

# A Denitrification System for Domestic Wastewater Using Sulfur Oxidizing Bacteria

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## ABSTRACT

Nitrate loadings from on-site residential wastewater treatment facilities continue to contribute to groundwater contamination. A mound-like denitrification system using a packed bed reactor containing elemental sulfur and dolomitic limestone, was constructed in order to create a passive, inexpensive and long lasting denitrification system. Under anaerobic conditions, sulfur oxidizing bacteria metabolize elemental sulfur as an electron donor, reducing  $\text{NO}_3^-$  as an electron acceptor, transforming the  $\text{NO}_3^-$  ion to  $\text{N}_2$  gas. Wastewater has been applied to a mound containing a  $0.1044 \text{ m}^3$  ( $3.75 \text{ ft}^3$ ) saturated zone amended with industrial grade elemental sulfur ( $<2.5 \text{ mm}$  diam.) and dolomite chips ( $<6 \text{ mm}$  diam.). The saturated zone is created by an impermeable (trough shaped) barrier built of PVC plastic that restricts flow in the mound. A saturated zone containing only coarse sand was used for a control. The mound was divided by  $0.95\text{-cm}$  ( $3/8\text{-inch}$ ) PVC plastic sheets into eight discrete cells of  $45.72 \text{ cm}$  ( $18 \text{ inch}$ ) width. Two textural mixes of  $\text{S}^0$  and dolomite were compared with the coarse sand control. Results show mean total nitrogen levels of  $8.7$ ,  $3.0$ , and  $23.2 \text{ mg N/L}$  at the outlets of the flow barrier in the cells of the  $\text{S}^0$ /dolomite mixtures and control, respectively, when applying septic tank effluent (STE) with mean total nitrogen concentration of  $25.7 \text{ mg N/L}$ . Total nitrogen concentrations of  $2.5$ ,  $0.2$ , and  $43.0 \text{ mg N/L}$  were reported in the  $\text{S}^0$ /dolomite mixtures and control, respectively, when applying STE containing additional  $\text{NH}_4\text{Cl}$  and a total N concentration of  $51.7 \text{ mg N/L}$ . Mean pH levels in the STE and controls were  $7.1$  and  $6.8$ , respectively, while the treated cells averaged  $6.5$  for mixtures 1 and 2, an indication of a buffered system due to the limestone amendment. Elemental sulfur can provide an inexpensive, relatively long lasting energy source for the denitrification of domestic wastewater in a passive treatment system. This sulfur/dolomite amendment, under anaerobic saturated flow conditions, is capable (without additional bacterial inoculation) of consistently reducing the concentration of total nitrogen below  $10 \text{ mg N/L}$  in a mound-type system in the absence of extreme winter temperatures. Questions remain. Research is continuing regarding the levels of sulfate produced by the system.

**Keywords:** Sulfur-oxidation, Nitrogen removal, Domestic wastewater treatment

## INTRODUCTION

Septic systems have been identified as a source of nitrate contamination to groundwater, particularly those systems placed in areas of sandy soils with high water tables (Walker et al., 1973; Robertson et al., 1991).

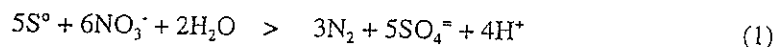
The biological reduction of nitrate (denitrification) has been identified as a feasible method for the removal of nitrate from domestic wastewater. The biological reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  gas is dependent on an anaerobic environment in the presence of an electron donor

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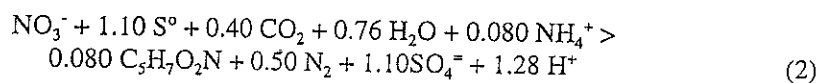
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source. The majority of research on the denitrification of domestic wastewater has focused on the use of organic carbon as an electron donor source. Less attention has been put on the use of elemental sulfur as an energy source. At present, designs that facilitate autotrophic denitrification in domestic wastewater have been limited to laboratory studies (Sikora and Keeney, 1976; Xiushan et al., 1993) or oriented towards municipal scale treatment facilities (Batchelor and Lawrence, 1978; Schippers et al., 1987).

Denitrification is facilitated by a suite of facultative aerobes whom in the absence of oxygen incorporate the  $\text{NO}_3^-$  ion as an electron acceptor in their process of energy production (catabolism). Heterotrophic bacteria require a source of labile organic carbon in their process of cell biomass production (anabolism). Denitrification by sulfur-oxidizing autotrophs is commonly associated with the species *Thiomicrospira denitrificans*, and *Thiobacillus denitrificans*. In the presence of elemental sulfur, these species reduce the nitrate ion to nitrogen gas according to the following reaction (Schippers et al., 1987):

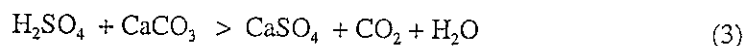


Taking into account ammoniacal nitrogen and the empirical formula for cell biomass production ( $\text{C}_5\text{H}_7\text{O}_2\text{N}$ ), the following reaction is carried out (Schippers et al., 1987).



