The Effect of Salinity on Bacterial Denitrification of San Joaquin Valley Agricultural Drainage Water

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Abstract The accumulation of salt, nitrate, and selenium in drainage water by intensive agricultural activity in the San Joaquin Valley is problematic to aquatic organisms and to agricultural production. The use of bacteria under anaerobic conditions has been demonstrated to be an effective method for nitrate and selenium removal. Nitrate removal is necessary for selenium removal. While it has been shown that denitrification occurs at the same rate in agricultural drainage wastewaters containing 8 to 22 g L^{-1} salt, it is unknown whether denitrification can be effectively performed at higher salt concentrations of 66 to 140 g L^{-1}. Wastewater produced during reverse osmosis treatment is expected to contain up to 70 g L^{-1} salt and up to 120 mg L^{-1} N-nitrate. Two laboratory experiments were conducted with agricultural drainage water and reverse osmosis wastewater. First, denitrification was observed in agricultural drainage wastewater with NaCl additions. The denitrification rates at 66, 100, and 140 g L^{-1} of NaCl were significantly lower than at 22 g L^{-1} of NaCl (95% confidence of t-test). Second, similar procedures were used to determine the relative denitrification rate in reverse osmosis wastewater. Calculation of zero order kinetic constants showed that the reverse osmosis wastewater with its 33g L^{-1} salt content denitrified at a similar rate as 66 g L^{-1} NaCl. Although soluble reactive phosphorus and pH are considered as confounded factors to denitrification, the presence of both a significant level of phosphate and a near neatural pH level indicates both weren't factors to denitrification.

Introduction

The San Joaquin Valley in California is home to large-scale agriculture and produces more than 70% of the nation's fruit and vegetables (Zhang and Frankenberger 2003). Because the Valley is largely in an arid climate, water for irrigation must be pumped from wells or channeled from the Sierra Nevada mountain range (Quinn et al. 1998). In the western San Joaquin Valley, agricultural drainage water is either discharged to sloughs, which drain into the San Joaquin River and then into the Delta, or it is evaporated in terminal ponds (AARG 2000). As shown in Fig 1, current agricultural water disposal schemes do not treat NO_3^- and selenium in drainage water.

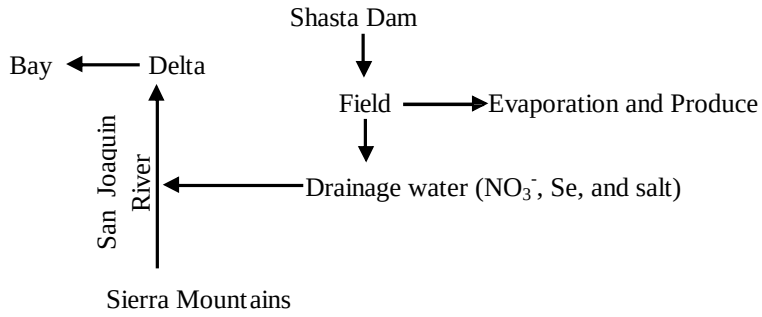

Figure 1. Flowchart of major parts of the current water cycle in San Joaquin Valley.

Common agricultural and irrigation practices result in the accumulation of high concentrations of nitrate (NO_3^-), selenium, and salt in subsurface drainage in some areas of the San Joaquin Valley. A high concentration of NO_3^- in drainage water ranging from 20-120 mg L^{-1} (12 times higher than the 10 mg L^{-1} safe drinking standard established by the EPA) is the result of heavy use of fertilizer in agricultural crops (AARG 2000; Zhang and Frankenberger 2003; US EPA 2005). Nitrates are considered detrimental because they continue to be a fertilizer in surface water, causing excess vegetation and algae blooms, i.e., eutrophication (Botkin and Keller 2000; Johansson el at. 2001). Naturally occurring high selenium levels from the leaching of soils in agricultural irrigation runoff in the San Joaquin Valley ranging from 50-1200 μg L^{-1} is 24 times higher than EPA maximum contaminant level for selenium in drinking water, and this high level of selenium has been linked to birth defects in aquatic birds due to selenium bioaccumulation in the food chain (Lemly 1999; AARG 2000; Zhang and Frankenberger 2003; US EPA 2005). The most notable example of

selenium bioaccumulation was the deformation of birds at Kesterson Reservoir, California. (Ohlendorf 2002; Dungan et al. 2003). Due to evaporation of irrigation water, ranging from 5 to 20 g L^{-1} (20 times higher than the state-recommended upper maximum contaminant level drinking water standard), a high concentration of salt (total dissolved solids) comprised primarily of sulfate, sodium, calcium, magnesium, and chloride, and many crops cannot tolerate in the salt accumulated soil (Meng and Moyle 1995; Green et al. 2003; CCC 2005).

