

## Nitrogen balance and cycling in an ecologically engineered septage treatment system

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Received 12 May 2000; received in revised form 14 December 2000; accepted 15 December 2000

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### Abstract

Septage disposal presents technical difficulties due to its high concentrations of solids and nutrients. Many existing treatment options create excessive waste solids or fail to remove nutrients, which, upon discharge, impact groundwater and aquatic systems. This study evaluated nitrogen removal in an ecologically engineered wastewater treatment system (the Solar Aquatic System™ [SAS]) that combines aeration and activated solids recycling with aquatic and constructed wetlands treatment components. A septage-treating SAS in coastal Massachusetts, USA, produced an effluent of 113 mg/l chemical oxygen demand, 16 mg/l total suspended solids (TSS), 0.56 mg/l ammonium nitrogen, 6.1 mg/l total nitrogen and 1.5 mg/l total phosphorus. Waste solids production was low at 0.81 g/g influent TSS, including harvested plant biomass. Primary treatment was by biological oxidation with activated solids, followed by gravity clarification. Further oxidation and ammonium removal occurred in planted aerated aquatic tanks enclosed in a greenhouse, followed by secondary gravity clarification. Nitrogen removal in the aquatic tanks was limited by denitrification, which was limited by the availability of anaerobic microsites. Mineralization of particulate organic nitrogen, followed by nitrification and denitrification, occurred simultaneously during primary treatment and in the aquatic tanks. Final solids polishing and denitrification occurred in a constructed wetland. Although 88% of the influent nitrogen was removed during primary treatment, further treatment in the aquatic tanks and wetlands was required to reduce effluent nitrogen to tertiary standards. Nitrogen removal in the whole system was primarily by sedimentation of waste solids, accounting for 57.6% of the influent nitrogen, followed by denitrification, at 40.9%. Direct uptake of nitrogen by plants was insignificant, at 0.5%. 1.0% of the total influent nitrogen left the facility in the effluent water. The SAS has the capability to treat septage to tertiary standards with low solids production in small, decentralized facilities. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Constructed wetland; Denitrification; Ecological engineering; Nitrification; Nitrogen; Septage; Wastewater

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## 1. Introduction

Septic tanks are used for on-site disposal of about 25% of U.S. domestic wastewater (U.S. EPA, 1987). Inexpensive, reliable and suited to areas of low population density, septic tank use may reach 100% in some non-urban areas. Nonetheless, septic disposal of wastewater can be an important contributor of nutrients to natural waters (Weiskel and Howes, 1991). Regionally, septic leachate adds 320 MT nitrogen per year to coastal ecosystems of Buzzards Bay, Massachusetts, USA, 26% of the total municipal wastewater loading (Howes and Goehringer, 1996).

In on-site septic treatment, wastewater undergoes anaerobic biodegradation and sedimentation in an underground tank, and the clarified wastewater, high in inorganic nitrogen and phosphorus, is discharged to a subsurface leaching field (Wilhelm et al., 1994). Biologically labile organic material is degraded within the tank, resulting in the accumulation of recalcitrant solids, which must be periodically removed. Solids accumulate within the tank at a rate of approximately 40–73 l per person per year (Philip et al., 1993). Upon removal, this mixture of accumulated solids and

liquid is termed septage. Septage contains a very high concentration of organic matter (total volatile solids 8200–27 600 mg/l), and biodegradation of these solids results in the production of high levels of dissolved nutrients (Canter and Knox, 1985). In coastal areas, nitrogen (N) is the most important contributor to eutrophication. Nitrogen loads to the environment from septage disposal are not well studied, but may contribute an amount equal to septic tank leachate (Weiskel and Howes, 1991). Although in many areas, septic treatment may eventually give way to alternatives, the prevalence of septic treatment in the U.S. means that septage waste disposal will continue to be an issue for some time.

The pattern of generation and composition of septage differs from sewage in many ways, requiring different approaches to management and treatment. Unlike sewage, which is continuously collected from relatively densely populated regions, septage is collected periodically by tanker truck from less densely populated areas (1–103 septic systems/km<sup>2</sup>; Canter and Knox, 1985). In addition, septage production tends to be highly seasonal, with septage removal concentrated during the warmer months. Septage is also many

Table 1  
Composition of influent and effluent at the Marion SAS<sup>a</sup>

| (mg/l)             | Marion septage <sup>b</sup> | Minimum | Maximum | Providence sewage <sup>c</sup> | Marion effluent <sup>d</sup> | Minimum | Maximum | Orleans effluent <sup>e</sup> |
|--------------------|-----------------------------|---------|---------|--------------------------------|------------------------------|---------|---------|-------------------------------|
| COD <sup>f</sup>   | 11 700                      | 1950    | 22 300  | 323                            | 113                          | 1.6     | 254     | –                             |
| TSS                | 7460                        | 2520    | 23 600  | 165                            | 16                           | 1       | 45      | 54                            |
| NH <sub>4</sub> -N | 32.8                        | 1.6     | 128     | 10.1                           | 0.56                         | 0.05    | 1.98    | 28.2                          |
| NO <sub>x</sub> -N | 1.1                         | 0.5     | 2.1     | –                              | 1.7                          | 0.5     | 6.2     | 10.9                          |
| PON                | 444                         | 150     | 1400    | –                              | –                            | –       | –       | –                             |
| TN                 | 483                         | 160     | 1540    | –                              | 6.1                          | 4.4     | 11.8    | 52.6                          |
| TP <sup>g</sup>    | 51.5                        | 16.5    | 88      | 3.8                            | 1.5                          | 0.2     | 1.6     | 0.7 <sup>h</sup>              |

<sup>a</sup> Screened and dewatered sewage (Providence) shown for comparison to septage, and effluent from a nearby septage-treating rotating biological contactor (Orleans) shown for comparison with Marion effluent.

<sup>b</sup> Composition of screened and dewatered septage from the Equalization tank (EQ) at the Marion SAS. Flow-weighted mean concentration during 6 months operation,  $n = 22$ .

<sup>c</sup> Screened and dewatered sewage from Field Point Sewage Treatment Facility, Providence, RI (source: EEA personal communication). 1 year average,  $n = 64$ .

<sup>d</sup> Flow-weighted concentrations over 6 months operation,  $n = 22$ .

<sup>e</sup> Tri-Town Septage Treatment Facility, Orleans, Mass. Average over 2 years,  $n = 59$  (DeSimone and Howes, 1995).

<sup>f</sup> Chemical oxygen demand.

<sup>g</sup> Total phosphorus.

