



# Control of denitrification in a septage-treating artificial wetland: the dual role of particulate organic carbon

M. Robert Hamersley<sup>a,\*</sup>, Brian L. Howes<sup>b</sup>

<sup>a</sup>Department of Biology, Woods Hole Oceanographic Institution, MS#33, Woods Hole, MA 02543, USA

<sup>b</sup>School for Marine Science and Technology, University of Massachusetts, 706 S. Rodney French Blvd., New Bedford, MA 02744-1221, USA

## Abstract

We examined the factors controlling organic carbon (C) cycling and its control of nitrogen (N) removal via denitrification in an aerated artificial wetland treating highly concentrated wastewater to nutrient-removal standards. Processing of organic material by the septage-treating wetland affected the biological reactivity (half-life, or  $t_{1/2}$ ) of organic C pools through microbial degradation and gravity fractionation of the influent septage. Primary sedimentation fractionated the initial septage material ( $t_{1/2} = 8.4$  d) into recalcitrant waste solids ( $t_{1/2} = 16.7$  d) and highly labile supernatant ( $t_{1/2} = 5.0$  d), allowing this reactive fraction to be further degraded during treatment in aerobic wetland tanks until a less labile material ( $t_{1/2} = 7.3$  d) remained. Organic C contributions from in situ fixation by nitrifying bacteria or algae in these tanks were small, about 1% of the C degradation rate.

In the aerated tanks, denitrification was correlated with particulate organic C loading rates, although the average C required ( $0.35 \text{ mg C L}^{-1} \text{ h}^{-1}$ ) to support denitrification was only 12% of the total C respiration rate ( $2.9 \text{ mg C L}^{-1} \text{ h}^{-1}$ ). Additions of plant litter ( $2.5 \text{ g C L}^{-1}$ ) to the aerated tanks under normal operating conditions doubled denitrification rates to  $0.58 \text{ mg N L}^{-1} \text{ h}^{-1}$ , and reduced effluent nitrate levels by half, from  $12.7$  to  $6.4 \text{ mg N L}^{-1}$ . However, C degradation within the plant litter ( $0.15 \text{ mg C L}^{-1} \text{ h}^{-1}$ ) was sufficient to have accounted for only 35% of the additional denitrification. Evidence from laboratory and full-scale plant litter additions as well as process monitoring indicates that the stimulation of denitrification is due to the respiration-driven formation of anaerobic microsites within particulate organic C. In this aerated highly C-loaded septage-treating wetland, anaerobic microsite, rather than C substrate availability limits denitrification. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Anaerobic microsites; Artificial wetlands; Denitrification; Nitrogen; Particulate organic carbon; Septage

## 1. Introduction

Wastewater treatment in coastal areas requires effective and efficient nitrogen (N) removal because N compounds can lead to the eutrophication of coastal

waters, while nitrate ( $\text{NO}_3^-$ ) can contaminate drinking water supplies [1,2]. Biological treatment is the most effective means of N removal, converting biologically available N compounds into  $\text{N}_2$  gas through a sequence of microbial transformations within aerobic and anaerobic environments [3]. Degradation of organic material by invertebrates and microbes releases ammonium ( $\text{NH}_4^+$ ), which is converted to nitrate by oxygen ( $\text{O}_2$ )—requiring, carbon dioxide-fixing nitrifying bacteria. In the absence of  $\text{O}_2$ , organic carbon (OC)-consuming denitrifiers then reduce  $\text{NO}_3^-$  to  $\text{N}_2$  gas. This sequence is also an important part of the functioning of natural aquatic systems [4].

\*Corresponding author. Tel.: +1-508-910-6365; fax: +1-508-999-8197.

E-mail address: rhamersley@umassd.edu (M.R. Hamersley).

<sup>1</sup>Current address: School for Marine Science and Technology, University of Massachusetts, 706 S. Rodney French Blvd., New Bedford, MA 02744-1221, USA.