A cost-effective solution to these problems may be a combination of reverse osmosis and bacterial bioremediation. Reverse osmosis (RO) treatment followed by the bacterial bioremediation of RO wastewater proposed by US Bureau of Reclamation and California Department of Water Resources would remove nitrate, selenate, and salinity as shown in Fig. 2 (AARG 2000). The RO treatment is a proven way of extracting fresh water from agricultural drainage wastewater. However the RO wastewater, which is a byproduct of reverse osmosis, still needs to be treated to remove selenium before the RO wastewater is discharged to evaporations ponds that inevitably attract fowl. The RO wastewater from RO treatment of agricultural drainage wastewater is expected to contain between 10 g L^{-1} and 70 g L^{-1} salt measured as total dissolved solids (TDS) and up to 120 mg L^{-1} N-nitrate (Lundquist 2005 pers. comm.).

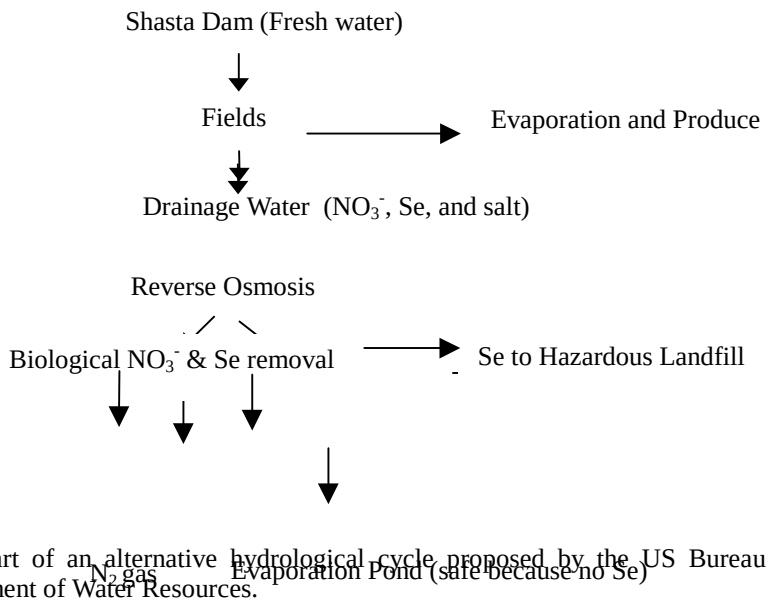

Figure 2. Flowchart of an alternative hydrological cycle proposed by the US Bureau of Reclamation and California Department of Water Resources.

A bioremediation method using bacteria under anaerobic conditions has been demonstrated to be an effective method to remove NO_3^- and selenium. Carbon substrate such as molasses is added to the drainage water allowing growth of denitrifying bacteria that reduce nitrate to nitrite and then elemental nitrogen gas (Oswald 1996). In this method, past studies have shown that NO_3^- hinders selenate removal. Bacteria prefer reducing NO_3^- before converting selenate to the more easily removable selenite and elemental selenium (Huang 2003); that is; NO_3^- needs to be removed before selenium can be removed in drainage waters (Green et al. 2003).

Huang's study (2003) found that denitrification occurred without inhibition with up to 22 g L^{-1} of NaCl added. Some studies have shown that high salinity is not compatible with bioremediation of nitrates and selenium, while others show a certain concentration is tolerable, though the salt concentration can affect the rate of denitrification (Glass and Silverstein. 1999; Ucisik and Henze 2004). Thus, a question remaining is: At what NaCl level is NO_3^- removal inhibited?

The first objective of this study is to determine the correlation between NaCl concentration in agricultural drainage wastewater and the denitrification rate. Between 22 g L^{-1} and 140 g L^{-1} of NaCl the denitrification rate was predictably slow. This study seeks to ascertain the tradeoff between salinity and the denitrification rate.

Reverse osmosis wastewater from San Joaquin Valley drainage treatment is not readily available for denitrification research since the only successful pilot RO plant shut down

several years ago. Thus, the second objective of this study is to see whether the RO wastewater solution at a given salinity has the same denitrification rate as agricultural drainage wastewater with NaCl addition. If so, agricultural drainage wastewater with NaCl addition can be a substitute in research for RO wastewater. Because the rate of denitrification in RO wastewater is predicted to be similar to that of agricultural drainage wastewater with NaCl addition, successful nitrate reduction of RO wastewater provides further evidence of the possibility of economical selenium removal as proposed in Fig. 2. However, it is hypothesized that high sodium sulfate and/or high selenium concentrations in the RO wastewater might be toxic (inhibitory) to denitrifying bacteria. Thus, the large amount of nitrate and other salts existing in RO wastewater solutions were thought to require a longer time and/or a higher bacterial concentration to achieve complete denitrification.