<sup>h</sup> Precipitated with ferric chloride.

times more concentrated than sewage, in both dissolved and solid constituents (Table 1). The high organic and nitrogenous strength of septage requires different approaches to treatment than are used for sewage (Kersten, 1994). Given the dispersed nature of the source, the associated transport costs, and the interposition of jurisdictional boundaries, smaller, local facilities may be more practical than large, centralized septage treatment facilities.

A number of treatment strategies have been applied, ranging from those which treat septage as concentrated wastewater, to those which treat it as waste solids. Perhaps the most commonly employed alternative to older infiltration lagoons is co-treatment with sewage in municipal facilities. However, this approach is limited by the anaerobic and highly concentrated nature of septage (biological oxygen demand [BOD<sub>5</sub>] typically 30–70 times and ammonium nitrogen [NH<sub>4</sub>-N] 3–6 times sewage levels, respectively). Consequently, co-treatment requires a large dilution with sewage influent to prevent disruption of system operations (Canter and Knox, 1985; Kersten, 1994). Septage is also treated as solids, for example by precipitation with lime and ferric chloride and dewatering with a filter press.

Ecologically engineered wastewater treatment systems are one option which may have the potential to address the special treatment requirements of septage. Ecologically engineered systems utilize the properties of natural wetlands, which contain diverse microenvironments capable of supporting high metabolic and species diversity which may aid in the degradation of recalcitrant organic material and support nutrient removal processes. The biological N removal processes of wetlands have low environmental impact, converting biologically-available N compounds into N<sub>2</sub> gas. Organic N is first mineralized into ammonium (NH<sub>4</sub><sup>+</sup>) via the degradative actions of invertebrate and microbial communities. NH<sub>4</sub><sup>+</sup> is then used as an energy source by obligately aerobic chemosynthetic nitrifying bacteria, producing nitrate (NO<sub>3</sub><sup>-</sup>) as an endproduct (Focht and Chang, 1975). Finally, denitrification, an obligately anaerobic heterotrophic respiratory pathway, uses NO<sub>3</sub><sup>-</sup> as a terminal electron acceptor, reducing it to N<sub>2</sub> gas.

One new system for treating septage that addresses both the solids and nutrient issues is the Solar Aquatic System (SAS)<sup>TM</sup> (Ecological Engineering Associates [EEA], Marion, MA., USA). This system uses an ecologically engineered process that treats septage and other high-strength organic wastewaters to tertiary standards. The SAS incorporates aeration and activated solids recycling into a design that combines aquatic and constructed wetlands components, to enhance N removal through microbial mineralization, nitrification and denitrification reactions (Teal and Peterson, 1991, 1993; Teal et al., 1994; Peterson and Teal, 1996). However, the factors that control N removal within these systems remained unclear (Teal et al., 1994).

Progress in developing design criteria for ecologically engineered wastewater treatment systems such as constructed wetlands has been limited by the lack of experimentally-verified understanding of removal mechanisms. This study applies methods and analysis derived from biogeochemical studies of wetlands and aquatic systems to develop a N balance based upon measured inputs and outputs of N over 6 months of system operation. Denitrification as a N removal mechanism is evaluated relative to overall N loading and other N outputs. Nitrogen cycling within each component of the SAS, particularly the ecologically engineered components, was determined to identify the primary sites of N removal.

## 2. Methods

### 2.1. Abbreviations

DO, dissolved oxygen; DON, dissolved organic nitrogen; NH<sub>4</sub>-N, ammonium; NO<sub>x</sub>-N, nitrate plus nitrite; ON, organic nitrogen (PON + DON); POC, particulate organic carbon; PON, particulate organic nitrogen; SAS, Solar Aquatic System; TN, total nitrogen (ON + NH<sub>4</sub>-N + NO<sub>x</sub>-N); TSS, total suspended solids.

#### 2.1.1. System description

Research was conducted at a demonstration-scale septage-only SAS in Marion, MA., USA,

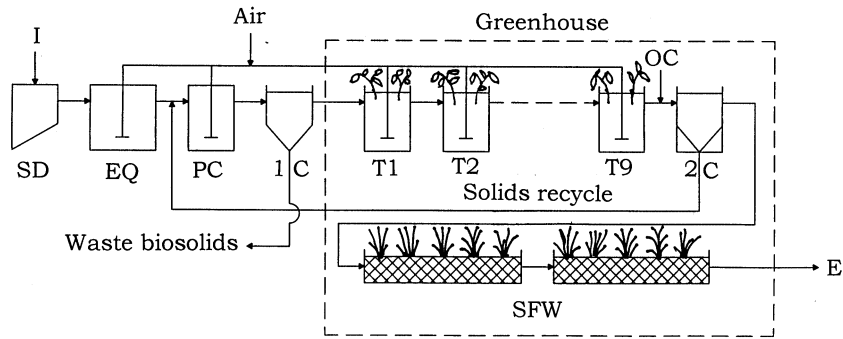


Fig. 1. Flow diagram of Marion Solar Aquatic septage treatment facility. I, influent from pump trucks, SD, screening and degritting; EQ, equalization tank; PC, preliminary conditioning tank; 1C and 2C, primary and secondary clarifiers, T1...T9, aquatic tanks 1–9 with floating plants; SFW, subsurface flow constructed wetlands, E, effluent. Aquatic tanks and wetlands are enclosed in a greenhouse.

receiving 2200 l septage per day (Fig. 1). The facility, operating since 1993, is similar to a former larger facility in Harwich, Mass. (Teal and Peterson, 1993). Raw septage from domestic and commercial sources is tankered to the site. The influent septage is bar screened (2 cm) and degritted before entering a 19 000 l aerated equalization tank. The equalization tank allows the blending of septage loads, reducing the variability of the influent. The equalization tank is regularly seeded with a commercial bacterial preparation containing species extracted from natural wetland environments (Aquaresearch Inc., North Hatley, Quebec, Canada). These species include *Aerobacter aerogene*, *Bacillus subtilis*, and *Cellulomonas biazotea* (for reducing solids formation), *Pseudomonas denitrificans*, *P. stutzeri*, *Rhodopseudomonas palustris*, *Nitrosomonas sp.*, and *Nitrobacter winogradskyi* (for promoting nitrification and denitrification), and *Acinetobacter* (for reducing grease content, bulking and foaming) (Ehrlich et al., 1991).

The equalization tank discharges via a grinder pump to an aerated 3800 l preliminary conditioning tank where influent is mixed 3:1 with an activated biosolids suspension from the secondary clarifier in the greenhouse portion of the facility (see below; Fig. 1). Effluent from the preliminary conditioning tank undergoes solids removal in the 3000 l primary gravity clarifier, and the clarified primary effluent is treated by the greenhouse system. Waste biosolids from the primary clarifier are pumped to an aerated holding tank for stabi-

lization (average 12 days) and disposal. The flow of primary effluent into the greenhouse averaged 2600 l per day (including the secondary biosolids recycle) over the 6 month period of study.