The physical and chemical heterogeneity of natural wetlands permits simultaneous nitrification-denitrification [5]. In aerobic waters, biofilms containing heterotrophic and nitrifying bacteria form on the surfaces of sedimentary or suspended organic matter. Anaerobic conditions may form as little as 100  $\mu\text{m}$  below the surface of this actively degrading detrital material because aerobic OC consumption removes  $\text{O}_2$  faster than it can diffuse from the surrounding water [6,7].  $\text{NO}_3^-$  produced aerobically by nitrification diffuses into these anaerobic microsites, where it becomes available to denitrifiers. As in nature, simultaneous biological nutrient removal (nitrification–denitrification) in wastewater is enhanced by redox heterogeneity [8]. However, wastewater sedimentation removes organic particles, leaving a supernatant with homogeneous redox conditions and low OC, making denitrification difficult [9].

Exogenous sources of dissolved OC, such as acetate and methanol are often used to promote denitrification of wastewater nitrate [10,11]. In wetland treatment systems, plant biomass has been investigated as a carbon source in order to utilize wetland-generated biomass [12–14]. In low-oxygen wastewater treatment systems, both forms of OC act as a substrate for denitrification. However, in aerated systems, plant biomass (a form of particulate organic carbon (POC) causes the formation of anaerobic microsites, supporting simultaneous nitrification and denitrification. POC therefore may play a dual role in denitrification, since it supports the heterotrophic metabolism of denitrifying bacteria as well as the  $\text{O}_2$  consumption which creates anaerobic microsites necessary for denitrification.

Like natural wetlands, artificial wetlands designed for wastewater treatment are high in microscale heterogeneity, promoting simultaneous nitrification–denitrification [5,15]. However, low  $\text{O}_2$  availability typically limits their use to the treatment of relatively oxidized waste streams with low biological oxygen demand and  $\text{NH}_4^+$  levels [16]. Nitrification of wastewater in

constructed wetlands can take place with long retention times or fill-and-draw cycles to increase aeration [17–19]. Nevertheless, artificial wetlands have also been used to treat high-strength organic waste streams such as septage, a byproduct of on-site septic wastewater treatment. Septage is typically very high in recalcitrant organic matter and dissolved nutrients (total volatile solids 8170–27 600  $\text{mg L}^{-1}$ , dissolved N 7.9–135  $\text{mg L}^{-1}$ ), making treatment and disposal problematic [20], and in rural areas is a major contributor of N to coastal ecosystems [21]. Wetland treatment of septage, with its high concentration of organic matter, requires preliminary sedimentation to prevent the accumulation of recalcitrant organic material within the wetland, and mechanical aeration to promote biological oxidation and nitrification [22]. A number of studies have demonstrated the ability of artificial wetlands to treat septage to nutrient removal standards with low solids production [22–25].

An earlier study of nitrogen cycling in such a septage-treating wetland described the fate of septage N during treatment [26]. Primary biological oxidation (Fig. 1), resulted in the removal of 31% of the influent organic N through simultaneous mineralization, nitrification, and denitrification. A further 57% of the influent N was removed as waste solids by gravity clarification, generating only 0.81 g waste solids  $\text{g}^{-1}$  influent total suspended solids (TSS). The supernatant which then flowed into the wetland treatment system had TSS and  $\text{NH}_4^+$  concentrations (940 and 9.3  $\text{mg L}^{-1}$ , respectively) similar to raw sewage, and was first treated in aerated tanks containing floating plants. In these aquatic treatment tanks, nitrification exceeded denitrification, resulting in the removal of  $\text{NH}_4^+$  and the accumulation of  $\text{NO}_x^-$ . Nevertheless, half of the total N entering the aquatic tanks was denitrified. After secondary clarification, the supernatant, containing N predominantly as  $\text{NO}_x^-$ , was treated in the anaerobic environment of a gravel wetland bed, where denitrification reduced its