Methods

Sodium chloride addition experiment in agricultural drainage wastewater

Materials Subsurface agricultural drainage wastewater was collected from the Panoche Drainage District, Fresno County, California near the city of Firebaugh on December 27th, 2004. The agricultural drainage wastewater was brought to Lawrence Berkeley National Laboratory in a 20 L plastic container and stored in the dark at room temperature. In addition, water-saturated soil samples were collected from Red Rock Ranch near Five Points in the Central Valley. This agricultural drainage wastewater recycling area presumably supports salt-adapted bacteria that were used as inoculum in this study.

Drainage preparation One month later, five salinity levels of NaCl (Fisher Certified sodium chloride (Appendix 1)) were prepared: 0, 22, 66, 100, and 140 g L^{-1} of NaCl were each added to 1.5 L aliquots of the agricultural drainage wastewater. To all salinity levels, the following was added: 0.5 ml L^{-1} of 5 % (W/V) molasses solution, 0.1 ml L^{-1} of trace metal nutrient solution (Appendix 2), and 2 ml L^{-1} of inoculum solution. One hundred ml from each of the five NaCl addition levels were poured into 150-mL serum bottles; three replicates of 0 g L^{-1} NaCl level and 15 replicates of each of the other four NaCl addition levels for a total of 63 bottles. The headspace of each bottle was flushed with nitrogen gas for 10 seconds to lessen the oxygen reservoir in each bottle. The liquid was not sparged so that all bottles would contain nearly equal dissolved oxygen concentrations. The bottles were sealed with rubber sleeve stoppers and incubated in the dark at 21 °C beginning January 25th, 2005. During incubation, the each bottle was inverted once each a day, and the incubator

temperature recorded.

Sampling and Preservation On day 0, 2, 4, 6, 8, and 13 of the incubation, three replicates of each of the four NaCl addition levels were analyzed for nitrate whereas the 0 g L^{-1} NaCl addition level was measured on days 0 and 13. On day 4, 6, 8, and 13 of the incubation, three replicates of each of the four NaCl addition levels were analyzed for nitrite whereas the 0 g L^{-1} NaCl addition level was measured on day 13. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured on day 0, 6, 8, and 13, and total dissolved solids (TDS) were measured on days 0 and 13. Phosphorus was measured on day 13 for all NaCl addition levels and pH was measured on day 0 and 13 for all NaCl addition levels.

Nitrate was measured, using the ultraviolet spectrophotometric screening method (APHA 1995), and matrix spike recoveries within 85%-115% indicated accurate results in each batch of samples tested. The pH was measured using a Denver Instrument Model 215 pH meter on day 0 and day 13 of the incubation. Phosphate was determined by a colormetric method (APHA 1995).

For total suspended solids (TSS), total dissolved solids (TDS) and volatile suspended solids (VSS), the filtration method outlined in the *Standard Methods* was used (APHA 1995). To start, 50 ml from each bottle were filtered through a 1.2 μm- pore Whatman GF/C filter, using a vacuum pump attached to a 250 ml sidearm flask. The mass of TDS, TSS, and non-volatile suspended solids (NVSS) for each 50 ml aliquot was determined according to the outline in Figure 3. Volatile suspended solids concentration was the proxy for bacteria concentration, and it was calculated as TSS – NVSS = VSS. Each filtered water sample was then collected into a 250 ml flask and 10 ml was poured into aluminum trays and placed in a 180 °C oven for 2 hours to 12 hours. The difference between final weight and initial weight determined the amount of total dissolved solids in each sample.

For each sample, half of the filtered water (5 ml) was refiltered through a 0.2 µm membrane filter attached to a syringe. The 5 ml of 0.2-µm-membrane filtrate solution was used for NO_2^- determination using the high range ferrous sulfate method (Hach Co. Method 8153). The remaining 35 ml was poured into a 59 ml Fisher wide mouth bottle and stored in the refrigerator.