The subsequent evolution of hydrogen ions will cause a significant lowering in pH in the absence of a buffered system. Thus, the addition of a limestone amendment is required to neutralize the acidic effects of this reaction. This is important both in terms of preventing the acidification of groundwater and for maintaining a suitable environment for denitrification. The optimal pH range for growth of *T. denitrificans* is 6.8 to 7.4. Carbonate species evolving from the limestone addition also serve as a source of inorganic carbon for autotrophic metabolism. Precipitation of gypsum is of minimal concern as the solubility of  $\text{CaSO}_4$  is 1.93 g/L at 20°C (Wenst, 1971).

In a buffered system rich in limestone, the resulting acid can be neutralized in a reaction similar to Eq. 3:



Laboratory research carried out in a steady-state, continuous-flow, closed-column system using a mixture of sulfur and dolomite, resulted in nearly complete  $\text{NO}_3^-$  removal in 3.3 hours at 23°C from a solution containing 40 mg/L  $\text{KNO}_3$  (Sikora and Keeney, 1976). Based on the stoichiometry from Equation (2), 2.5 mg/L  $\text{S}^0$  are required to reduce 1.0 mg/L of  $\text{NO}_3^-$ -N. A concentration of 2.13 mg/L  $\text{SO}_4^{2-}$ -S (6.39 mg/L  $\text{SO}_4^{2-}$ ) was produced per mg/L  $\text{NO}_3^-$ -N reduced, indicating the production of  $\text{SO}_4^{2-}$  concentrations in excess of 250 mg/L. The objective of this research was to develop a passive low-cost, low-maintenance, denitrification system, using sulfur oxidizing bacteria.

The work presented in this text represents a portion of the research being carried out with the objective of applying the principles reported by (Sikora and Keeney, 1976) to the field conditions of an on-site wastewater treatment system.

## MATERIALS AND METHODS

### Experimental Design

The experiment was carried out, receiving wastewater from the residence of the Sheep Research Center at the Arlington Research Station of the College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison. The house was served by a 3787-L (1000-gal.) septic tank and an in-ground dosed absorption bed. The average wastewater generated was 1050 L (277 gal.)/day. A 1893-L (500-gal.) concrete pump chamber was installed to the existing system. A Myers ME40 4/10 Horsepower, submersible effluent pump was installed in the pump chamber to deliver pressurized dosings to the experimental mound.

A series of eight treatment "cells" were constructed using 0.9525-cm (3/8-inch) PVC plastic sheets. Each cell contains a zone of restricted saturated flow. Flow is restricted within each cell by a "trough"-shaped barrier constructed with 0.635-cm (1/4-inch) PVC plastic, to create a volume of 0.1044 m<sup>3</sup> (3.75 ft<sup>3</sup>) of saturated conditions within the mound (Fig. 1). The saturated zone of each cell was filled with one of the textural mixtures to be examined for capacity to remove nitrogen: (1) 4.536 kg (10 lb) S and 4.536 kg dolomite within a coarse sand matrix, (2) 4.536 kg S in a matrix of dolomite, and (3) a saturated zone containing only coarse sand serving as a control. The mound was divided by 0.95-cm (3/8-inch) PVC plastic sheets into eight discrete cells. Three cells were made up of mixture 1, three of mixture 2, and two for controls. The mass of sulfur used was calculated based on the following criteria: the stoichiometry of Eq. 2, NO<sub>3</sub><sup>-</sup>-N concentration of 50 mg/L, and a loading rate based on one-half the design load for a 10-year period as prescribed by the *Wisconsin Mound Soil Absorption System: Siting, Design, and Construction Manual* (Converse et al., 1990). An additional sheet of 0.95-cm (3/8-inch) PVC was installed within the trough for the purpose of creating a tortuous flow path to insure flow throughout the entirety of the reactive zone. The materials of this saturated reactive zone were separated from the overlying sand by drainfield cloth that was draped over the outflow edge of the trough.

Specifications for the mound were adapted from Converse et al. (1990) (Fig. 1). The height of the mound was increased by 38.1 cm (15 inches) from the prescribed dimensions, to increase travel time for aerobic treatment of the effluent. The mound was designed and constructed according to Converse et al. (1990).

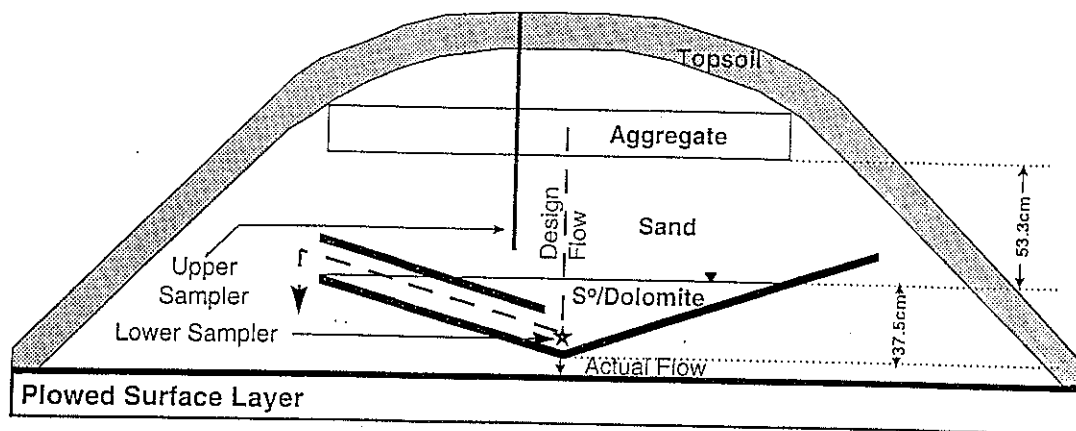


Figure 1. Mound system cross-section (not to scale).