The clarified primary effluent is gravity fed into the greenhouse through two parallel trains, each consisting of nine aerated aquatic tanks (0.75 m dia.  $\times$  1.5 m high, 620 l; total vol. 11 200 l; total tank surface area 11.6 m<sup>2</sup>). Aeration averages 1 m<sup>3</sup>/h per tank, and was adjusted to maintain DO > 5.0 mg/l (mean  $7.2 \pm 0.2$  mg/l) in all tanks. The tanks are planted with a mixture of floating plants including willows (*Salix nigra* Marshall), water hyacinth (*Eichhornia crassipes* (Mart) Solms), pennywort (*Hydrocotyle umbellata* L.), primula (*Primula veris* L.) and mint (*Mentha arvensis* L.). Root penetration into the water column is 20–40 cm or  $\sim 20\%$  of the water column. Translucent tank walls allow the development of an algal-bacterial mat inside. The tanks also support large populations of zooplankton, nematodes and snails. The tanks are designed to support a large degree of microhabitat diversity, with fixed biofilms found on roots and tank walls, root-associated detritivorous invertebrates, and aerobic and anaerobic zones in close proximity. Effluent from the final tank in each train, T9, flows to secondary gravity clarifiers (Fig. 1; 620 l each). Settable solids comprising  $\sim 30\%$  of the flow are recycled back to the preliminary conditioning tank, which allows microorganisms and invertebrates from the aquatic treatment tanks to

seed themselves in primary treatment. The supernatant discharges into subsurface flow gravel bed wetlands (22.3 m<sup>2</sup> × 0.6 m deep) planted with cattails (*Typha latifolia* L.), sedges (*Cyperus spp.* L.) and primula, where final solids polishing and denitrification occur before discharge. The wetland is designed to promote denitrification through plant-derived organic carbon, as well as trap residual organic particles.

## 2.2. Analytical methods

All samples were collected in duplicate and transported to the laboratory on ice. Upon reaching the laboratory, the first sample was filtered through a 0.22 μm Millipore™ filter and the filtrate used for determinations of NH<sub>4</sub>-N, NO<sub>x</sub>-N and total dissolved N. NH<sub>4</sub>-N was analyzed immediately (to prevent ammonia volatilization) by the indophenol colorimetric method (Scheiner, 1976). NO<sub>x</sub>-N was analyzed with azo dye after cadmium reduction (Wood et al., 1967) using a Lachat Automated Ion Analyzer™. DON was measured as the difference between total dissolved N and NH<sub>4</sub><sup>+</sup>-N and NO<sub>x</sub><sup>-</sup>-N. Total dissolved N was measured as NO<sub>3</sub><sup>-</sup> following persulfate oxidation (D'Elia et al., 1977). A second sample was vacuum-filtered through precombusted Whatman™ GFF filters and dried to constant weight (60°C) to collect solids for TSS (APHA, 1989) and POC/N determinations, which were made on a Perkin-Elmer Model 2400™ CHN elemental analyzer. Samples of waste biosolids, plant tissue and plant litter were dried to constant weight at 60°C and ground for POC/N analysis. All reported values are means ± standard error.

Concentrations of nitrifying bacteria in the equalization and preliminary conditioning tanks, the greenhouse influent, the aquatic tanks, and associated with water hyacinth roots were estimated using the most probable number (MPN) method (APHA, 1989) as part of another study (Hamersley et al., in preparation (a)). Samples were homogenized (Virtis™ blender) for 4 min (minimum time required to maximize MPN results). Test tubes containing sterilized ammonia oxidizer medium (Schmidt and Belser, 1982) mixed with phenol red indicator were inoculated

with serial 10-fold dilutions of root and wastewater homogenates.

## 2.3. Whole-system performance and nitrogen mass balance

Determinations of total solids, NH<sub>4</sub>-N and NO<sub>x</sub>-N, content were made on raw influent, waste biosolids, primary clarifier effluent, secondary clarifier effluent and final effluent 22 times over the course of 6 months (05/94–12/94) by EEA as part of the NPDES permit requirements using standard methods (APHA, 1989). We estimated ON from total solids by regression-based DON and PON determinations made in our laboratory (0.0595 ± 0.0004 mg ON/mg total solids, *n* = 174, *r*<sup>2</sup> = 0.98). A water balance for the facility was made using volume measurements of influent septage, effluent water and waste solids production, combined with periodic measurements of flow rates into the greenhouse, and of recycled secondary solids. Evapotranspiration ('missing' water in water balance) for the whole facility was less than 3%, and was therefore disregarded in calculations. Nutrient data were flow-weighted for the development of the N mass balance for the facility. All harvested plant materials were weighed and samples taken for N content determinations. Plant N uptake was functionally defined as harvested biomass N.

Denitrification during primary treatment was estimated as the 'missing' N determined from the difference between facility influent N and all measured facility outputs, including solids and plant waste, final effluent, and separate estimations of denitrification losses in the greenhouse.

## 2.4. Aquatic tanks

To study the controls on N transformations within the aquatic tanks, nitrogen species (PON, DON, NH<sub>4</sub>-N and NO<sub>x</sub>-N) were monitored during normal operation. Preliminary data indicated that N concentrations in each tank were temporally variable. To increase replication, in each of the two treatment trains, flow from tank T2 was redirected to an experimental tank system (replicate tanks) replacing tanks T3 and T4, after which

it was pumped back into tank T5. The experimental tank system inserted into each train consisted of two parallel sub-trains of two aquatic tanks each (186 l each) in series. The four replicate tanks within each train (eight tanks total) were planted and aerated like the main tanks. The distribution of water to the sub-trains was periodically calibrated using graduated flasks and a stopwatch, and flow was equalized by adjustments to the splitter box. This region of the tank system (after T2) was selected for intensive study of internal nitrogen cycling because: (1) it has high rates of nitrogen transformations; (2) the essential mechanisms of nitrogen transformation are the same throughout the tank system; and (3) wastewater characteristics vary so widely that we were able to quantify the full spectrum of conditions found throughout the aquatic tank system within the experimental tank system alone, but with higher replication. Wastewater from each tank in both trains, as well as the experimental tank system, was sampled eight times over a 3 week period under normal flow. Samples were immediately placed on ice in coolers and transported (45 min) to the laboratory at Woods Hole for analysis.