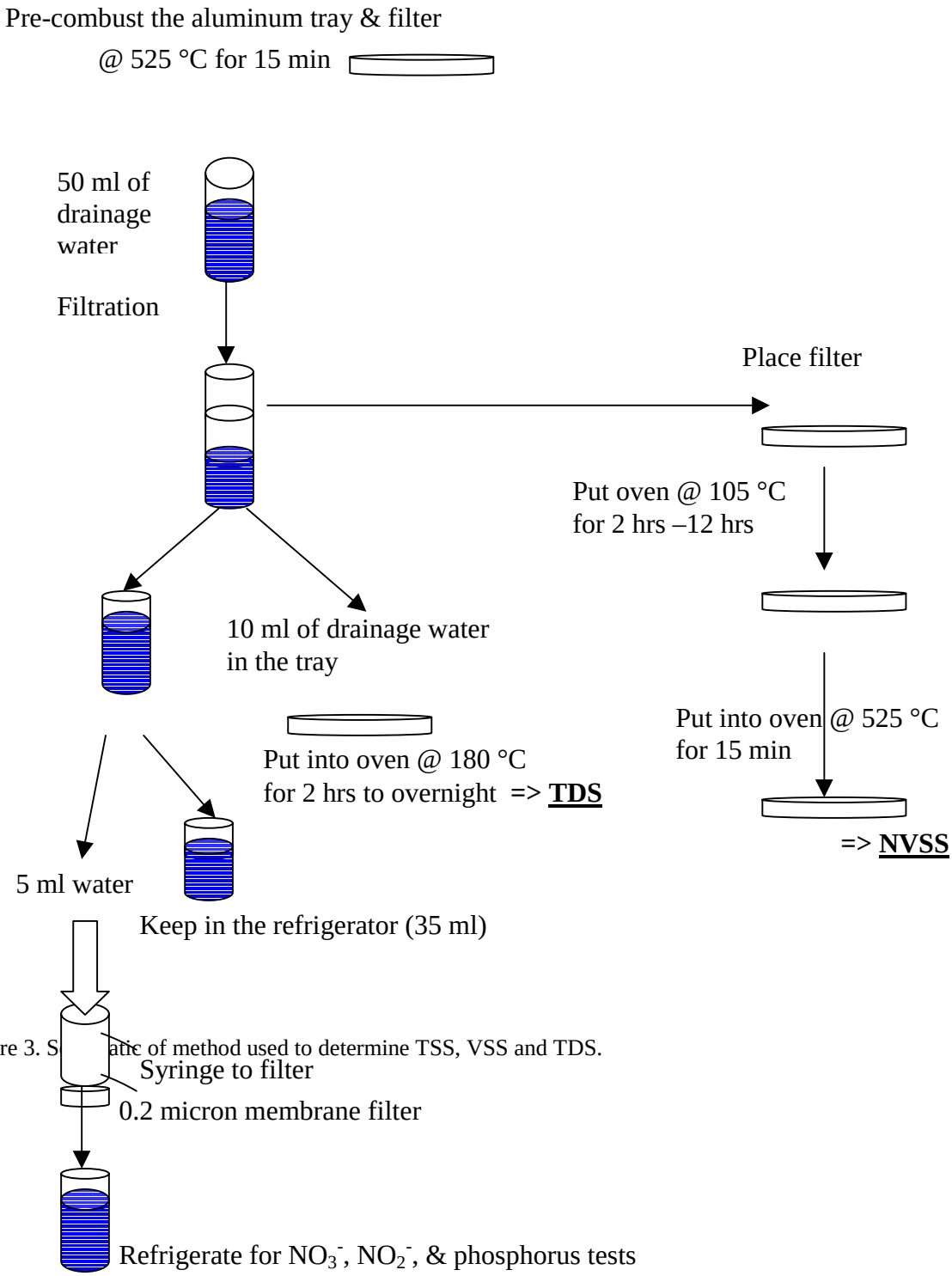

Figure 3. Schematic of method used to determine TSS, VSS and TDS.

RO wastewater experiment

Materials The RO wastewater from agricultural drainage wastewater that passed through a pilot reverse osmosis facility at Panoche Drainage District, Fresno County, California near the city of Firebaugh, was obtained from Water Partner's Incorporated on March 27th, 2003. The RO wastewater sample was brought to Lawrence Berkeley National Laboratory in a 0.7 L glass jar and stored at room temperature in the dark.

Primary measurements Before the RO wastewater solution was prepared, NO_3^- and TDS were measured as was done in the NaCl addition experiment.

RO wastewater preparation The 0.6 L of RO wastewater solution received 3 ml of molasses 5 % solution, 0.6 ml of trace metals nutrient solution, and 6 ml of inoculum solution, and 35 ml was poured into a 150-mL serum bottle to make 15 replicates. For the control, the 120 ml of unfiltered Panoche Influence drainage water collected December 27th, 2004 received 0.6 ml of molasses solution, 0.12 ml of nutrients solution, and 1.2 ml of inoculum solution, and 40 ml was poured into a 150-mL serum bottle to make three replicates. Total of 18 bottles were prepared. The volume incubated in this experiment was less than in the NaCl addition experiment because of the small volume of RO wastewater available, the reverse water system having been shut down in 2002. The rest of the procedure for the preparation was identical to the NaCl addition experiment. Incubated in the dark at 21°C began April 5th, 2005.

Sampling and preservation The same procedure was used as in the NaCl addition experiment. On day 0, 2, 4, 6, 8, and 13 of the incubation, nitrate, nitrite, TSS, VSS, and phosphorus for RO wastewater solution were sampled. Nitrate, nitrite, and TSS, and VSS for the control were measured on day 0 and 13, and pH for the RO wastewater solution and control were measured on day 0 and 13. To see whether RO wastewater has similar reduction rate to NaCl addition levels, the zero order kinetic model was used with the rate constant, k, determined by the following equation, $(N_0 - N_t) = k*(t - t_0)$.