Effluent was dosed through 0.635 cm (1/4 inch) threaded brass elbows and 0.635 cm (1/4 inch) PVC hosebarb elbow, mounted to the top of the distribution lateral. The hosebarb fitting was included to enable measurement of the dose volumes from the mound surface by attaching a length of Tygon tubing. The lateral was placed in the center of the PVC sheets.

Samplers made of 3.175 cm (1.25 inch) diam. slotted pipe 0.15 mm (0.006 inch) were installed in each cell at the bottom of the saturated zone (Fig. 1). Samplers were fitted with 0.635 cm x 0.953 cm (1/4 inch x 3/8 inch) male brass elbows, connected to 0.635 cm (1/4 inch) diam. Polyflo plastic tubing (fitted with PVC hosebarbs) that extended to the soil surface. Sampler ports were housed in plastic irrigation valve boxes, with heat tape installed to prevent freeze-up at the surface. Porous ceramic cup lysimeters, 1.58 cm (5/8 inch) diam., were installed in the unsaturated, untreated portion of the cells to confirm that the nitrification process was proceeding. The lysimeters were placed 12.7 cm (5 inch) above the uppermost portion of the saturated zone (Fig. 1). The ceramic cups were glued with epoxy to 1.91 cm (3/4 inch) schedule 40 pipe that extended to the surface through the 15.24 cm (6 inch) access tubes. An electric vacuum pump was used with a 3.66 m (12 ft) long, 3.81 cm (1.5 inch) diam. schedule 40 manifold, to obtain samples from the lysimeters.

Heavy duty 50-watt light bulbs were installed within the PVC access tubes to prevent freeze-up of samples drawn from the lysimeters, as well as providing heat to the system during the winter months to insure nitrification of the effluent. A wood enclosure was constructed to house the lysimeter vacuum pump, a Campbell Scientific datalogger, and electrical outlets.

#### Hydraulic Loading Characteristics

Loading rates were based on the criteria recommended by Converse et al. (1990), for a system using sand that meets C-33 specifications (ASTM, 1984) sited on a silt loam textured soil, with a moderate or strong structure. The applied loading rate was based on an approximate doubling of the "average" loading rate described for a three-bedroom house. This rate translates to approximately 60% the design loading rate of 450 gal./day. Each cell received an average dose of 3429 mL (0.905 gal.) at 6-hour intervals. Total daily loading rate was 109.7 (29 gpd) L/day, linear loading rate 30.0 L (2.4 gpd/Lf)/day/meter and sand loading rate was 24.6 L/day/(m<sup>2</sup>).

A spike of 189 g of NH<sub>4</sub>Cl was added to the pump chamber for the purpose of examining the system's retention time and its capacity to treat an increased concentration of nitrogen. The pump chamber was filled to its 1893 L (500 gal.) capacity with STE on April 29, and received no additional effluent during the 15-day monitoring period.

#### System Monitoring

System start-up occurred on November 30, 1996 (Julian day 335). Sampling was carried out for a period of 124 days, beginning December 1, 1996 and ending April 3, 1997. Wastewater samples were taken on a daily basis during the initial 16 days of operation. The system was sampled on a twice weekly basis from December 29 through January 14. A weekly sampling schedule was carried out following this date. No samples were taken during the first 3 weeks of March due to pump failure. Two series of samples were taken for fecal coliform analysis. Three series were taken for BOD<sub>5</sub>. An additional series of seven samples were taken between April 29 and May 13 to observe the effects of the NH<sub>4</sub>Cl addition.

Copper-constantan thermocouples were installed at each sampling point to monitor temperatures within the system and the atmosphere. Temperature was recorded in every 6 hours with a Campbell Scientific datalogger.

Effluent analyses were performed for the following parameters: nitrate-N (NO<sub>3</sub><sup>-</sup>-N), ammonium-N (NH<sub>4</sub><sup>+</sup>-N), organic-N, chloride Cl<sup>-</sup>, pH, and organic carbon. Nitrogen species were analyzed in accordance with *Methods of Soil Analysis* (Page et al., 1982); organic nitrogen by Kjeldahl digestion, ammonium-N and nitrate-N by steam distillation methods using MgO

and Devarda's alloy. The pH was measured in the field at the time of sampling. Chlorides were measured with a Buchler Instruments Chloride Station. Organic carbon was measured using a Tekmahr Dohrman DC-190 Total Organic Carbon Analyzer. Samples from the pump chamber (STE) were filtered through a 1- $\mu$  filter prior to analysis for organic carbon, yielding concentrations for dissolved organic carbon. Fecal coliforms and BOD<sub>5</sub> were measured in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995).