Chloride tracer studies showed that flow in both the main and the replicate tanks conformed to the tanks-in-series model (Ruzicka and Hansen, 1988) and that the effective mixed volume averaged 73% of the total tank volume (unpublished data). Rates of nitrogen transformations were determined from the changes in concentration of nitrogen species between one tank and the previous one, divided by the tank's hydraulic retention time (based on concurrently measured flow rate and effective mixed volume).

Nitrogen mineralization rates were determined by the above method from the sum of the changes in PON and DON concentrations. Because we did not consider the measurements of N mineralization made in Tanks T5–T9 to be well replicated, the highly replicated measurements from the replicate tanks inserted after T2 were used. This rate was extrapolated to all the aquatic tanks using an empirically-derived exponential organic matter decay constant (determined using changes in oxy-

gen uptake rates during 6-week bottle incubations) from which we obtained an estimate of the mean mineralization rate throughout the aquatic tanks (Hamersley et al., in preparation (a)). Nitrification rates were determined from the sum of mineralization rates and changes in  $\text{NH}_4\text{-N}$ . Denitrification in the aquatic tanks was determined using an in situ approach during normal system operation. The rate of dissolved inorganic N ( $\text{NH}_4\text{-N} + \text{NO}_x\text{-N}$ ) increase over the entire series of tanks was subtracted from the mineralization rate to obtain an estimate of denitrification in all of the aquatic tanks by mass balance. Temperature, dissolved oxygen and flow rate were measured concurrently with water sampling. Dissolved oxygen was measured using a calibrated YSI™ Model 58 oxygen meter equipped with a self-stirring probe.

### 3. Results

#### 3.1. Whole system performance

During the 6-month interval of study, influent chemical oxygen demand averaged 11 700 mg/l, TSS averaged 7460 mg/l, TN averaged 483 mg/l,  $\text{NH}_4\text{-N}$  averaged 32.8 mg/l and total phosphorus averaged 51.5 mg/l (Table 1). Influent TN was primarily (92%) in the form of PON, with only minimal levels of  $\text{NO}_x\text{-N}$  (1.1 mg/l).

The septage-treating SAS reduced mean influent TN, 483 mg/l, by 99% to produce a mean effluent TN of 6.1 mg/l (Table 1). Of the N remaining in the effluent, 47% was DON, 28%  $\text{NO}_x\text{-N}$ , 16% PON and 9%  $\text{NH}_4\text{-N}$  (0.56 mg/l). Effluent TSS averaged 16 mg/l. Although no precipitants were used to reduce phosphorus, the system reduced total phosphorus by 97%, yielding an effluent averaging 1.5 mg/l. Of the 187 kg of total influent N, 57.6% was removed by sedimentation of biosolids (3.6% TS by weight; 98% as PON), and only 1.0% was discharged in the effluent stream. The production of waste solids (including plant biomass) was low: 5.9 g/l septage treated or 0.81 g/g influent TSS. Uptake of N into harvested plant biomass in the SAS accounted for only 0.5% of the total septage N input. Denitrification, as determined from missing N in the mass

balance, accounted for 40.9% of the influent N. Significant volatilization of ammonia was ruled out, since at the mean pH of 7.2 and temperature of  $\sim 20^{\circ}\text{C}$ , ammonia accounts for only 0.35% of the ammonium/ammonia pool.

Of the TN (66.3 mg/l average) entering the greenhouse after primary treatment, 94% was removed by the aquatic tanks and the wetland before discharge (Table 2, Fig. 2a). Removal was via recycled secondary biosolids, denitrification, sedimentation in the wetland, and harvested plant biomass. Nitrogen removal efficiency in the greenhouse components was relatively insensitive to the variations in loading rate resulting from change in flow or influent concentration over the measured loading range of 4.5–15.7 g N/m<sup>3</sup> per day. (Fig. 2b). There was an indication of a trend toward slightly higher N removal efficiencies of 0.5% for every 1 g N/m<sup>3</sup> per day increase in loading throughout this range (Fig. 2a). Mean N removal for another septage-treating SAS operating within a similar N loading range (Harwich, Mass.) showed comparable efficiencies (Fig. 2; Wright-Pierce, 1993).

### 3.2. Mineralization of organic nitrogen

Nitrogen mineralization is the microbial conversion of organic N to ammonium. ON concentrations declined 93% during primary treatment from an average of 449–55.9 mg/l discharged to the aquatic tanks. The aquatic tanks further reduced the ON to 7.9 mg/l after which treatment in the wetland resulted in the system final effluent ON of 3.8 mg/l (Table 2). A mass balance of ON during primary treatment indicated that 23% of influent ON was mineralized during the 6.7 day hydraulic retention time of primary treatment, yielding a mean N mineralization rate of 0.60 mg/l per hour (Table 2). Waste biosolids accounted for most of the ON removal, 62%, with the balance (15%) discharged to the greenhouse aquatic tanks.

Monitoring of both the aquatic tanks and the experimental tanks within the greenhouse showed that dissolved inorganic N rose over the course of treatment from tanks T2 to T9, consistent with the biodegradation of solids continually releasing dissolved N (Fig. 3). DON was constant in all of

Table 2

Nitrogen species concentrations and rates of nitrogen transformations in the Marion SAS facility components<sup>a</sup>

|                                 | Primary treatment <sup>b</sup> | Aquatic treatment tanks <sup>c</sup> | Wetland        |
|---------------------------------|--------------------------------|--------------------------------------|----------------|
| Mean concentrations (mg/l)      |                                |                                      |                |
| ON                              | 449                            | 30.4                                 | 5.9            |
| NH <sub>4</sub> -N              | 20.6                           | 2.7                                  | 0.64           |
| NO <sub>x</sub> -N              | 1.1                            | 12.5                                 | 8.3            |
| TSS                             | 5830                           | 428                                  | 15             |
| DO                              | 2.14 ± 0.89 <sup>d</sup>       | 7.2 ± 0.2                            | 0.69 ± 0.06    |
| Mean rates (mg/l per hour)      |                                |                                      |                |
| N mineralization                | 0.60                           | 0.451 ± 0.042                        | – <sup>e</sup> |
| Nitrification                   | 0.71                           | 0.606 ± 0.059                        | – <sup>e</sup> |
| Denitrification                 | 0.75                           | 0.260 ± 0.048                        | 0.18           |
| Hydraulic retention time (days) | 6.7                            | 4.8                                  | 3.5            |
| Temperature (°C) <sup>f</sup>   | 23.3                           | 22.3                                 | 23.7           |

<sup>a</sup> These environments are not homogenous; usually a downward gradient exists from the beginning of the component to its end. Rates and concentrations have been averaged over the whole component volume in order to summarize differences in conditions between components. All data determined by mass balance using EEA permit data ( $n = 22$ ) unless otherwise noted.

