatment system [1,2]. Centrate treatment results in a biomass highly concentrated with nitrifying bacteria that can be used as a seed source for bioaugmentation of main-stream bioreactors [3,4].

Numerical analysis and models have been developed to study the theoretical benefits of bioaugmentation with nitrifying bacteria. Kos [3] showed that the apparent solids retention time (SRT) (Eq. (1)) of a nitrifying wastewater treatment system could be decreased from 13–18 to 7–10 d by nitrifying NH_3 from centrate in a side-stream and then recycling the excess biomass into the main bioreactors. Rittmann [5] calculated that the apparent SRT, could be decreased from 15 to 1.5 d to achieve effluent with NH_4-N concentrations less than 1 mg/l, if at least 15 mg/l of active nitrifying biomass was added with the influent stream of a chemostat treating 33 mg NH_4-N/l d.

$$\text{Apparent SRT} = \frac{V_r X_r}{Q^w X_w + Q^e X_e}. \quad (1)$$

Recently, bioaugmentation has been shown to be an effective means of maintaining nitrification in situations of stress or in systems with an apparent SRT near or less than SRT_{min} for nitrification [6]. A few full-scale case studies have been documented where biomass grown within an existing treatment plant (either intentionally or unintentionally) acts as seed for nitrification. In one example, Neethling et al. [7] describe a full-scale wastewater treatment system operating with two trains; one with an apparent SRT of 12 d and another with an SRT of 4.6 d. Transferring biomass from the 12-d system

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Nomenclature

μ_{max}	maximum growth rate of ammonia oxidizers, /d
μ_T	growth rate of ammonia oxidizers at a given temperature, /d
μ	growth rate of ammonia oxidizers, /d
θ	hydraulic retention time, d
θ_x	seeded SRT for ammonia oxidizers, d
b_T	decay rate of ammonia oxidizers at a given temperature, /d
$-\Delta N/\Delta t$	nitrification rate, mg NH_3-N/l h
K	half saturation coefficient, mg NH_3-N/l
P	proportion of ammonia oxidizers in the seeded system, mg VSS/mg VSS
Q^w	flow rate of waste stream, l/d
Q^e	flow rate of effluent, l/d
Q^i	flow rate of influent, l/d
Q^s	flow rate of seed stream, l/d
S	substrate concentration in the effluent, mg NH_3-N/l
S^o	substrate concentration in the influent, mg NH_3-N/l
SRT	solids retention time, d
SRT_{min}	minimum SRT for nitrification, d
X_a^e	concentration of ammonia oxidizers in the effluent, mg VSS/l
X_a	concentration of ammonia oxidizers in the reactor, mg VSS/l
X_a^o	concentration of ammonia oxidizers in the influent stream, mg VSS/l
X_a^w	concentration of ammonia oxidizers in the waste stream, mg VSS/l
X_e	concentration of volatile suspended solids in the effluent stream, mg/l
X_r	concentration of volatile suspended solids in the reactor, mg/l
X_w	concentration of volatile suspended solids in the waste stream, mg/l
T	temperature in the reactor, °C
T_o	original temperature in the reactor, °C
ΔT	change in temperature, °C
V_r	volume of reactor contents, l
Y	yield of ammonia oxidizers, g VSS/g NH_3-N

to the 4.6-d system allowed nitrification to be established in the 4.6-d system where none existed before. Daigger et al. [8] found that nitrification occurred in an aerobic bioreactor tank as a result of sloughing of nitrifying biomass from an upstream trickling filter.

Limitations to bioaugmentation can occur when the biomass to be seeded is grown in one environment and then seeded into another. Changes in environmental conditions, such as, temperature, substrate and biomass composition may affect the nitrifying capabilities of the seeded biomass. The objective of this research was to determine whether a nitrifying biomass treating warm centrate could act as a seed source to induce nitrification in cold sequencing batch reactors (SBRs) operating with an apparent SRT less than SRT_{min} for nitrification. The aim was to demonstrate that, with seeding, full nitrification could be achieved in reactors otherwise operating under conditions not conducive to nitrification.