## RESULTS and DISCUSSION

After running the system with tap water, wastewater was applied on November 30, 1996, and an acclimation period occurred. During this time tap water was displaced by wastewater and a bacterial flora was being established. The acclimation phase lasted for about 30 days and is shown in (Fig. 2 and 3).

Daily averages of total nitrogen (expressed as N) found in the system are shown in (Fig. 3). High percentage levels of nitrogen removal were observed in the samples taken from both sulfur treated mixtures (as shown by the dashed lines with circles and triangles). This contrasts with the low levels of removal that were seen in the sand controls (as shown by the dotted line with diamonds), and the unsaturated upper portions of the cells (expressed by thick black line with squares). The negative figures reported here indicate the possible accumulation of N (as NH<sub>4</sub><sup>+</sup> or organic N) by adsorption. Or else its the result of incongruities between the

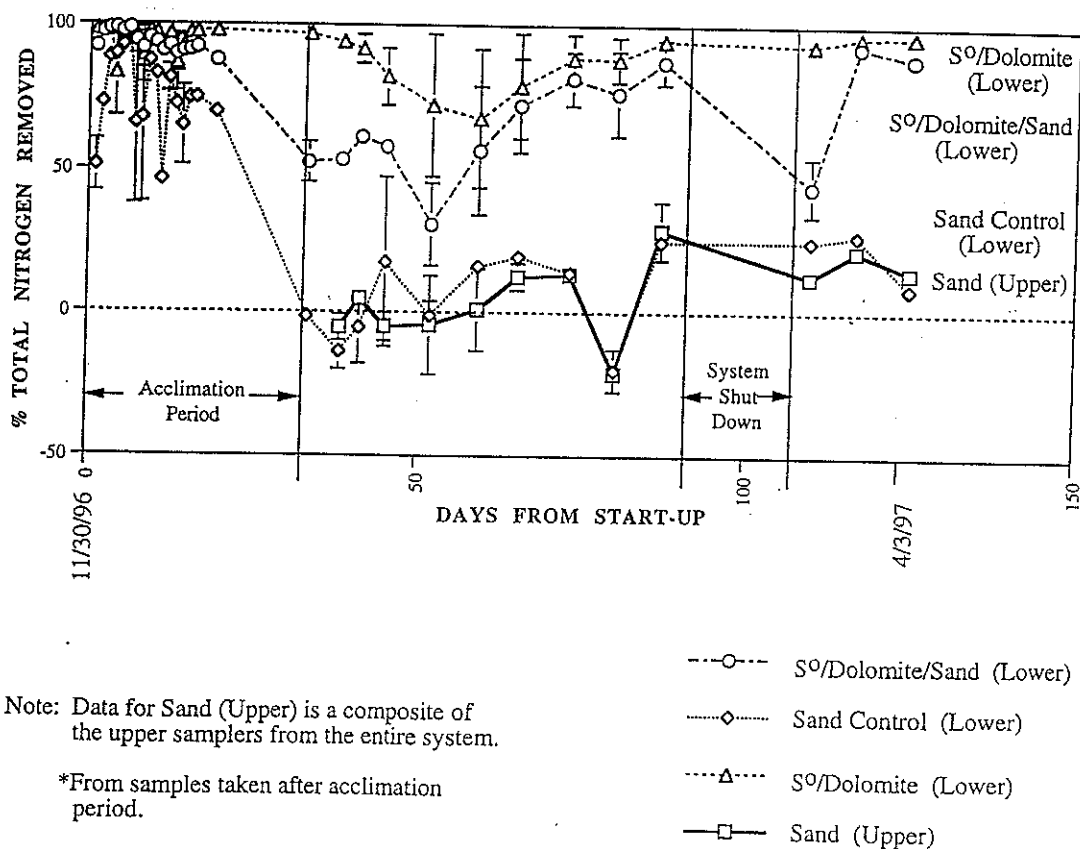


Figure 2. Percent total nitrogen removed at upper and lower samplers. Mean total N from STE = 25.7 mg/L.

samples and the STE caused by the travel time differential. Concentrations of total N (Fig. 3) and percent total nitrogen removal in the upper portions of the cells (as shown by the thick black line with squares) and the lower sand controls (dotted line with diamonds) were similar throughout the regular (post acclimation) sampling period.