<sup>b</sup> Assuming equalization tank is on average half full.

<sup>c</sup> Determined using intensive monitoring data from aquatic tanks ( $n = 8$ ).

<sup>d</sup> Concentration in the preliminary conditioning tank. DO is near zero in the equalization tank and primary clarifier.

<sup>e</sup> Assumed to be zero, since DO in the wetland was 0.69 mg/l, inhibiting nitrification, and no increase in either NH<sub>4</sub>-N or NO<sub>x</sub>-N was detected.

<sup>f</sup> Mean for the period of June through September.

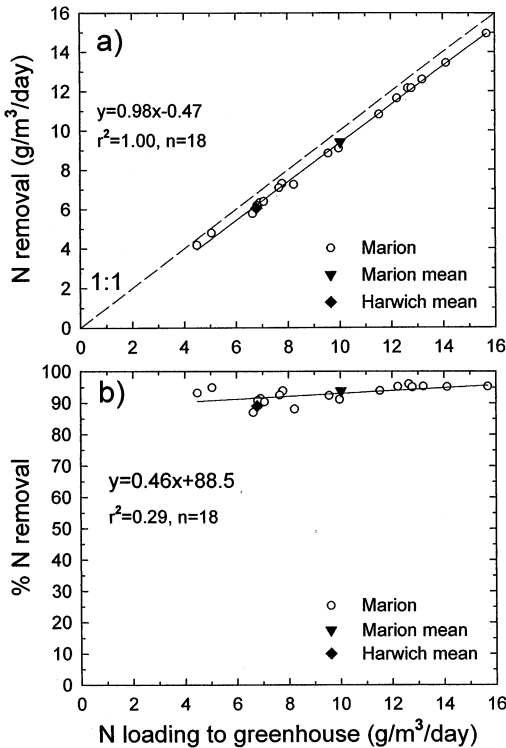


Fig. 2. Response of greenhouse components of Marion SAS (aquatic tanks and wetland) to various nitrogen loads over 6 months operation based on mass balance of nitrogen. (a) Percent removal of nitrogen from greenhouse at differing nitrogen loads with linear regression line. Dashed line indicates 100% removal. (b) Increase in nitrogen removal in response to increased nitrogen loading to greenhouse. Line through points is linear regression. Flow-weighted mean data from another septage-treating SAS shown for comparison (6 months operation; Wright-Pierce, 1993).

the aquatic tanks, averaging  $4.94 \pm 0.12$  mg/l, indicating that rates of DON production and DON mineralization were similar. Within the aquatic tanks, the mean N mineralization rate was  $0.451 \pm 0.042$  mg/l per hour (Table 2). Mass balance showed that a mean of 69% of greenhouse influent ON was mineralized in the aquatic tanks during their 4.8 day hydraulic retention time, and 19% was recycled back to the preliminary conditioning tank (recycle TSS = 428 mg/l). The remainder, 12%, was discharged to the wetland. In passage through the subsurface flow wetland, DON remained relatively constant, while PON declined by 79%, resulting in a decrease in ON

from 7.9 to 3.8 mg/l. Since NH<sub>4</sub>-N remained relatively constant and we assumed no nitrification (due to low DO levels), the decrease in PON during wetland treatment probably resulted largely from sedimentation, since significant mineralization without nitrification would have resulted in an accumulation of NH<sub>4</sub>-N. Even assuming that all of the N required for plant growth came from ammonium, rather than nitrate, the maximum decline in ON that mineralization could have accounted for would be 1.4 mg/l, indicating that sedimentation was the major process accounting for ON decline in the wetland.

### 3.3. Nitrification

Bacterial nitrification results in the conversion of ammonium to nitrate. Changes in NH<sub>4</sub>-N concentrations in wastewater reflect the balance of inputs from N mineralization with outputs through nitrification. During primary treatment, NH<sub>4</sub>-N declined from 32.8 to 9.3 mg/l, indicating nitrification in excess of mineralization. Adding the mean rate of NH<sub>4</sub>-N decrease (0.11 mg/l per hour from NH<sub>4</sub>-N mass balance) to the rate of NH<sub>4</sub>-N production through N mineralization (0.60 mg/l per hour) results in a nitrification rate of 0.71 mg/l per hour (averaged over the whole of primary treatment; Table 2). The high ambient concentrations of NH<sub>4</sub>-N indicate that nitrifica-

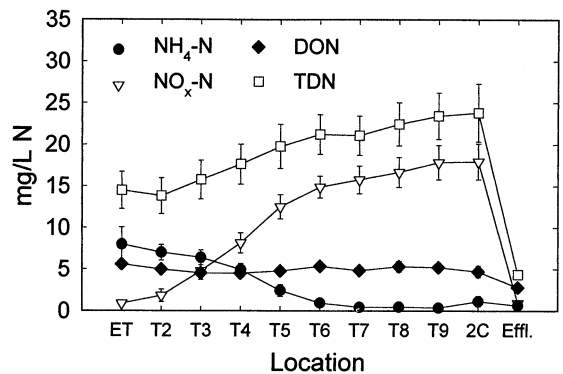


Fig. 3. Average concentrations of dissolved nitrogen species in equalization tank (ET), aquatic tanks T2–T9, secondary clarifier (2C) and final facility effluent over 3-week monitoring period.  $N = 8$ ; error bars represent standard errors.

tion was not limited by ammonium availability, but was likely limited either by the supply of oxygen ( $2.14 \pm 0.89$  mg/l in the preliminary conditioning tank) or by the slow growth of nitrifiers.

$\text{NH}_4\text{-N}$  also decreased over the course of treatment in the aquatic tanks, declining from 6.4 mg/l in tank T2 to less than 1 mg/l by T5 (Fig. 3). Lower  $\text{NH}_4\text{-N}$  production (mineralization =  $0.451 \pm 0.042$  mg/l per hour) than consumption (nitrification =  $0.606 \pm 0.059$  mg/l per hour) resulted in depletion of the  $\text{NH}_4\text{-N}$  pool during tank treatment (Figs. 3 and 4a). However, when  $\text{NH}_4\text{-N}$  concentrations were less than 3.0 mg/l (Fig. 4a), the nitrification rate ( $0.461 \pm 0.042$  mg/l per hour) was lower than the mineralization rate ( $0.541 \pm 0.075$  mg/l per hour). Under these conditions of lower  $\text{NH}_4\text{-N}$  concentration, nitrification was probably limited by ammonium supply. In the wetland, the low concentration of  $\text{NH}_4\text{-N}$  (0.64 mg/l) and low DO level ( $0.69 \pm 0.06$  mg/l) suggest inhibition of nitrification. The very small decline in  $\text{NH}_4\text{-N}$  observed (0.74–0.56 mg/l) represents only 13% of that required to support wetland plant growth, and so could be accounted for by plant uptake alone.