2. Materials and methods

2.1. Operation of warm nitrifying seed source reactors

Three 2.4 l SBRs were fed centrate from the dewatering of mesophilically (38°C) digested mixed primary and waste activated sludges from the North End Water Pollution Control Centre (NEWPCC) in Winnipeg, Manitoba, Canada. The SBRs were operated under continuous aeration at 20°C, 25°C, or 30°C with an SRT and hydraulic retention time (HRT) of 5 d. Feeding consisted of adding 160 ml of centrate 3 times per day, every 8 h. Wasting occurred once per day after the third cycle by removing one-fifth of the reactor volume. The pH was automatically controlled; a peristaltic pump metered in a concentrated solution of sodium bicarbonate ($NaHCO_3$) to maintain the pH at or above 7.2. The mean NH_3-N concentration of the dewatering liquor was 638 ± 41 mg/l and was always within the range of 600–700 mg/l.

Sampling of SBR effluent was conducted at least 3 times weekly. Analysis included total and volatile suspended solids (TSS and VSS), NH_3-N, chemical oxygen demand (COD), nitrite nitrogen and nitrate nitrogen (NO_x-N).

2.2. Effects of sudden decrease in temperature on nitrification rates

On four occasions waste biomass (480 ml) from the warm nitrifying reactors was cooled quickly to 10°C in an ice water bath. Stirring was provided to ensure even cooling throughout the mixture. A volume of centrate (35 ml) was added to the cooled biomass and the mixture was then aerated. Ammonia removal rates were determined by sampling directly from the reactors over a period of 6.5 h. At the same time, the warm nitrifying

Table 1
Temperature dependence of nitrifying bacteria growth rates

Reference	Equation for growth rate, μ (/d)	Temperature correction factor (/°C)
Downing and Hopwood [9]	$(0.18)e^{0.12(T-15)}$	1.127
US EPA [10]	$(0.47)e^{0.09(T-15)}$	1.103
Barnard [11]	$(0.33)1.27^{(T-15)}$	1.127
Painter and Loveless [13]	$(0.18)e^{0.0729(T-15)}$	1.0756
Biowin Default [12]	$\mu_{max}e^{0.0917(T-To)}$	1.096
Jones [14]	$\mu_{max}e^{0.0695(T-To)}$	1.072
Observed data	$\mu_{max}e^{0.0844(T-To)}$	1.088

reactors were sampled over a period of at least 2 h after feeding and the NH_3-N removal rates determined.

The decrease in nitrification rate for each temperature range was determined by Eq. (2). Table 1 shows a comparison of growth rate expressions published by other researchers and that obtained in this study. The percent decrease in nitrification rate is the same as the percent decrease in growth rate as shown by the relationship in Eq. (3). The values for X_a and Y need not be known since they are eliminated as Eq. (2) is calculated:

$$\text{Decrease in nitrification rate} = \frac{\Delta N/\Delta t_T - \Delta N/\Delta t_{10^\circ C}}{\Delta N/\Delta t_T} \times 100\% = \frac{\mu_T - \mu_{10^\circ C}}{\mu_T} \times 100\%, \quad (2)$$

$$\mu_{max} = \frac{Y(-\Delta N/\Delta t)}{X_a}. \quad (3)$$

2.3. Seeding nitrifying biomass into non-nitrifying SBRs

Four SBRs (2 l each) were fed synthetic wastewater (Table 2) and operated at 10°C with HRTs of 43.6, 53.3, 68.6 and 96 h. The initial biomass for the start-up of these reactors was from a non-nitrifying reactor fed similar substrate at 5°C with an SRT of 10 d. Aeration was provided by a diffuser stone with additional mixing by a magnetic stirrer. Feeding, wasting, settling (1 h), and decanting were once per day. The SRT was controlled by wasting one-quarter of the reactor volume daily during aeration to have an apparent SRT of approximately 4 d. The reactors were operated for 8 d before sampling commenced and after 16 d of operation (corresponding to day-9 in Figs. 4–6) the SBRs were seeded daily with 100 ml of the nitrifying biomass produced from the warm nitrifying reactor treating centrate at 20°C. The reactor configuration is shown in Fig. 1.