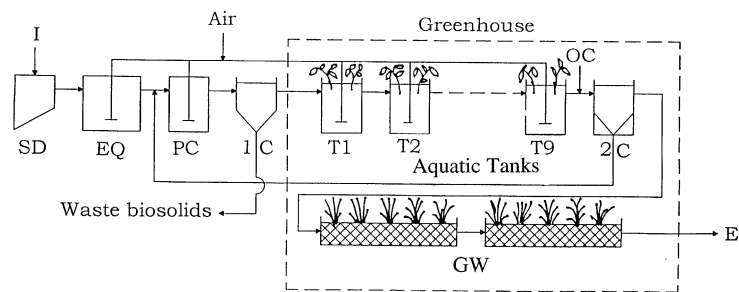


Fig. 1. Flow diagram of the Marion, MA septage-treating wetland. I—influent from pump trucks, SD—screening and degritting, EQ—equalization tank, PC—preliminary conditioning tank, 1C and 2C—primary and secondary clarifiers, T1–T9—aquatic treatment tanks 1–9 with floating plants (single train only shown), OC—site of acetate addition, GW—gravel wetland bed, E—effluent. Primary treatment consists of all steps prior to the greenhouse containing the aquatic tanks and gravel wetland bed.

concentration in the final facility effluent to 1.7 mg  $\text{NO}_x^- \text{L}^{-1}$ .

The goal of the present study, was to determine the factors controlling the efficiency of  $\text{NO}_3^-$  removal through denitrification in this wetland septage treatment facility using techniques and analysis derived from the biogeochemical study of natural wetlands. Our process monitoring and experimental manipulations focused primarily on interactions between C and N cycling and were concentrated on the redox-diverse environment of the aquatic tanks, although we evaluated denitrification during primary treatment and in the gravel wetland for comparison. We quantified both the sources and availability of OC throughout the process stream and evaluated the response of denitrification to manipulation of POC levels using wetland biomass in laboratory and full-scale experiments.

## 2. Materials and methods

### 2.1. System description

The study system was a 2200  $\text{L d}^{-1}$  demonstration-scale septage-only artificial wetland in Marion, MA, USA (the Solar Aquatic System, designed and operated by Ecological Engineering Associates, Weston, MA), described in detail in an earlier study [26]. Septage (primarily residential) is first treated by biological oxidation in a 3800 L tank, followed by gravity clarification. The total hydraulic retention time (HRT) of this primary treatment step is 6.7 d (Fig. 1). The clarified primary effluent is gravity fed into a greenhouse which contains the wetland treatment systems, where the flow is split to feed two parallel trains of nine aerated aquatic treatment tanks each. Each tank maintains 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.). Effluent from the final tank in each train (T9) flows to a secondary clarifier, and settleable solids comprising ~30% of the flow are recycled back to primary biological oxidation. The total volume of all of these tanks is 11200 L and the HRT for this treatment step is 4.8 d. The clarified supernatant is discharged into a subsurface flow gravel wetland bed ( $22.3 \text{ m}^{-2}$ , HRT = 3.5 d) planted with cattails (*Typha latifolia* L.), sedges (*Cyperus* spp. L.), and primula. Retention time of the whole system averaged 16.3 d.

Throughout the 6 month study period, the influent septage had a high organic matter and N content, with TSS concentrations averaging 45 times and  $\text{NH}_4^+$ -N more than 3 times local sewage levels (Table 1) [26]. The final effluent averaged 2.3 mg inorganic  $\text{N L}^{-1}$ , while total N averaged 6.1 mg  $\text{L}^{-1}$ , well below that of a nearby

Table 1

Composition of influent septage and effluent at the septage-treating wetland<sup>a</sup>

(mg $\text{L}^{-1}$ )	Influent septage <sup>b</sup>	Final effluent	Sewage <sup>c</sup>
TSS	7460	16	165
Organic C	2890	24.8	—
Organic N	449	3.8	—
$\text{NH}_4^+$ -N	32.8	0.56	10.1
$\text{NO}_x^-$ -N	1.1	1.7	—
Total N	483	6.1	—
Total P	51.5	1.5	3.8

<sup>a</sup> Flow-weighted concentrations over 6 months operation.  $n = 22$ . Adapted from [26].

<sup>b</sup> Composition of screened and dewatered septage from the equalization tank (Fig. 1).

<sup>c</sup> Fields Point, Providence, RI.

septage-treating rotating biological contactor (52.6 mg  $\text{L}^{-1}$ ; [27]).