### 3.4. Denitrification

Denitrification is the bacterial conversion of nitrate to  $\text{N}_2$  gas.  $\text{NO}_x\text{-N}$  concentrations were relatively low and remained unchanged during primary treatment, at 1.1 mg/l (Table 2). In contrast, within the greenhouse,  $\text{NO}_x\text{-N}$  increased rapidly with passage through aquatic tanks T2 to T6, and then more slowly until the anaerobic secondary clarifier located after tank T9 (Fig. 3). After passage through the aquatic tanks, 75% of the total dissolved N was in the form of  $\text{NO}_x\text{-N}$ , hence available for removal by denitrification in the wetland. The wetland removed 89% of its influent concentration of  $\text{NO}_x\text{-N}$ , 14.9 mg/l, discharging 1.7 mg/l  $\text{NO}_x\text{-N}$ .

Since  $\text{NO}_x\text{-N}$  concentrations did not change during primary treatment, all of the  $\text{NO}_x\text{-N}$  produced by nitrification must have been denitrified. The additional  $\text{NO}_x\text{-N}$  input from the secondary clarifier recycle stream was also denitrified, resulting in a total mean denitrification rate of 0.75

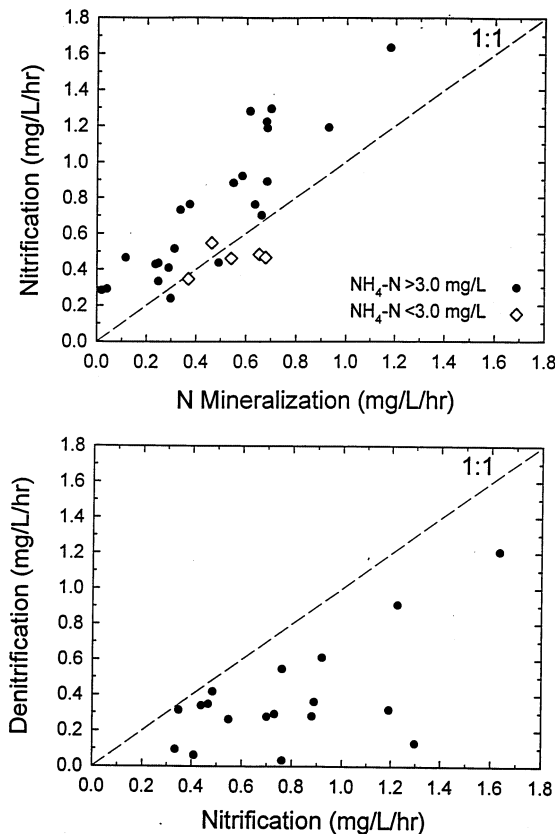


Fig. 4. (a) Relationship between nitrogen mineralization and nitrification rates in the experimental replicate aquatic tanks following T2. (a) Transformation rates at high ( $> 3.0$  mg/l) and low ( $< 3.0$  mg/l)  $\text{NH}_4\text{-N}$  shown by closed circles and open diamonds, respectively. Dashed line indicates complete nitrification of mineralized N. (b) Relationship between nitrification and denitrification rates in the experimental replicate tanks placed after T2. Dashed line indicates nitrification = denitrification; points below the line indicate nitrification rates greater than denitrification.

mg/l per hour (calculated from the mass balance of all N inputs and outputs to primary treatment). Neither organic carbon nor anaerobic microsite availability are likely to have limited denitrification during primary treatment, since DO was low (2.14 mg/l), POC concentrations averaged 2300 mg/l and the high respiration rate (inferred from the N mineralization rate) and particle abundance (5800 mg/l TSS) likely would have resulted in a large volume of anaerobic microsites within the particles.

Within the aquatic tanks, denitrification was the primary N loss mechanism, accounting for 7.6% of facility influent N versus 8.6% to other sinks and discharge. Denitrification rates ( $0.260 \pm 0.048$  mg/l per hour) within the aquatic tanks were always lower than nitrification rates ( $0.606 \pm 0.059$  mg/l per hour) (Fig. 4b), consistent with the observed accumulation of  $\text{NO}_x\text{-N}$  from tank T2 to T9 (Fig. 3). The high concentration of  $\text{NO}_x\text{-N}$  suggests that some other factor (oxygen or organic carbon) limits N removal via denitrification within this component of the SAS. Organic carbon availability is at least a partial factor, since denitrification rates were found to increase with increasing POC loading rates, (denitrification [mg/l per hour] =  $0.018 \times \text{POC loading [mg/l per hour]} - 0.094$ ;  $r^2 = 0.63$ ,  $n = 25$ ). In the wetland, the absence of nitrification means that no new nitrate was produced; the reduction of nitrate therefore accounted for all of the denitrification, at a rate of 0.18 mg N/l per hour.

## 4. Discussion

### 4.1. System performance

Over the 6 months of monitoring, the septage-treating SAS reduced influent chemical oxygen demand and TSS by an average of greater than 99%, and total phosphorus by more than 97%. Total N removal efficiency averaged 99% and was > 92.6% over an N loading range of 4.8–54.4 g N/m<sup>3</sup> per day. On average, the final treated effluent contained 1.0% of total influent N (Fig. 5). Removal as solids was the major pathway of N removal, at 57.6%, with denitrification being second at 40.9% of the total TN load. Uptake by plants accounted for only a very small proportion of the N removal, 0.5% of influent TN.

Septage comes from a variety of sources, and is delivered to treatment facilities in tanker loads. At Marion, the mean size of a load was ca 5000 l, but ranged from 246–15 700 l. TN concentrations in the influent septage ranged from 160–1540 mg/l. Due to the variability between septage loads, the treatment units of the facility must be scaled to cope with large variations in the strength of in-

coming wastewater flow. In its present configuration, the greenhouse portion of the SAS system was able to maintain a consistently low effluent N level despite a fourfold variation in the influent N loading (Fig. 2). This overcapacity in the present facility provides a buffer against the high variability of the influent (Table 1).

The high loss of N due to denitrification during primary treatment (30.9% of the total N system input of 187 kg over 6 months; Fig. 5) was similar to that reported for another septage treating SAS (21.8%) under similar loading (Wright-Pierce, 1993). Biological mineralization and oxidation of septage during primary treatment produced a TSS-specific denitrification rate of 3.1 mg N/g TSS/d, comparable to that obtained for sewage in an activated sludge facility (2.3–3.1 mg N/g volatile suspended solids per day; Drury et al., 1995).