Table 2
Synthetic wastewater recipe for SBRs at 10°C

Ingredient	Concentration (mg/l)
Beef extract powder	150
Yeast extract powder	150
$MgSO_4 \cdot 7H_2O$	50
$MnSO_4 \cdot 7H_2O$	5.0
$FeSO_4 \cdot 7H_2O$	2.2
KCl	7.0
NH_4Cl	150
K_2HPO_4	196
$NaHCO_3$	556
$CaCl_2$	3.8
NH_3-N	25
COD	250

The HRTs used for the seeded SBRs were much longer than those that would be employed in full-scale application because it was known that the nitrification rate would be extremely slow due to temperature and dilution effects. The long HRTs would allow ample time for significant NH_3-N removal to occur.

The TSS and VSS, COD, NH_3-N, and NO_x-N were measured in all of the reactors at least 3 times per week.

2.4. Analyses

All analyses were conducted as per Standard Methods [15]. Ammonia was measured by the automated phenate method (4500-NH_3 G), and NO_x-N was measured by the automated cadmium reduction method (4500-NO_3^- F). Soluble COD (SCOD) samples were prepared by filtering through a 0.45 μm nylon membrane filter and analyzed by the closed reflux, colorimetric method (5220 D). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to methods 2540 D and 2540 E, respectively.

2.5. Determination of seeded sludge age

Development of equations for the estimation of SRT of seeded systems has been done elsewhere (e.g. [8,5]). The seeded SRT was calculated by first estimating the concentration of ammonia oxidizers in the influent stream (X_a^o) (Eq. (4)). S^o in this case is the substrate concentration of the centrate and S is the effluent substrate concentration from that reactor. The seeded SRT of the cold SBRs can then be determined from Eq. (5) [5,16] which accounts for ammonia oxidizers entering and leaving the system:

$$X_a^o = \frac{Q^s}{Q^i + Q^s}\left[\frac{Y(S^o - S)}{1 + b_{20}\theta_x}\right], \quad (4)$$

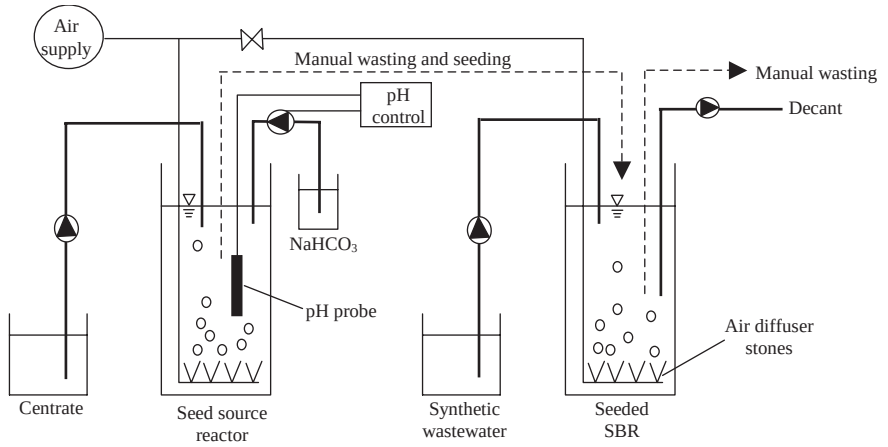

Fig. 1. Reactor configuration for seeding nitrifying bacteria into cold SBRs.