### 2.2. Analytical methods

All wastewater samples were collected in duplicate and immediately transported on ice (45 min) to the laboratory. Upon arrival, the first sample was pressure filtered (0.22  $\mu\text{m}$ ) and the filtrate assayed for  $\text{NH}_4^+$ -N,  $\text{NO}_x^-$ -N (nitrate + nitrite) and dissolved organic N (DON).  $\text{NH}_4^+$ -N was analyzed immediately (to prevent ammonia volatilization) by a colorimetric indophenol method [28].  $\text{NO}_x^-$ -N was analyzed with azo dye after cadmium reduction on a Lachat Automated Ion Analyzer [29]. Total dissolved N was measured as  $\text{NO}_3^-$  following persulfate oxidation [30]. DON was determined from the difference between total dissolved N and  $\text{NH}_4^+$ -N and  $\text{NO}_x^-$ -N. The second sample was vacuum filtered through precombusted Whatman GFF filters and dried to constant weight (60°C) to collect solids for TSS measurement [31], after which the particulate organic C and N (POC and PON) content of the solids was assayed on a Perkin-Elmer Model 2400 CHN elemental analyzer. Plant biomass was dried to constant weight at 60°C, ground, and analyzed for POC and PON content. All reported values are means  $\pm$  standard error.

### 2.3. C and N mass balance in the septage treating wetland facility

In the experimental portions of this investigation, both POC and TSS were measured on parallel samples. From these data, a linear relationship between POC and TSS concentrations emerged ( $0.383 \pm 0.002 \text{ mg POC mg TSS}^{-1}$ ,  $n = 174$ ,  $r^2 = 0.98$ ) which was found to be constant throughout the treatment process and

over the range of TSS concentrations. For calculation of the system-wide carbon mass balance only, POC was estimated from measured TSS levels using this empirically derived relation. The TSS content of raw influent, waste solids, primary clarifier effluent, secondary clarifier effluent, and final effluent was determined on 22 dates over the 6 month (05/94–12/94) study period by Ecological Engineering Associates as part of the National Pollutant Discharge Elimination System (NPDES) permit requirements [31]. Dissolved OC was estimated from DON based upon the conservative assumption of a C/N ratio for dissolved material equal to that measured for particulate septage solids (mass ratio = 6.4). Since POC represented from 86% to 99% of the total OC pool up to the secondary clarifier, errors in the estimation of dissolved OC are unlikely to affect our conclusions. In addition, since the C/N ratio of solids did not appreciably change during treatment, we have no reason to think that organic material released into dissolved forms during degradation differed in its C/N ratio from the solid material. Regularly measured influent, waste solids and effluent flow rates, along with flow rates measured at various points throughout the facility were used in the construction of a water balance for the facility and to flow-weight concentration data for the construction of the mass balance. The rate of OC oxidation during primary treatment was calculated from the “missing” OC (from the mass balance) and the measured hydraulic retention time.

#### 2.4. C and N cycling in the aquatic treatment tanks

In order to study C and N metabolism in the aquatic treatment tanks, wastewater from each tank in both trains was sampled 8 times over a 3 week period under normal flow conditions. Metabolic rates were determined from the changes in concentration of constituents between one tank and the previous one, divided by the tank hydraulic retention time. HRT was calculated from the concurrently measured flow rate and a determination of the effective mixed volume of the tanks made using a  $\text{Cl}^-$  tracer (73%; [26]). The tracer studies showed that wastewater flow through these tanks conformed to the tanks-in-series model [42]. Carbon oxidation rates were determined from the sum of the changes in the concentration of POC and dissolved OC. Similarly, N mineralization rates were determined by the sum of measured changes in the concentrations of PON and DON. Nitrification rates were determined from the sum of mineralization rates and changes in the  $\text{NH}_4^+$ -N pool. The rate of increase in dissolved inorganic N ( $\text{NH}_4^+$ -N +  $\text{NO}_3^-$ -N) over the entire series of tanks was subtracted from the mineralization rate to yield denitrification (as missing N) by mass balance. The validity of this mass balance approach for determination of denitrification rates has been confirmed in previous

studies of a septage-treating wetland where parallel in situ direct measures of denitrification gave comparable results [25]. Temperature, dissolved oxygen (DO), and flow rate were measured concurrently with water sampling. DO was measured using a calibrated YSI Model 58 oxygen meter.