Results

The NaCl addition experiment showed NO_3^- removal occurring for all five NaCl addition levels (Fig. 4). After 13 days of incubation, agricultural drainage wastewater at the 0 and 22 g L^{-1} NaCl levels had approximately 8 mg L^{-1} of nitrate remaining, and at the 66-140 g L^{-1} NaCl addition levels, approximately 16 mg L^{-1} of nitrate remained. Looking at each denitrification rate, denitrification was inhibited at the100 and 140 g L^{-1} NaCl addition levels; that is, the rate slowed shown in Fig. 4, but on day 13 there was not a significant difference (95 % confidence level; t-test) in the amount of nitrate remaining in any of the NaCl addition

levels. In the primary measurement for the RO wastewater experiment, the RO wastewater solution contained 229 mg L^{-1} of nitrate-N and 33 g L^{-1} TDS. For the RO wastewater experiment, denitrification occurred at a roughly similar rate to the 22 g L^{-1} NaCl addition level (Fig. 4 in the second y-axis). The NO_3^- reduction in the RO wastewater was 24 % with 13 days of incubation. After day 8, NO_3^- reduction stagnated presumably due to carbon limitation. Total nitrate removal for the RO wastewater control was similar to the control in the NaCl addition experiment.

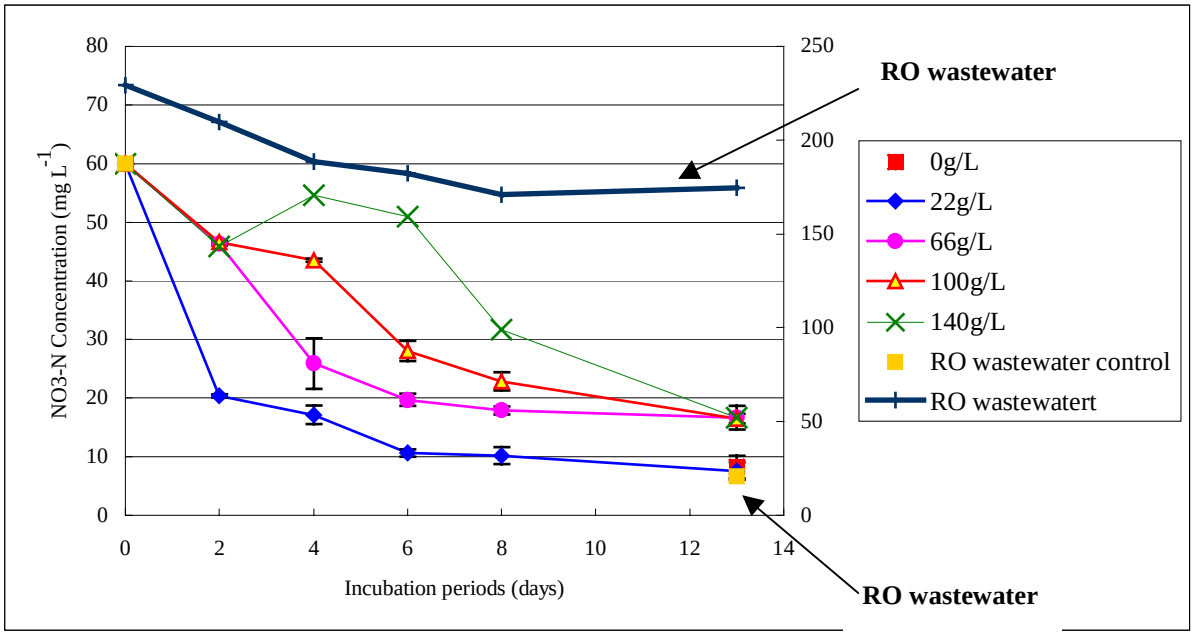

Figure 4. Nitrate-N concentrations over time with various NaCl additions. Second y-axis is for RO wastewater experiment. Bars represent one standard error. Where bars are not seen, they are smaller than the marker point.

As nitrate was reduced (Fig. 4), an increase in nitrite was observed (Fig. 5). The amount of nitrite at the 22 - 100 g L^{-1} NaCl addition levels reached maximum on day 6, but from day 6 to 8, NO_2^- was reduced rapidly at 22- 100 g L^{-1} NaCl addition levels. These results indicated the similar trends of NO_2^- reduction among these NaCl addition levels. On the other hand, on day 4 to 13, NO_2^- increased rapidly at the 140 g L^{-1} NaCl addition level and did not decrease within the 13 days of incubation. This delayed NO_2^- production follows the delayed NO_3^- reduction at the 140 g L^{-1} NaCl level. For the RO wastewater experiment, the peak NO_2^- concentration occurred on day 4 of incubation which was earlier than the peak at the 22 g L^{-1} NaCl addition level (Fig. 5). However, NO_2^- reduction was not complete probably due to carbon limitation.