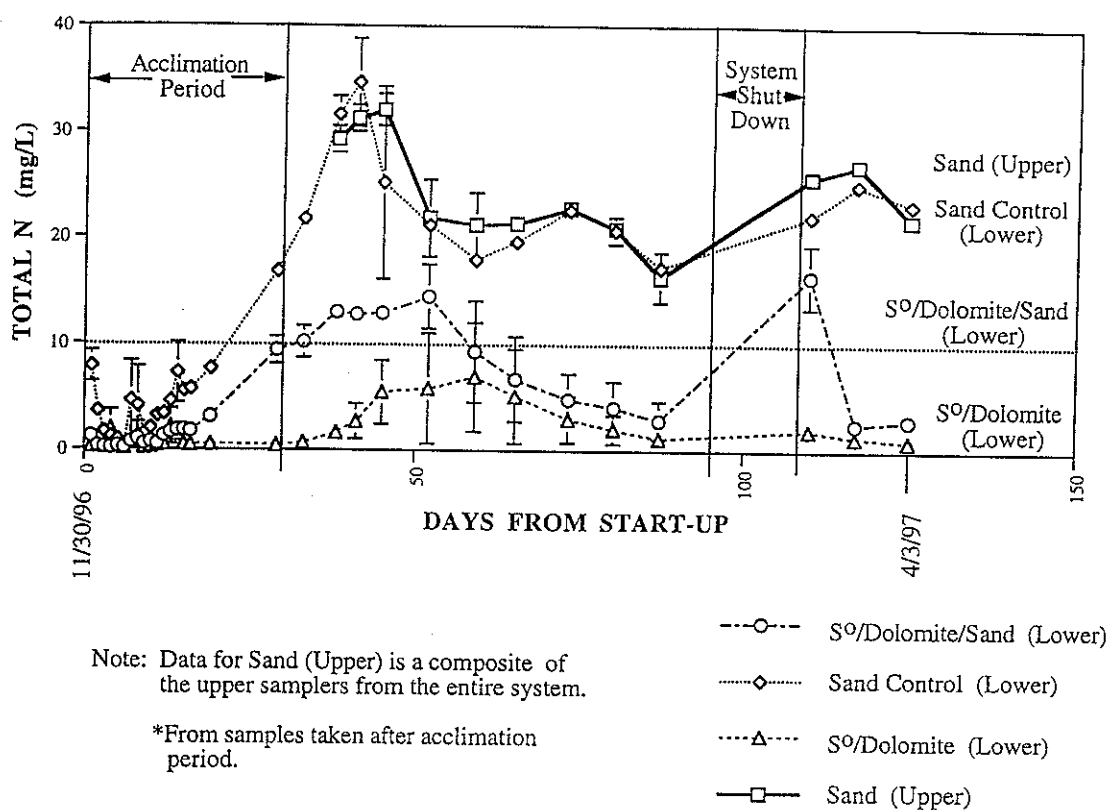


Figure 3. Total nitrogen concentrations (mg/L) at upper and lower samplers. Mean total N from STE = 25.7 mg/L

There was a decrease in the percentage rate of nitrogen removal in the treated cells 52 days after system start-up (Fig. 2). This drop coincided with a prolonged cold period (ambient temperatures -20 to -25 C°) in mid-January.

After running the system for an initial period, it became evident that the flow restricted portions of the cells were not maintaining the designed water level. Leaks in the troughs were confirmed through water level measurements taken through access tubes. Leakage rates in the troughs containing the sand matrices were found to occur at approximately three times the rate of those troughs containing the dolomite matrix. This difference could be a function of the packing of the platy shaped dolomite chips. Though leakage has significantly affected the system's flow path, it has had little effect on the system's overall capacity to remove nitrogen as the samples were taken from the bottom of the trough and not from the originally intended outlet.

A 2-week shut down period due to pump failure is indicated in (Fig. 2 and 3). Prior to re-dosing of the system, it was noticed that the cells containing the dolomite matrix still contained wastewater at the bottom of the troughs, while the remaining cells had fully drained. The effect of this shut down is evident in cells containing the S<sup>2</sup>/dolomite/sand mixture by the drop in % total-N removed (as seen in Fig. 2), and a subsequent increase in the concentrations

of total-N (as seen in Fig. 3). The lack of a subsequent drop in the cells containing the S<sup>0</sup>/dolomite mixture and the consistently lower concentrations of total-N seen in this mixture in comparison with the S<sup>0</sup>/dolomite/sand mix, is probably an effect of this difference in retention times found in the system's cells. Nitrogen removal rates stabilized within the following week as the S<sup>0</sup>/dolomite/sand cells re-acclimated.

The mean percent nitrogen removal from January 2 to April 3, 1997 was reported as 66.0 and 87.9 in the lower portions of the S<sup>0</sup>/dolomite/sand and S<sup>0</sup>/dolomite treatments, respectively (Table 1). This contrasted with the rate of 8.6% reported in the lower portions of the sand controls. Septic tank effluent had average total-N concentrations of 25.7 mg N/L and only 0.5 mg N/L as nitrate. This total is nitrified in the sand and NO<sub>3</sub><sup>-</sup> concentrations in the upper sample points of 23.5, 20.5, and 22.0 mg NO<sub>3</sub><sup>-</sup>/N/L were present. Nitrification was confirmed by the NO<sub>3</sub><sup>-</sup>-N concentrations observed in the upper (suction lysimeter) samples taken from the aerated unsaturated portion of the system (Table 1). The decreases in the total-N/Cl<sup>-</sup> ratios of the STE (1.6) from the overlying unsaturated regions to those observed in the saturated portions of the treated cells (0.6 and 0.2), respectively, indicate the removal of NO<sub>3</sub><sup>-</sup>-N by biological denitrification rather than dilution. Chloride concentrations were relatively constant with mean concentrations 13 to 17 mg Cl<sup>-</sup>/L throughout the system during the main sampling period.