#### 2.5. Organic carbon decay constants

To determine the relative lability of the septage OC, aerobic biodegradation rates of OC at various stages of treatment were measured. Long term (~5 week) laboratory biodegradation incubations were conducted on septage from the equalization tank, water from tanks T1 to T9 and waste biosolids (Fig. 1). For comparison to septage, screened and dewatered sewage from the Field's Point Sewage Treatment Facility, Providence, RI, was also assayed. Dilutions (4.5–60 mL wastewater  $\text{L}^{-1}$  deionized water, depending on the sample's POC concentration) of each sample were placed in each of 4 opaque ground glass-stoppered bottles (300 mL) and incubated at 20°C. N-Serve (2-chloro-6-[trichloromethyl] pyridine, Dow) was added ( $10 \text{ mg L}^{-1}$ ) to inhibit oxygen uptake by nitrification. DO was measured approximately every 2 d. Samples were reaerated to saturation whenever DO fell below  $4.0 \text{ mg L}^{-1}$ . The decline in the  $\text{O}_2$  consumption rate over time could be described by the exponential decay equation:

$$R_t = R_0 e^{(-jt)}, \quad (1)$$

where  $R_0$  is the initial rate of  $\text{O}_2$  consumption ( $\text{mmol L}^{-1} \text{ h}^{-1}$ ),  $R_t$  is the  $\text{O}_2$  consumption rate at time  $t$  (d), and  $j$  is the reaction rate constant ( $\text{d}^{-1}$ ). Taking the integral of Eq. (1) with respect to  $t$  and converting  $\text{O}_2$  consumption to C oxidation (using a stoichiometry of 1 mol  $\text{O}_2/\text{mol C}$  or 32 g  $\text{O}_2/12 \text{ g C}$ ) gives

$$C_t = C_0 - \frac{R_0}{-k} e^{(-kt)} - \frac{R_0}{k}, \quad (2)$$

where  $C_0$  is the initial C concentration ( $\text{mmol L}^{-1}$ ),  $C_t$  is the C concentration ( $\text{mmol L}^{-1}$ ) at time  $t$ , and  $k$  is the decay constant of OC ( $\text{d}^{-1}$ ). The value of  $k$  was determined by curve-fitting Eq. (2) to  $\text{O}_2$  consumption data. The half-life ( $t_{1/2}$ ) of OC in the samples was determined from  $k$  by the formula

$$t_{1/2} = \frac{\log_e 2}{k}. \quad (3)$$

#### 2.6. Carbon fixation rates

In order to determine the contribution of algal and microbial C fixation to this wetland system,  $\text{CO}_2$  uptake in the aquatic tanks was determined by carbon-14 ( $^{14}\text{C}$ ) incorporation under light and dark conditions [32]. Incorporation of  $^{14}\text{CO}_2$  into organic forms under dark conditions measures chemosynthetic activity (mainly

nitrification), while the additional fixation under light is due to photosynthetic activity. These two measures gave a measure of the availability of non-septage C as well as an independent measure of nitrification rates for comparison to those determined from the system mass balance. Carbon fixation rates were determined in aquatic tanks T2, T5 and T9 (Fig. 1). For each tank, three 300 mL bottles (two clear and one dark) were filled with septage, injected with 0.25 mL of 8.5  $\mu\text{Ci}$   $^{14}\text{C}$  as  $\text{NaH}^{14}\text{CO}_3$ , and suspended against the clear-sided walls for 6 daylight hours. After incubation, samples were analyzed for  $^{14}\text{C}$  incorporated into organic matter on a United Technologies Packard Minaxib Tri-Carb 4000 series liquid scintillation counter. Dissolved  $\text{CO}_2$  levels in the tanks were determined on water samples using headspace equilibration and infrared analysis (Beckman Model 15A).