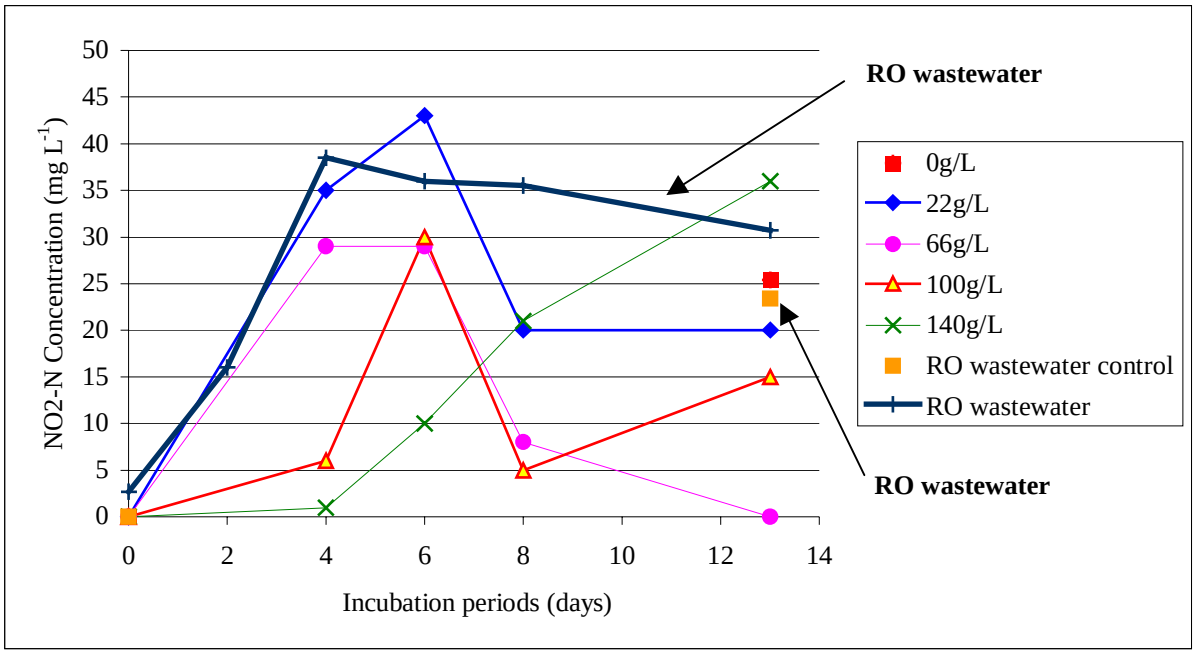

Figure 5. Nitrite-N concentrations over time at various NaCl level and RO wastewater experiment.

Volatile suspended solids (VSS) concentration represents the bacterial biomass in each sample (Lundquist 2005 pers. comm.). Between day 4 and 6, NO_3^- was reduced (Fig. 4), while NO_2^- increased quickly (Fig. 5), and this implies bacterial growth occurred rapidly at the 22-100 g L^{-1} NaCl addition levels (Fig. 6). In contrast, the growth rate of bacterial biomass was slow at 140 g L^{-1} NaCl addition level shown in Fig. 6 as NO_3^- reduction was slow until day 6. At the 140 g L^{-1} NaCl addition level, NO_3^- was reduced into NO_2^- quickly from day 6 to 8, as the same time as the growth rate of bacterial biomass occurred rapidly. These results support the notion that the lag period in bacterial growth was extended at the 140 g L^{-1} NaCl addition level. For the RO wastewater experiment, the VSS measurement results showed that suspended bacterial biomass did not increase in either the RO wastewater or the control, while denitrification was observed as shown in the Fig. 6.