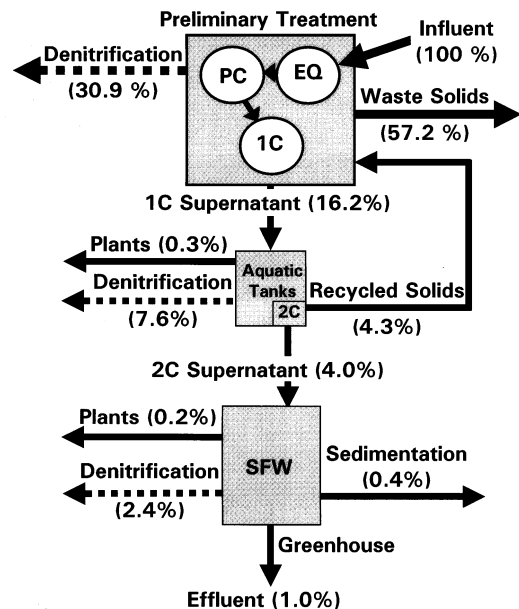


Fig. 5. Nitrogen balance in the Marion SAS integrated over 6 months operation. Box sizes show relative area of treatment components. EQ, equalization tank; PC, preliminary conditioning tank; 1C and 2C, primary and secondary clarifiers; aquatic tanks-aquatic tanks T1 through T9; SFW, subsurface flow wetlands. Total influent nitrogen = 187 kg = 100%.

#### 4.2. Whole-system Nitrogen budget

57.6% of SAS influent TN left the facility as settled solids (Fig. 5). This is lower than a nearby conventional facility in Orleans, Mass. where septage is dewatered by lime and ferric chloride precipitation followed by filter pressing, causing 86% of influent N to be removed as solids (Slater et al., 1987). Primary sedimentation in the SAS resulted in the production of 0.15 l waste solids/l septage treated, and the total waste solids production of the facility was 0.81 g/g influent TSS (including harvested plant biomass), less than the 1.3 g/g TSS produced by primary septage treatment by chemical precipitation (Orleans, Mass.; Slater et al., 1987). Lower solids production in the SAS resulted from the lack of chemical precipitants combined with biological treatment of raw septage before sedimentation. Removing particulates by chemical precipitation early in the treatment process prevents PON from becoming available for nitrification and denitrification, and inhibits denitrification.

In addition to the large loss of TN via waste solids during primary treatment, denitrification also removed a significant amount (30.9%) of the TN load. The high loss through denitrification resulted from the aeration of the particle-rich, high-ammonium septage and the influx of nitrate-rich water in the greenhouse recycle stream. Only 16.2% of the SAS influent TN remained in the wastewater after primary treatment for further treatment in the greenhouse system. However, the primary effluent is still high in N and further treatment in the wetland portions of the facility is required to nitrify the remaining ammonia and remove nutrients to tertiary standards.

In contrast to the primary treatment component where settling of solids is the dominant TN removal mechanism, most of the TN loss in the aquatic tanks resulted from denitrification. Denitrification within the aquatic tanks accounted for nearly half of the TN processed by this system component or 7.6% of the SAS influent (Fig. 5). 4.3% of SAS influent TN was recycled as settled solids to the preliminary conditioning tank. Thirty percent of this recycled N, equaling 1.3% of influent TN, was as nitrate ( $\text{NO}_x\text{-N} = 19.2 \text{ mg/l}$ ). 4.0%

of the SAS influent N flowed onward to the wetland; 62% of this as  $\text{NO}_x\text{-N}$ . More than half of the wetland N input (2.4% of total influent N or 91% of wetland influent  $\text{NO}_x\text{-N}$ ) was denitrified in the wetland, with 0.4% of the septage influent N retained in the marsh as solids. Only 1.0% of the TN entering the SAS was discharged in the effluent from the greenhouse, mostly as DON (Table 1).

Plant uptake of N played a insignificant role in N removal in both the aquatic tanks and the wetland, totaling 0.5% of the total influent N or 3% of the N entering the greenhouse. Plant uptake was nearly equal in the aquatic tanks and the wetland (Fig. 5). Nevertheless, plant roots may still have a role, perhaps in providing a haven for bacteria against washout or poisoning by toxic compounds. Nitrogen mineralization, nitrification and denitrification occurred simultaneously during preliminary conditioning as well as in the greenhouse aquatic tanks. However, the factors controlling their relative importance differed among the three SAS treatment subunits.

#### 4.3. Mineralization

In the anaerobic environment of a septic tank, there is an accumulation of organic matter not susceptible to anaerobic degradation. Within the aerobic environment of the SAS, much of this material becomes available for degradation. The rate of decomposition of this organic matter is controlled by its concentration and lability, oxygen availability and temperature. During treatment in the SAS, the more labile fractions are degraded, reducing the lability of the remaining fraction. Although abundant organic matter was available for mineralization during primary treatment, oxygen availability was low (2.14 mg/l), and may have limited mineralization rates (Table 2).

The rate of N mineralization in the aquatic tanks, despite constant DO levels and temperatures, was highly variable, averaging 0.451 mg N/l per hour. While some of the variation may have been due to variation in ON concentrations, ON concentration alone could not account for the observed variation in rates (linear regression of mineralization against ON concentration;  $r^2 =$

Table 3

Most probable number (MPN) of nitrifying bacteria in water from various sources in the Marion SAS and on plant roots (adapted from Hamersley et al., in preparation (b))

| Source                        | Nitrifying Bacteria |                    |                         |
|-------------------------------|---------------------|--------------------|-------------------------|
|                               | (MPN/l)             | (MPN/l/mg TSS)     | (MPN/tank) <sup>a</sup> |
| Equalization tank             | $0.80 \times 10^8$  | $1.1 \times 10^4$  |                         |
| Preliminary conditioning tank | $1.4 \times 10^8$   | $1.9 \times 10^4$  |                         |
| 1° clarifier supernatant      | $2.0 \times 10^8$   | –                  |                         |
| Aquatic tanks <sup>b</sup>    | $3.7 \times 10^8$   | $0.82 \times 10^4$ | $2.3 \times 10^{11}$    |
| 2° clarifier recycle          | $5.0 \times 10^8$   | $0.58 \times 10^4$ |                         |
|                               | (MPN/g [wet])       |                    |                         |
| Plant roots <sup>c</sup>      | $0.19 \times 10^8$  |                    | $0.25 \times 10^{11}$   |

<sup>a</sup> Total MPN per aquatic tank indicates relative contribution of water and plants to total nitrifier supply.

<sup>b</sup> Aquatic tanks T1–T9,  $n = 15$ .

<sup>c</sup> Floating plants from aquatic tanks,  $n = 13$ .