### 2.7. Particulate organic carbon effects on denitrification

Denitrification is typically limited by  $\text{O}_2$  or by the availability of  $\text{NO}_3^-$  or labile OC. The potential for the limitation of denitrification by OC availability in the aquatic tanks (Fig. 1) was tested using biomass POC additions in laboratory incubations and as full-scale modifications of the septage-treating system. In the laboratory, denitrification rates were measured using the acetylene block method [33] on samples amended with POC (wheat straw) which had been pre-conditioned by submersion (3–5 d) in the aquatic tanks to promote microbial colonization. Substrate pre-conditioning allowed the effects of DO, POC, and  $\text{NO}_x^-$  on denitrification to be studied independently of the effects of microbial colonization rates. Sewage from aquatic tanks 1, 8, and 13 at the similarly designed Field's Point sewage-treating wetland (Providence, RI, USA) was used because septage was unavailable. The chemical oxygen demand of the sewage ranged from 25 to 1010  $\text{g L}^{-1}$ , compared with 180–2010  $\text{mg L}^{-1}$  in the aquatic tanks of the septage-treating wetland (EEA, personal communication). The serum bottles (160 mL) received 0, 0.35, 0.7, 1.4, 2.1 or 3.5  $\text{g POC L}^{-1}$  as straw and 75 mL of sewage, and were sealed with an air headspace using gas-tight septa. A second identical set of bottles were made anaerobic prior to incubation by sparging with  $\text{N}_2$ . To test the effect of  $\text{NO}_x^-$  availability, sewage samples with 4  $\text{NO}_x^-$ -N concentrations ranging from 0.13 to 12.05  $\text{mg L}^{-1}$ , similar to concentrations found in septage in the aquatic tanks, were tested at each POC addition level. Each bottle was injected with 9 mL of acetylene and incubated at in situ temperature (20°C). Acetylene inhibits the conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$  during denitrification, so that denitrified N can be easily measured as  $\text{N}_2\text{O}$ . Duplicate bottles were run for the lower two  $\text{NO}_x^-$ -N concentrations (0.13 and 2.93  $\text{mg L}^{-1}$ ), while the higher two concentrations (5.74

and 12.05  $\text{mg L}^{-1}$ ) were run in quadruplicate. Both alkaline-killed ( $\text{pH} > 11$ ) and sterile-filtered (0.22  $\mu\text{m}$ ) samples were run as controls. Nitrous oxide ( $\text{N}_2\text{O}$ ) evolution in the headspace of the bottles was measured at 2 h intervals over 10 h by withdrawing samples of headspace gas in valved gas-tight syringes for analysis on a Shimadzu GC-8A gas chromatograph with an electron capture detector.  $\text{N}_2\text{O}$  production rates were determined by linear regression of  $\text{N}_2\text{O}$  concentration over time.

### 2.8. Full-scale biomass addition

Biomass POC addition experiments were also conducted in situ within the septage-treating wetland during otherwise normal operations. Aquatic tanks T7, T8 and T9 (Fig. 1) each received three mesh bags (0.5 cm mesh) filled with dried plant litter and suspended below the root zone of the floating plants for 7 weeks. A total of 5.1 kg dry wt. of biomass was added to each tank (8.2  $\text{g biomass L}^{-1}$  or 2.5  $\text{g POC L}^{-1}$ ), 33% as dried cattails (*Typha*) harvested from the subsurface flow wetland and 67% as wheat straw [34,35]. The parallel tank series was left unamended as a control, since a preliminary 2 week monitoring found no significant differences in  $\text{NO}_x^-$ -N levels (2-tailed *t*-test,  $p > 0.10$ ,  $n = 5$ ) between the paired series of tanks (T7, T8, T9). During the 7 week amendment experiment, water samples were collected for C and N analysis 8 times from each of tanks T5–T9 in both the amended and unamended trains. The bags were then retrieved, rinsed gently with distilled water to remove any loose floc, reweighed, and analyzed for POC/N content to determine the rate of decomposition and leaching of the plant material.