Table 1. Total N, NO<sub>3</sub><sup>-</sup>-N and % total N removal total N/Cl<sup>-</sup> ratio from septic tank effluent (STE) and samples from upper and lower samplers. Means for each treatment from samples taken January 2 to April 3, 1997.

Statistics	STE Pump chamber	Sand (control) upper	Sand (control) lower	Sand upper trt. 1	S <sup>0</sup> /dolomite sand lower trt. 1	Sand upper trt. 2	S <sup>0</sup> /dolomite lower trt. 2
Total N (mg/L)							
Mean	25.7	24.4	23.2	24.3	8.7	23.5	3.0
Range	34-4-17.2	36.5-16.0	38.9-16.1	43.2-2.3	22.5-0.5	31.9-16.8	17.0-0.15
n	13	24	26	34	39	24	39
NO <sub>3</sub> <sup>-</sup> -N (mg/L)							
Mean	0.5	23.5	21.1	20.5	4.8	22.0	1.2
Range	1.4-0.1	36.0-15.3	33.6-10.90	36.3-1.7	20.8-0.1	30.5-15.9	11.7-0.1
n	13	24	26	34	39	24	39
Total N/Cl <sup>-</sup> ratio (mg/L)							
Mean	1.6	1.4	1.5	1.5	0.6	1.5	0.2
Range	1.8-1.9	2.4-1.1	2.5-1.0	3.9-0.1	1.7-0.0	2.2-1.1	1.3-0.1
n	13	20	26	34	39	23	39
% Total N removed (mg/L)							
Mean	NA†	6.2	8.6	3.9	66	9.6	87.9
Range	NA	23.3-24.9	47.3-27.6	90.1-106.9	98-5	23.2-19.2	99.1-20.3
n	NA	24	26	34	39	24	39

† NA = not available.

The low concentrations of organic carbon found in the control cells (Table 2) and the lack of NO<sub>3</sub><sup>-</sup> removal, suggested that there is not enough C to allow denitrification with carbon. Therefore the NO<sub>3</sub><sup>-</sup> loss in the treatment cells is considered to be the result of sulfur oxidizing/nitrate reducing bacteria. No inoculation of the system with sulfur oxidizing bacteria was carried out at any time, thus the assumption can be made that these bacteria are indigenous to the sulfur or other materials that were introduced to the system.

Table 2. Organic carbon and pH from septic tank effluent (STE) and samples from upper and lower samplers. Means for each treatment from samples taken January 2 to April 3, 1997.

Statistics	STE pump chamber	Sand (control) upper	Sand (control) lower	Sand upper trt. 1	S <sup>0</sup> /dolomite sand lower trt. 1	Sand upper trt. 2	S <sup>0</sup> /dolomite lower trt. 2
Organic carbon (mg/L)							
Mean	53.1†	4.0	2.8	4.1	4.0	3.7	4.6
Range	74.2-37.5	8.3-1.0	4.8-0.0	7.4-0.3	9.2-0.0	7.7-0.6	11.5-0.6
n	11	20	20	28	30	20	28
pH							
Mean	7.1	7.4	6.8	7.2	6.5	7.3	6.5
Range	7.3-6.8	7.8-7.1	7.0-6.6	7.5-6.9	6.7-6.2	7.7-7	6.6-6.3
n	12	22	23	31	36	21	36

† Samples of STE were filtered prior to analysis, thus this figure represents the concentration of total *dissolved* organic carbon.

Although the pH readings observed indicate a slight increase in acidity in the sulfur treated cells, the readings of 6.5 indicate the effectiveness of the dolomite in maintaining a buffered system. In the absence of a liming material such as dolomite, this drop can be expected to be much more dramatic.

Intermittent sampling for fecal coliform bacteria and BOD<sub>5</sub> indicate that the system's ability to remove fecal indicator bacteria and BOD<sub>5</sub> to levels similar to those achieved in traditional mound systems.