$$\theta_x = \frac{X_a V_r}{Q^w X_a + Q^e X_a^e - Q^i X_a^o}. \quad (5)$$

Because the concentration of ammonia oxidizers in the effluent of the seeded SBR was unknown, it was assumed that the proportion of ammonia oxidizers in the effluent was equal to the proportion in the reactor (Eq. (6)). The proportion of ammonia oxidizers in the seeded SBR can be expressed as:

$$P = \frac{X_a}{X_r}. \quad (6)$$

Eq. (5) then becomes:

$$\theta_x = \frac{X_a V_r}{Q^w X_a + Q^e P X_e - Q^i X_a^o}. \quad (7)$$

The concentration of ammonia oxidizers in the seeded SBR can then be estimated by Eq. (8). In this case S^o is the substrate concentration of the synthetic wastewater fed to the cold SBRs, S is the lowest and final achievable substrate concentration in the effluent from these reactors and b_{10} is the decay rate at 10°C. Due to the variability in feed NH_3-N concentrations, the total amount of NH_3-N available for nitrification was assumed to be the concentration of NH_3-N in the effluent from the SBRs before nitrification was initiated in these reactors (i.e., the mean NH_3-N concentration between day 0 and day 9 in Fig. 4):

$$X_a = \frac{\theta_x}{\theta}\left[\frac{Y(S^o - S)}{1 + b_{10}\theta_x}\right]. \quad (8)$$

Eqs. (6)–(8) were solved simultaneously to determine the seeded SRT of the nitrifying biomass (θ_x) in the seeded SBRs at 10°C. The values for b and Y were assumed to be 0.10/d and 0.24 g VSS/g NH_3-N [17], respectively, at 20°C. The temperature dependency of the decay rate was assumed to be the same as that for the growth rate [18,19] and following the Arrhenius equation (Eq. (9)), where k is the temperature correction factor for a particular nitrifying biomass:

$$b_T = 0.10\, e^{k(T-20)}. \quad (9)$$

3. Results and discussion

3.1. Determination of cold shock effects

Removal rates were significantly decreased by sudden cooling and the magnitude of the decrease was dependent on the change in temperature (ΔT). Fig. 2 provides an example where the nitrification rates in the warm nitrifying reactors were compared with the rates at 10°C. A direct comparison can be made because the initial concentration of biomass, substrate, pH, and aerobic conditions in the warm and cold reactors were similar. The average decreases in nitrification rates with the sudden decreases in temperature were 58 ± 8.2% for the 20°C biomass, 71 ± 4.7% for the 25°C biomass, and 82 ± 1.4% for the 30°C biomass (Fig. 2).

Temperature dependency factors for nitrifier growth rates have been published elsewhere and several are shown in Table 1. Details of the derivation of these equations are unclear since little information is given concerning whether or not the equations were created from rapid or gradual changes in temperature. The decrease in nitrification rates observed in this study were similar to the decreases previously found by the studies listed in Table 1. Fig. 3 illustrates the similarities between the observed data and previous research. The temperature dependency factor (k) was found to be equal to 0.0844°C (Eq. (9)).

3.2. Seeding nitrifying biomass into non-nitrifying SBRs

The average mass of nitrifying seed added to each reactor was 13.4 ± 3.7 mg VSS/d or 6.7 mg VSS/V_r d.