## 3. Results

### 3.1. Carbon and nitrogen cycling

Biological treatment of septage in the wetland facility removed 99.3% of influent C and 99% of influent N from the waste stream (Fig. 2). Removal was via oxidation of organic matter, denitrification, and sedimentation. TSS and OC fell together during primary treatment: POC concentration was much higher than dissolved OC, and primary sedimentation therefore reduced both TSS and OC concentration by an equal amount, 87%. However, in the secondary clarifier, solids removal had a greater impact on TSS than on OC (83% versus 61%) because dissolved OC (not removed by sedimentation) accounted for a larger fraction of the total OC pool than during primary sedimentation. Dissolved OC levels remained roughly constant during treatment, ranging from 32 to 20  $\text{mg L}^{-1}$ . Thus OC concentration in the final effluent exceeded TSS

concentration. Total N followed the same pattern as OC during primary treatment, however the removal of total N (34%) in the secondary clarifier was even smaller than the OC removal, since a larger proportion of the total N

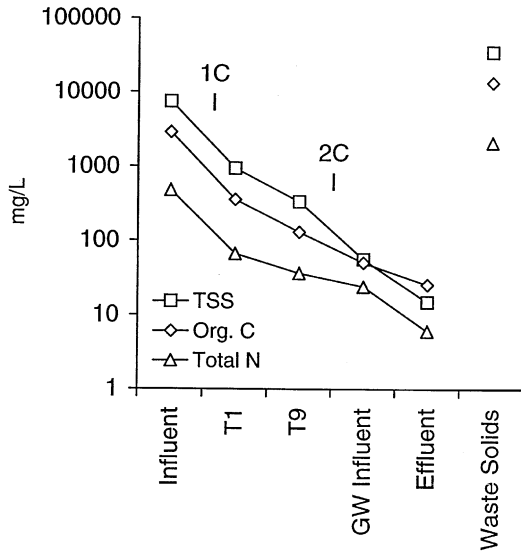


Fig. 2. Changes in the mean concentration of TSS, OC, and total N (TN) during treatment based on mass balance. Refer to Fig. 1 for locations. The declines in concentration between the influent and aquatic tank T1, and between tank T9 and the gravel wetland (GW) influent mainly result from sedimentation of solids in the primary and secondary clarifiers (1C and 2C). Other declines are due to degradation, oxidation, and denitrification of organic material. TN values from [26].

was composed of dissolved forms. Total N concentrations underwent a proportionally larger drop compared to OC in the course of treatment in the gravel wetland (75% versus 49%), as a result of denitrification of  $\text{NO}_x^-$ -N in the wetland influent. Since the removal of TSS in the gravel wetland was due primarily to sedimentation rather than oxidation [26], the decline of TSS and total N in this subsystem, although proportional, were causally unlinked.

Although OC concentrations in the raw septage averaged  $2890 \text{ mg L}^{-1}$ , biological oxidation and primary sedimentation removed 23% and 62% of the C, respectively, resulting in an influent to the aquatic tanks of only  $360 \text{ mg CL}^{-1}$  (Table 2). Oxidation within the aquatic tanks removed 64% of this influent C, while sedimentation in the secondary clarifier removed 19%, further reducing the OC concentration to  $50.6 \text{ mg L}^{-1}$  (Table 2). The measured rates of C oxidation within the aquatic tanks were similar to those during primary treatment ( $2.90$  versus  $3.86 \text{ mg L}^{-1} \text{ h}^{-1}$ ) in spite of the large difference in POC concentration. In contrast, rates of denitrification in the aquatic tanks were only about one third,  $0.26 \text{ mg L}^{-1} \text{ h}^{-1}$ , of those during primary treatment,  $0.75 \text{ mg L}^{-1} \text{ h}^{-1}$ .