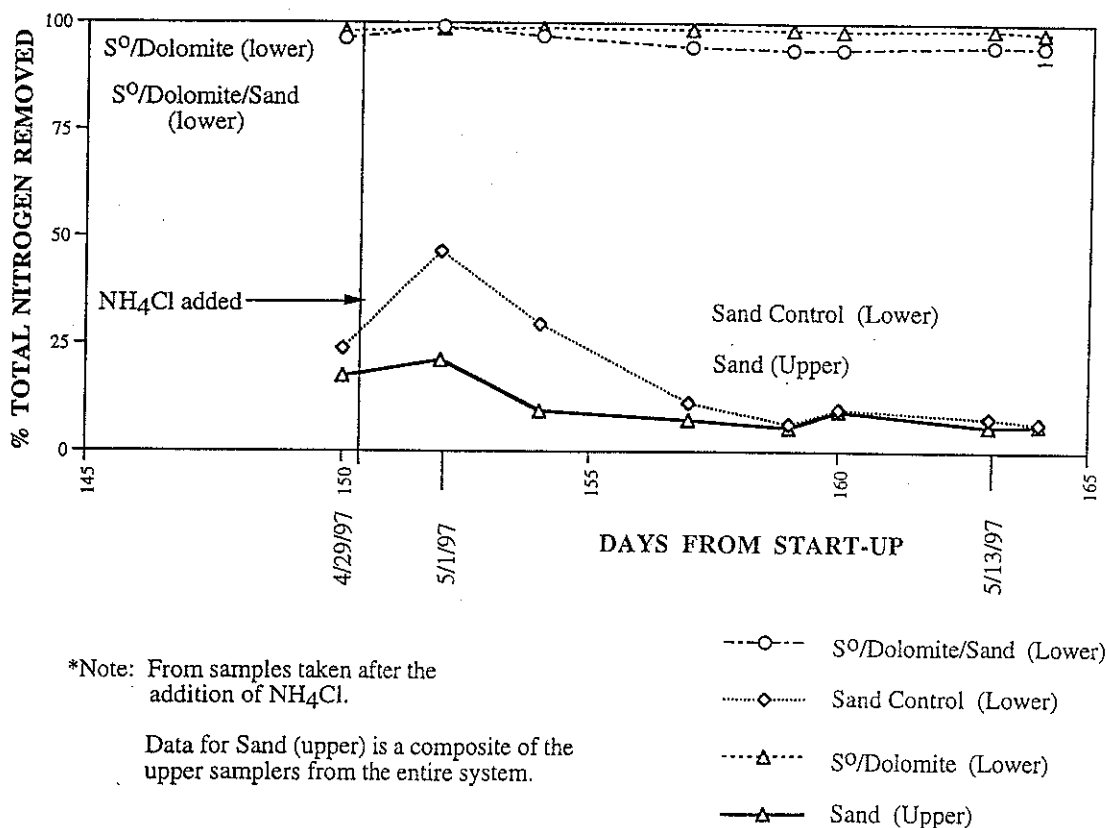


Figure 4. Percent total nitrogen removed at upper and lower samplers with NH<sub>4</sub>Cl added. Mean total N from STE = 51.7 mg/L.



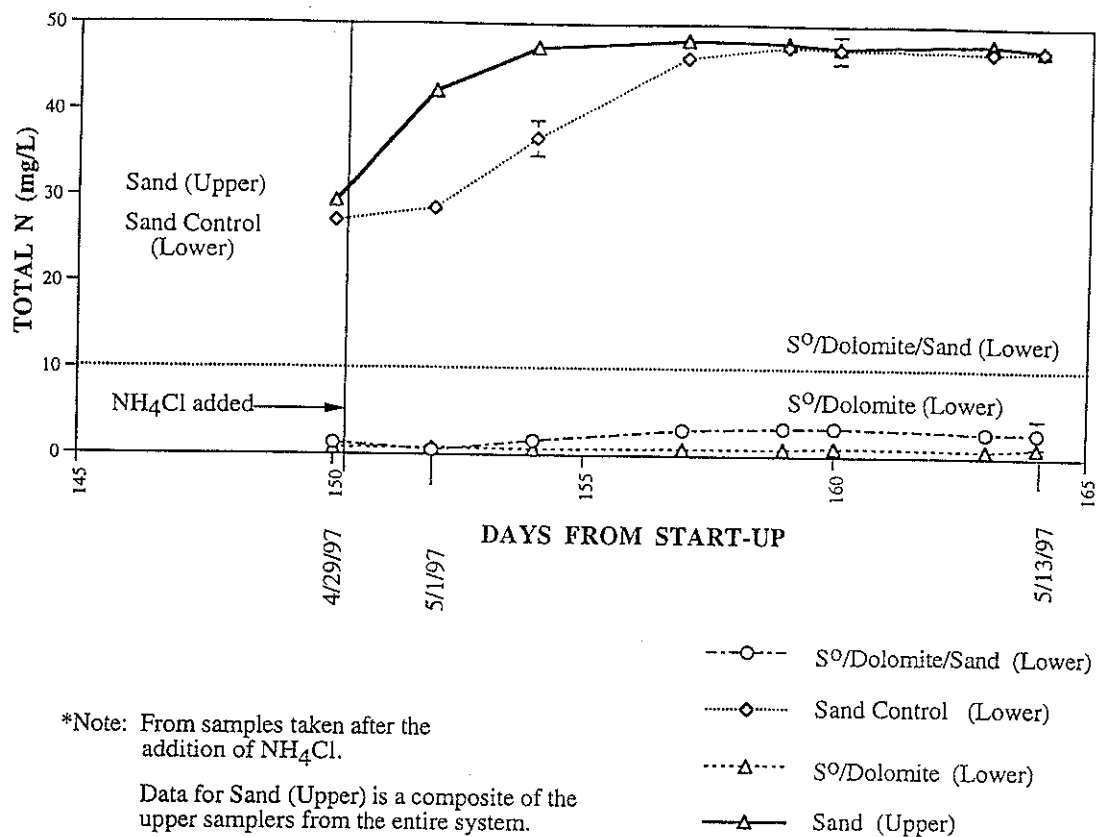


Figure 5. Total nitrogen concentrations (mg/L) at upper and lower samplers with  $\text{NH}_4\text{Cl}$  added. Mean total N from STE = 51.7 mg/L.