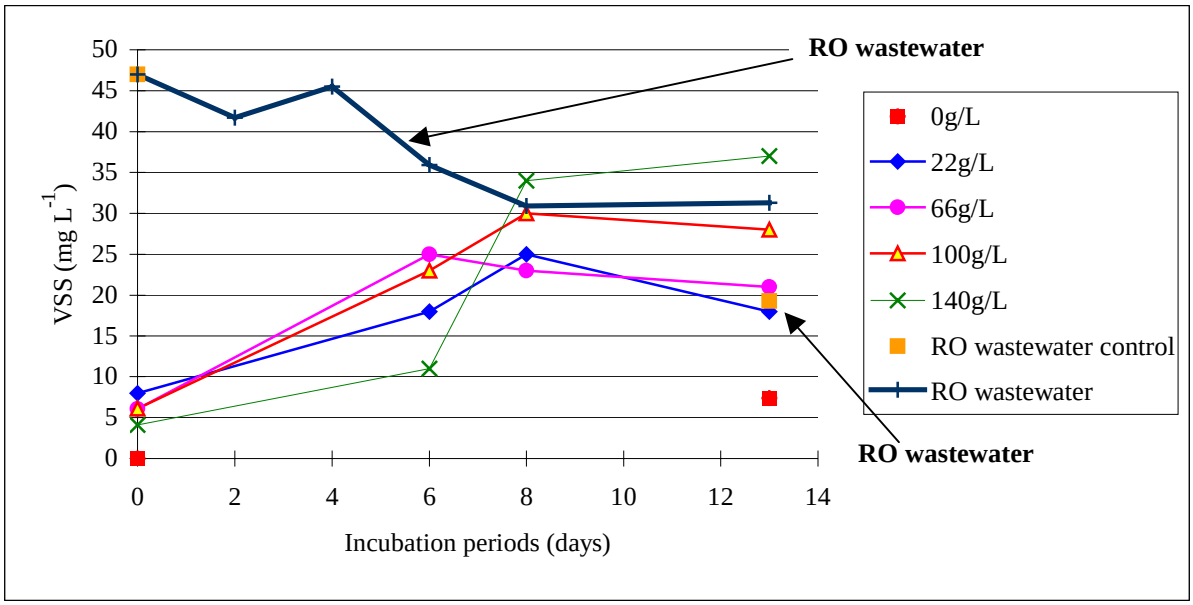

Figure 6. Volatile suspended solids (VSS) over time at various NaCl addition levels and RO wastewater experiment.

Above 5 mg L^{-1} nitrate-N, denitrification in drainage water with molasses is a zero order kinetic process; that is the rate of the reaction is independent of the concentration of any reactant (Lundquist 2005 pers. comm.). Based on this, zero order kinetic constant, k, was calculated. These results showed that k was lower for the RO wastewater compared to the 22 g L^{-1} NaCl addition level, and similar to the 66 g L^{-1} NaCl addition level, indicating some additional inhibition of denitrification in the RO wastewater compared to the 22 g L^{-1} NaCl addition level (Table 1) (Appendix 3).

Table 1. The zero order denitrification constant over the period which nitrate was reduced from initial levels to 40 mg L^{-1} of Nitrate-N concentrations reduction.

22 g L^{-1} NaCl	$20.0 \leq k$
66 g L^{-1} NaCl	$10.0 \leq k < 20.0$
100 g L^{-1} NaCl	$5.0 \leq k < 6.7$
140 g L^{-1} NaCl	$3.1 \leq k < 5.0$
RO wastewater	$10.0 \leq k < 20.0$

Discussion

Denitrification is the reduction of nitrate (NO_3^-) to nitrogen gas or to organic nitrogen compounds (Corbin 1998) according to the following equation.

NO_3^- -----> NO_2^- -----> NO -----> N_2O -----> N_2 gas

The observed decreases in NO_3^- (Fig. 4) where to some extent balanced by the formation of nitrite production was measured at all NaCl levels (Fig.5). Nitrite production was delayed at the 140 g L^{-1} NaCl addition level compared to the others also indicating delayed denitrification. The slow increase of VSS at the 140 g L^{-1} NaCl addition level indicates that it took time for bacteria to adapt to this highest NaCl level. Considering the results as a whole, it is concluded that this experiment supports my hypothesis that high salt concentrations such as the100 and 140 g L^{-1} NaCl addition levels affect the rate of denitrification in the intermediate steps. This is consistent with the findings of both Glass and Silverstein (1999) and Ucisik and Henze (2004).

In the RO wastewater experiment, the result of VSS showed that bacterial biomass did not increase at the high salt concentration. However, the fact that denitrification occurred in this experiment implies that bacterial biomass increased, using all available carbon from the molasses. In both experiments, molasses plays an important role for food source of bacteria (Huang 2003). The result that total reduction of NO_3^- in the RO wastewater experiment was similar to NaCl addition experiments (50-60 mg L^{-1} removed) means bacteria had an access to molasses to grow to do denitrification. Likely reason bacteria biomass in the RO wastewater appeared not to grow might be due to the small volume of solution incubated. The solution volume in the RO wastewater experiment was 35 m L whereas the volume was 100 m L in the NaCl addition experiment. Thus, the volume to bottle surface area was greater in the NaCl addition experiment. Also, a film was seen on all the bottles of the RO wastewater experiment. Such a film was not noticed during the NaCl addition experiment. The films presumably were cells attached to the walls that could not be measured in the VSS test. The decline in VSS during the brine experiment might have been due to continued cell attachment to the bottle wall.

It was considered that soluble reactive phosphorus and the pH might also inhibit denitrification thereby confounding the test of salt inhibition. Lehtola et al. (1999) found that below 1 µg L^{-1} phosphorus limited microbial growth. The source of the phosphorus in these experiments was the molasses additions. Because over 13 days the phosphorus concentration of all samples except day 2 sample and the control contained > 0.4 mg L^{-1} of soluble reactive phosphorus, phosphorus was unlikely to limit denitrification. It was known that denitrification rate is hindered by extreme pH and the optimal level of pH is the range of 7.0-8.0 (Koenig and Liu 2004). But the near neutral pH measurements in all samples in these

experiments indicate that this was not a factor in the denitrification results (Appendix 4).