0.63). The additional variability may have resulted from variations in the biodegradability of the organic matter. ON concentrations in the aquatic tanks averaged about 15 times lower than those during primary treatment because of primary clarification, while the mean mineralization rate was almost half that measured during primary treatment (Table 2). Normalizing the N mineralization rate to the concentration of ON results in a rate of 1.3 mg N mineralized/g ON per hour during primary treatment, compared with 15 mg N mineralized/g ON per hour in the aquatic tanks. The increased oxygen availability and the more diverse microfauna in the aquatic tanks may be responsible for the rise in mineralization.

While 99.4% of the particulate organic matter entering the SAS was already removed before reaching the wetland, a further 79% of the particulates flowing into the wetland were removed, most likely by sedimentation. This conservative assumption was chosen since even if mineralization and nitrification of PON in the wetland were occurring, wetland denitrification would only have been increased by a maximum of 19%.

#### 4.4. Nitrification

Nitrification is dependent on the metabolic activities of nitrifying bacteria present in biofilms found on the inside walls of the tanks, on plant

roots and on suspended organic particles in the wastewater (Table 3). These biofilms contain both heterotrophic organisms (which get energy from organic carbon degradation) and autotrophic organisms, including nitrifiers (which get energy from the transformation of ammonium to nitrate). Nitrification requires oxygen and may be limited by ammonium availability at low concentrations. Nitrification can also be controlled by the size of the population of nitrifiers, which may in turn be limited by slow growth and/or biofilm surface area (Focht and Chang, 1975; Hamersley et al., in preparation (b)).

During primary treatment, nitrification rates were most likely limited by both oxygen and nitrifier availability. Raw septage is anaerobic, and does not support large populations of nitrifiers (Table 3). The concentration of nitrifiers increased during the course of primary treatment due to growth and the input via recycle of secondary biosolids from the secondary clarifier in the greenhouse, but was still low relative to the levels in the aquatic tanks (Table 3).

At the beginning of the aquatic tank series, mineralization was higher than nitrification approximately 80% of the time (Fig. 4a), which caused ammonium levels to rise. As treatment continued, the more labile solids were degraded, slowing mineralization rates and reducing respiratory oxygen demand. These changes shifted the

balance of mineralization and nitrification, resulting in a decrease in ammonium concentrations in 'downstream' aquatic tanks. Low concentrations of ammonium inhibit nitrification rates but these low levels were usually not found until the second half of the tank treatment stream (Fig. 3). Although organic material-loading in the aquatic tanks was not as high as in the primary treatment component, the oxygen requirements of the heterotrophic community in the tanks were still high, and competition for oxygen between heterotrophs and nitrifiers probably limited nitrification rates. Nitrifier concentrations in the aquatic tanks were the highest found anywhere in the facility, and were supplemented by plant root-associated nitrifiers (Table 3).

In the wetland, low concentrations of ammonium and DO make significant nitrification unlikely and we have assumed negligible nitrification in the wetland in the mass balance calculations. The potential error of this assumption would be the unlikely scenario of all the PON retained by the wetland being mineralized, nitrified and denitrified. The resulting nitrification rate still would be only 0.029 mg/l per hour, or less than 6.4% of the rate in the aquatic tanks.

#### 4.5. Denitrification

Although the equalization tank and preliminary conditioning tank were aerated, they remained relatively anaerobic (Table 2), with high POC levels (2300 mg C/l). These are favorable conditions for denitrification, but the low levels of  $\text{NO}_x\text{-N}$  (Table 2) indicate that denitrification was probably limited by the supply of nitrate through nitrification. Nitrate-limited denitrification during primary treatment is further supported by the disappearance of  $\text{NO}_x\text{-N}$  recycled to the preliminary conditioning tank from the secondary clarifier, which accounted for only a further 0.071 mg/l per hour of denitrification.

In the aquatic tanks, the accumulation of  $\text{NO}_x\text{-N}$  indicates that denitrification limits nitrogen removal (Figs. 3 and 4b). In an earlier study by our laboratory involving a different septage-treating SAS (Harwich), denitrification was measured using the acetylene inhibition technique (Teal et al.,

1994). Using this technique, we estimated that the Harwich aquatic tanks were denitrifying about 8% of the nitrogen entering them. In the present study, denitrification in the aquatic tanks was much higher, removing nearly half of the total nitrogen entering the greenhouse. One possible explanation for this discrepancy is that the acetylene inhibition measurements were made on septage without plants, whereas the mass balance measurements in the present study included the whole tank system. Plant roots were observed to have immobilized a large quantity of particulates (pers. obs.), and the circulation of oxygenated water within the plant root systems was diminished. Denitrification rates within the plant root systems may have been elevated by these factors, resulting in the higher denitrification N loss in the present study over the earlier study. This observation may support a role for plant roots in enhancing denitrification. However, the results of studies specifically addressing this issue showed little or no effect (Hamersley et al., in preparation (b)). Notably, mass balance calculations in the previous study suggested that denitrification in the Harwich greenhouse accounted for 17–35% of the total SAS denitrification, a range which encompasses our own measurement of 25% for the Marion SAS (Teal et al., 1994).

The wetland has highly anaerobic conditions and influent nitrate levels are high (14.9 mg/l; Table 2), but with little associated POC (37.7 mg POC/l). In 1994, soluble organic carbon (as acetate) was routinely added to the secondary clarifier by EEA facility operators. That winter, the wetland gravel was left intact without removal and cleaning, since there had been no decline in its hydraulic conductivity during the 1994 season. In 1995, adequate denitrification occurred in the wetland without the addition of acetate, presumably because of the contribution of labile organic material (POC) from the previous year's plant litter.

The performance of the SAS results from simultaneous N mineralization, nitrification and denitrification supported by microscale heterogeneity in oxygen availability, allowing nitrification and denitrification to occur simultaneously. During primary treatment, mineralization is the rate-lim-

iting process, due to low DO availability and low lability of organic particulates. In the greenhouse, denitrification limits N removal because of the low availability of anaerobic microsites after primary clarification. Although the SAS treats septage to tertiary standards with minimal chemical addition and with low waste solids production, economic evaluation relative to existing septage treatment options is necessary to determine appropriate applications (Stephens, 1998).

### Acknowledgements

Financial support was provided by the Island Foundation of Marion, MA, by the Canadian National Science and Engineering Research Council and by the Education Program of the Woods Hole Oceanographic Institution (WHOI). Bruce Strong and Debbie Hamel of Ecological Engineering Associates operated the SAS facility and cooperated in the experimental efforts. Susan Brown-Leger of WHOI provided laboratory technical support. WHOI contribution # 10324.

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