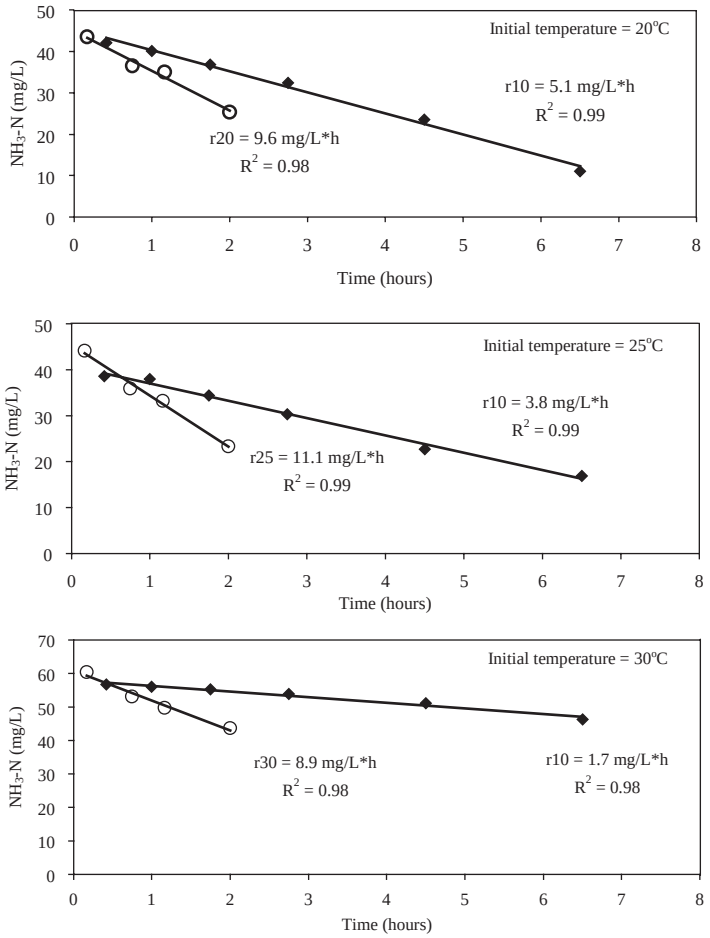

Fig. 2. Examples of nitrification rates for biomasses acclimated to 20°C, 25°C and 30°C before and after exposure to 10°C.

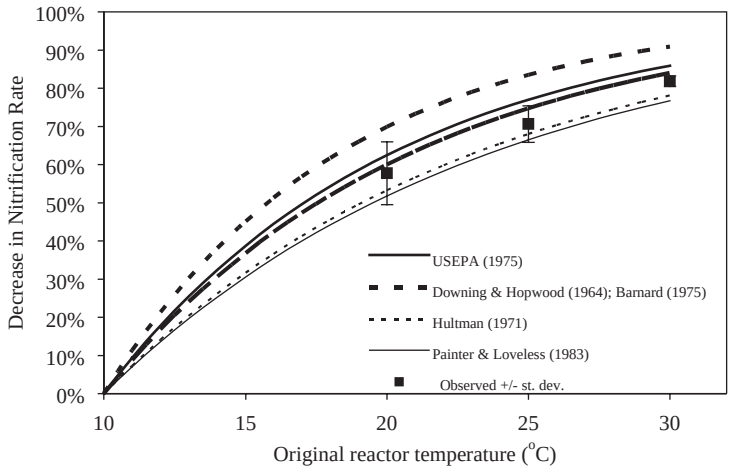

Fig. 3. Theoretical and observed decreases in nitrification rates after exposure to 10°C.

Table 3
Summary of observed and calculated seeded SBR characteristics during steady-state conditions

Input parameters	HRT (h)			
	43.6	53.3	68.6	96
θ (d)	1.817	2.221	2.858	4
Q^w (l/d)	0.5	0.5	0.5	0.5
Q^e (l/d)	0.6	0.4	0.2	0
Q^i (l/d)	1.0	0.8	0.6	0.4
Q^v (l/d)	0.1	0.1	0.1	0.1
X_r (mg VSS/l)	149	140	116	96.2
S_o (mg NH_3-N/l)	41.9	39.6	35.5	33.8
S (mg NH_3-N/l)	1.32	1.2	1.06	1.06
X_e (mg VSS/l)	20	20	20	0
Y (mg VSS/(mg NH_3-N))	0.24	0.24	0.24	0.24
b at 10°C (/d)	0.043	0.043	0.043	0.043
X_a^o (mg VSS/l)	8.65	10.6	13.6	19.0
Output calculations				
θ_x (d)	7.5	9.4	13.5	23.5
X_a (mg VSS/l)	30.4	27.7	24.7	23
P	0.204	0.198	0.213	0.239
$\mu = 1/\theta_x$ (/d)	0.13	0.11	0.074	0.043