A spike of  $\text{NH}_4\text{Cl}$  was added to the pump chamber, increasing the mean total nitrogen concentration from 25.7 to 51.7 mg N/L. Figures 4 and 5 indicate constant levels of nitrogen removal during this period. Samples taken from the upper unsaturated portions of the system (as shown by the thick black line with squares) and the lower saturated portions of the sand controls (as shown by the dotted line with diamonds) indicate an initial 6- to 7-day period for the full establishment of the spiked wastewater within the system. The information observed here indicate the system's ability to effectively remove nitrogen from wastewater at concentrations more representative of typical domestic wastewater than those applied during the regular sampling period. While the percent rate of nitrogen removal in the  $\text{S}^0/\text{dolomite}$  and  $\text{S}^0/\text{dolomite/sand}$  treated cells tended to fluctuate directly with increased total-N concentrations in the STE during the regular sampling phase (January 2 to April 3), relatively constant levels of nitrogen removal were reported in the  $\text{S}^0/\text{dolomite}$  and  $\text{S}^0/\text{dolomite/sand}$  treated cells during the period with additional  $\text{NH}_4\text{Cl}$  (April 29 to May 13). This suggests that the consistently high percentage rate of nitrogen removal is a function of temperature rather than concentration during the warmer months. The increase in the percent nitrogen removal seen on May 1, 1997 for the lower sand control (Fig. 4) (shown as a dotted line with diamonds) is due to an acclimation period. At this date, the spiked STE had not fully displaced all of the effluent remaining in the system from the pre-spike period.

Data presented in Table 3 indicate percent total N removal of 95.2, 98.4, and 16.7 for the  $\text{S}^0/\text{dolomite/sand}$ ,  $\text{S}^0/\text{dolomite}$ , and sand controls, respectively. Mean concentrations of total N were 2.5, 0.8, and 43.0 mg. N/L for the  $\text{S}^0/\text{dolomite/sand}$ ,  $\text{S}^0/\text{dolomite}$  and the sand controls

amendment, under anaerobic saturated flow conditions, is capable (without additional bacterial inoculation) of consistently reducing the concentration of total nitrogen below 10 mg N/L in a mound-type system in the absence of extreme winter temperatures. Limited acidity is produced due to the buffering capacity provided by the dolomite amendment. Questions remain regarding the levels of sulfate produced by the system, and the overall longevity of such a system. Research is continuing with regard to these issues.

## REFERENCES

1. American Public Health Association (APHA). 1995. *Standard Methods for the Examination of Water and Wastewater*. Nineteenth Edition. Am. Public Health Assoc., Washington, DC.
2. ASTM. 1984. *Standard Specific for Concrete Aggregate C-33*. Am. Soc. of Testing Materials, Philadelphia, PA.
3. Batchelor, B. and A.W. Lawrence. 1978. Autotrophic denitrification using elemental sulfur. *J. Water Pollut. Control Fed.* Aug. 1978, pp. 1986-2002.
4. Converse, J.C., E.J. Tyler, and J.O. Peterson. 1990. *Wisconsin Mound Soil Absorption System: Siting, Design and Construction Manual*. Small Scale Waste Management Project, Univ. of Wisconsin-Madison. Publication 15.22. 40 pp.
5. Page, A.L., R.H. Miller, and D.R. Keeney (ed.). 1982. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Second Edition. Agronomy no. 9. ASA and SSSA, Madison, WI. 1159 pp.
6. Robertson, W.D., J.A. Cherry, and E.A. Sudicky. 1991. Ground-water contamination from two small septic systems on sand aquifers. *Ground Water* 29(1):82-92.
7. Schippers, J.C., J.C. Kruithof, F.G. Mulder, and J.W. van Lieshout. 1987. Removal of nitrate by slow sulphur/limestone filtration. *Aqua* 5:274-280.
8. Sikora, L.J. and D.R. Keeney. 1976. Evaluation of a sulfur-*Thiobacillus denitrificans* nitrate removal system. *J. Environ. Qual.* 5:298-303.
9. Walker, W.G., J. Bouma, D.R. Keeney, and P.G. Olcott. 1973. Nitrogen transformations during subsurface disposal of septic tank effluent in sands: II Ground water quality. *J. Environ. Qual.* 2:521-525.
10. Wenst, R.G. (ed.). 1971. *CRC Handbook of Chemistry and Physics*. The Chemical Rubber Co., Cleveland, OH.
11. Xiushan, Y. G. Garuti, and A. Tiliche, 1993. Denitrification with *Thiobacillus denitrificans* in the Ananox process. *Biotechnol. Lettr.* 15(5):531-536.