Selenate reduction has been found to convert into selenite only after NO_3^- concentration has decreased below 5 mg L^{-1} (Gerhardt and Oswald 1990). The results that NO_3^- concentration stabilized presumably show that bacteria used up all available carbon from the molasses, and the remaining nitrate was more than 5 mg L^{-1} for both experiments as shown in Fig. 4. Thus, selenate was probably not removed to low levels in these experiments.

Zero order kinetic constants were calculated to see whether the RO wastewater had a similar reduction rate to that of NaCl addition level. To calculate the zero order rate constant, k, the point of 22 mg L^{-1} NO_3^- was used because it was assumed that some carbon was left at this point to proceed with denitrification. The results of k for the RO wastewater lies between the 22 and 66 g L^{-1} NaCl addition levels which is reasonable since the RO wastewater had 33 g L^{-1} TDS. These results suggest that NaCl addition level is reasonably representative of the RO wastewater. This result indicates that the NaCl addition experiment was useful for predicting the RO wastewater denitrification rates. The result that high salt concentrations slow denitrification is important since this slowing will make wastewater treatment more expensive than agricultural drainage treatment. This is because it takes about 2 days to reduce 40 mg L^{-1} nitrate at lower salt concentration like 22 g L^{-1} whereas it takes about 8 days to reduce small 40 mg L^{-1} nitrate at higher salt concentration like 140 g L^{-1}.

In future experiments, the interpretation of the results might be simplified if the carbon-to-nitrate ratio were made the same for the brine and control treatments either by fortifying the control with nitrate or adding additional molasses to the high nitrate brine. Also, more frequent sampling would improve the estimates of the denitrification rate constants.

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Appendix 1. Lot analysis for Fisher Certified NaCl used in experiments (Lot 796274).

Insoluble matter: 0.0025%	
Free acid as HCl: 0.002%	
Free alkali as NaOH: none	
Br: 0.001%	
I: 0.0005%	
N compounds as N: 3ppm	
PO_4: 2ppm	
SO_4: 0.0008%	
Ba:	0.001%
Fe:	0.5ppm
Ca, Mg, R_2O_3 ppt: 0.0035%	
Heavy metals as Pb: 1 ppm	
K:	0.002%
ClO_3: 0.001%	
NO_3: 0.003%	

Appendix 2. Composition of Trace Metals Solution with 2 ml concentrated HCl in 198 ml of DI water.

$MnSO_4 \bullet H_2O$	3.3 mg
$Cu\ SO_4 \bullet 5H_2O$	6.2 mg
$Zn\ SO_4 \bullet 7H_2O$	7.6 mg
$Na_2MoO_4 \bullet 2H_2O$	11.7 mg
$Fe\ SO_4 \bullet 7H_2O$	64.6 mg

Appendix 3. Zero order rate constant calculations.

Because Nitrate-N concentration was measured only every two days and it was not exactly known when 40 mg L^{-1} Nitrate-N concentration would be removed, a constant range was calculated. A 40 mg L^{-1} Nitrate-N concentration was chosen because there still seemed to be some carbon left that this point of concentration. That is, final nitrate removals were 50-60 mg L^{-1}.

Zero order: Rate = k => $N_0 - N_t = k*(t - t_0)$
(1) Latest possible days to reach 40 mg L^{-1} NO_3^- removed
N_0: Initial amount of NO_3^-
N_t: Final amount of NO_3^- when 40 mg L^{-1} NO_3^- reduction was achieved
t: Latest possible days to reach 40 mg L^{-1} NO_3^- removed
t_0: Initial day (that is 0)

	N_0	N_t	t	t_0	Zero order rate constant
22 g L^{-1} NaCl	60	20	2	0	$20.0 \leq k$
66 g L^{-1} NaCl	60	20	4	0	$10.0 \leq k$
100 g L^{-1} NaCl	60	20	8	0	$5.0 \leq k$
140 g L^{-1} NaCl	60	20	13	0	$3.1 \leq k$

RO wastewater	229	189	4	0	$10.0 \leq k$

(2) Earliest possible day to reach 40 mg L^{-1} NO_3^- removed

N_0: Initial amount of NO_3^-

N_t: Final amount of NO_3^- when 40 mg L^{-1} NO_3^- reduction was achieved

t: Earliest possible days to reach 40 mg L^{-1} NO_3^- removed

t_0: Initial day (that is 0)

	N_0	N_t	t	t_0	Zero order rate constant
22 g L^{-1} NaCl	60	20	0	0	-
66 g L^{-1} NaCl	60	20	2	0	<20.0
100 g L^{-1} NaCl	60	20	6	0	<6.7
140 g L^{-1} NaCl	60	20	8	0	<5.0
RO wastewater	229	189	2	0	<20.0

Appendix 4.

	Day	0	13		0	13
pH	22g/L	6.68	6.90	pH		
NaCl	66g/L	6.7	6.90	Brine	7.84	6.7
	100g/L	6.58	6.80	control	7.76	7.6
	140g/L	6.66	6.50			
	control		7.30			