Using the appropriate substrate concentrations and SRT for centrate treatment, the concentration of ammonia oxidizers in the seed source reactor was calculated to be at least 95.2 mg VSS/l (Eq. (10)). The values for X_a^o were determined for each seeded SBR by Eq. (11) and the results are listed in Table 3:

$$X_a = \frac{0.24\,\text{g/g} \times (600\,\text{mg/l} - 5\,\text{mg/l})}{1 + 0.1/\text{d}\,(5\,\text{d})} = 95.2\,\text{mg/l}, \quad (10)$$

$$X_a^o = \frac{95.2\,\text{mg/l} \times 0.1\,1}{Q^i + 0.1\,1}. \quad (11)$$

Effluent NH_3-N concentrations in the seeded SBRs decreased to less than 5 mg/l within 26–32 d of the start of seeding (Fig. 4). All four reactors achieved nearly complete NH_3-N removal while seeding continued, but once seeding was stopped, NH_3-N removal dropped off quickly. The rapid increase in effluent NH_3-N with the absence of seeding indicated that the nitrifying bacteria were being washed from the reactors rapidly.

Effluent NO_3-N concentrations increased sharply due to the input of seed and its associated nitrified liquor (Fig. 5). The NO_3-N concentration in the seed was approximately 600 mg/l which is equal to the centrate feed concentration of NH_3-N. As expected, the reactors with the longer HRTs had higher concentrations of NO_3-N in the effluent. The increases were due to a smaller fraction of liquid being exchanged per day in these reactors than those with shorter HRTs. It is unlikely that NO_3-N concentrations would reach such high values if this process for bioaugmentation was used

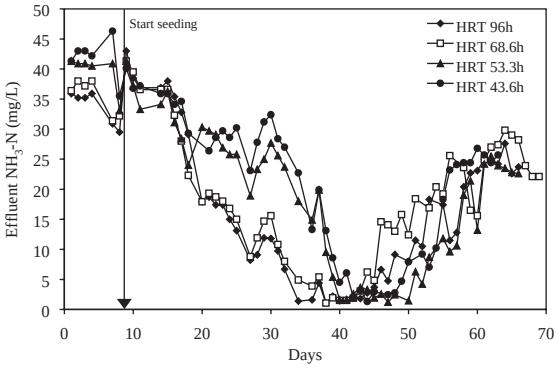

Fig. 4. Effluent NH_3-N concentrations for cold SBRs at various HRTs. Seeding was started on day 9 for all reactors. Seeding was stopped on days 47, 50, 47 and 45 for the SBRs with HRTs 43.6, 53.3, 68.6 and 96 h, respectively.

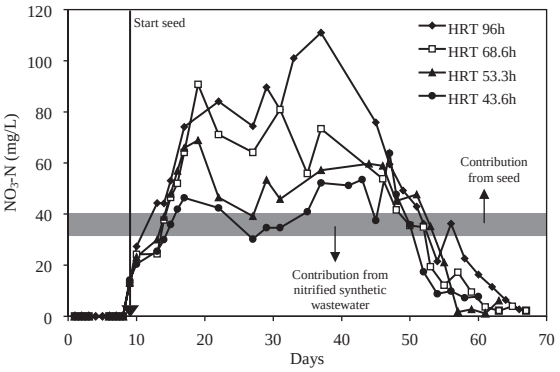

Fig. 5. NO_3-N concentrations for SBRs with various HRTs. Seeding was started on day 9 for all reactors. Seeding was stopped on days 47, 50, 47 and 45 for the SBRs with HRTs 43.6, 53.3, 68.6 and 96 h, respectively.

in full-scale systems, since, in this study the nitrified centrate seed made up 9–20% of the total flow entering the cold SBRs. In full-scale, the nitrified centrate would contribute only 1–2% to the influent flow [2]. The high NO_3-N concentrations in this study did not create any problems with settlability or floating biomass due to unintended denitrification.

The treated centrate contained significant levels of SCOD (325 ± 50 mg/l) although the liquors had already undergone 17 d of mesophilic anaerobic digestion (38°C) at the NEWPCC plant in addition to 5 d aeration in the laboratory nitrifying seed source reactors. As a result of seeding, effluent SCOD concentrations rose in the cold SBRs (Fig. 6). The rise in effluent SCOD followed a similar trend as NO_3-N, with higher effluent SCODs measured in the reactors with shorter hydraulic retention times.

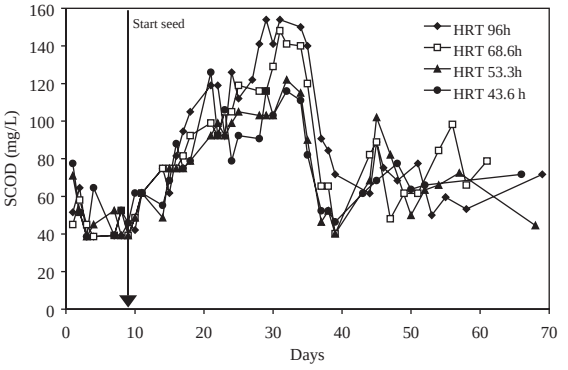

Fig. 6. Effluent soluble COD for SBRs at 10°C with various HRTs. Seeding was started on day 9 for all reactors. Seeding was stopped on days 47, 50, 47 and 45 for the SBRs with HRTs 43.6, 53.3, 68.6 and 96 h, respectively.

Influent NH_3-N variability was a problem despite the use of synthetic wastewater to eliminate this effect (data not shown). Feed was made on a weekly basis and stored at 4°C in a closed container. Degradation of the feed during storage resulted in an increased feed NH_3-N concentration likely due to the hydrolysis of organic nitrogen in the beef extract. Degradation of the feed during storage might have been minimized by making fresh feed every 2–3 days or by using sterilized water.

Full nitrification was achieved in the cold SBRs operating at an apparent SRT too short for nitrification to occur. Before seeding (days 0–9), nitrification was not occurring in the reactors as indicated by the high effluent NH_3-N concentrations and lack of NO_3-N production (Figs. 4 and 5). With seeding, the SRT of the nitrifying biomass was increased such that full nitrification could occur. This indicates that the seeded SRT is longer than the apparent SRT that was calculated based on the proportion of reactor solids wasted daily. The apparent SRTs ranged from 3.5 d for the SBR with HRT—43.3 h to 4 d for the SBR with HRT—96 h while the estimated seeded SRTs for the same SBRs ranged from 7.5 to 23.5 d, respectively (Table 3). Nitrification failure after seeding was stopped proves that seeding was the sole source of nitrifying bacteria in the cold SBRs.

The ability to achieve full nitrification without decreasing the proportion of biomass wasted daily (to increase the apparent SRT) suggests that the amount of solids wasted daily could be increased while still maintaining full nitrification. This is, in effect, short-SRT nitrification because the desired effluent quality is achieved without increasing the solids inventory [3].

4. Conclusions

The study evaluated the impact of sudden temperature shock on nitrification rates and compared them to other reported values in the literature. Nitrification continued after a sudden decrease in temperature as large as 20°C. Nitrification rates were decreased by an average of 58%, 71% and 82% for nitrifying biomasses cooled quickly to 10°C from 20°C, 25°C and 30°C, respectively. The observed decreases in nitrification rates due to sudden decrease in temperature were within the range found by other researchers. The temperature correction factor for nitrification was found to be equal to 0.0844/°C.

The study also tested the impact of seeding with warm nitrifying centrate biomass on cold reactors (10°C) operated at SRT below the minimum necessary for nitrification. Nitrification was induced in all seeded reactors. Effluent NH_3-N concentrations were reduced to less than 5 mg/l within 26–32 days as long as seeding was continued. Seeding as little as 6.7 mg VSS/l reactor volume was sufficient for full NH_3-N removal in all of the seeded reactors. Cessation of seeding led to rapid loss of nitrification in the cold SBRs.

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