Measurements of C lability yielded a longer half-life for septage OC (8.4 d) compared to sewage (6.9 d) (Table 3). The longer septage half-life results from oxidation of the more labile C fractions of raw sewage during septic tank treatment, leaving recalcitrant fractions behind. However, the small magnitude of the difference indicates that organic matter in septage is still relatively labile, in spite of the long intervals between pumping of septic tanks ( $\sim 1 \text{ yr}$ ). In contrast, both the biological activity

Table 2  
Composition of wastewater at locations within the septage-treating wetland (Fig. 1)

( $\text{mg L}^{-1}$ ) <sup>a</sup>	Primary treatment <sup>b</sup>	Primary effluent	Aquatic treatment tanks (mean) <sup>b</sup>	2° clarifier effluent
Organic C	2270	360	196	50.6
Organic N	352	55.9	30.4	7.9
$\text{NH}_4^+$ -N	20.6	9.3	2.7	0.72
$\text{NO}_x^-$ -N	1.1	1.1	12.5	14.9
Total N	373	66.3	45.6	23.5
( $\text{mg L}^{-1} \text{ h}^{-1}$ ) <sup>c</sup>				
C oxidation	3.86	—	$2.90 \pm 0.27$	—
Nitrification	0.71	—	$0.606 \pm 0.059$	—
Denitrification	0.75	—	$0.260 \pm 0.048$	—
Temperature ( $^{\circ}\text{C}$ )	23.3		22.3	

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

<sup>b</sup> Concentrations and rates are averaged over the whole component volume. Usually a downward concentration gradient (for all species except  $\text{NO}_x^-$ ) exists within the treatment component from its influent to its effluent.

<sup>c</sup> Rates for primary treatment determined by mass balance,  $n = 22$ . Rates for aquatic tanks determined from differences between the tanks. Error is standard error,  $n = 8$ .

Table 3  
Decomposition of biologically active OC in wastewater from the septage-treating wetland during aerobic degradation

Source	$k^a$ (d <sup>-1</sup> )	Half-life <sup>b</sup> (d)	$r^2$	O <sub>2</sub> uptake/OC <sup>c</sup> (mg O <sub>2</sub> g C <sup>-1</sup> h <sup>-1</sup> )
Septage				
Equalization tank	0.082	8.4	0.92	1.2
Waste biosolids	0.041	16.7	0.84	0.39
Tank T1	0.138	5.0	0.91	2.7
Tank T9	0.095	7.3	0.93	0.83
Sewage <sup>d</sup>				
	0.100	6.9	0.94	3.2

See Fig. 1 for sampling locations. Changes in the reactivity of the OC during treatment result from differential loss of labile and refractory forms through sedimentation or decay. The reactivity is relative to the total OC pool within a sample; total C concentrations decline with passage through the septage-treating wetland facility (Fig. 2).

<sup>a</sup>Decay constant.  $n = 4$  replicates per time point;  $n = 5-7$  time points.

<sup>b</sup>Half-life  $t_{1/2} = \ln(2)/k$ .

<sup>c</sup>At start of experiment.

<sup>d</sup>Fields Point, Providence, RI.

and the concentration of septage organic matter changed during treatment. Primary sedimentation resulted in the settling of relatively recalcitrant solids with a mean half-life of 16.7 d, producing a more labile supernatant with three times the reactivity ( $t_{1/2} = 5.0$  d; Table 3). During passage through the aquatic tanks, labile C fractions were most intensively degraded with the result that the half-life of the OC increased from 5.0 to 7.3 d between tanks T1 and T9.

Carbon inputs by algal photosynthesis and microbial CO<sub>2</sub> fixation (primarily nitrification) within the aquatic tanks were only a minor source of organic matter, supplying at most  $0.039 \pm 0.005$  mg C L<sup>-1</sup> h<sup>-1</sup> or only 1% of the measured POC degradation rate of  $2.90$  mg L<sup>-1</sup> h<sup>-1</sup>. Photosynthetic C fixation accounted for only about one quarter ( $0.009 \pm 0.001$  mg L<sup>-1</sup> h<sup>-1</sup>) of the total C fixation, with dark C fixation accounting for the remaining three quarters ( $0.030 \pm 0.004$  mg L<sup>-1</sup> h<sup>-1</sup>). Nitrification is the only dark process which could account for these rates. This rate of autotrophic dark fixation corresponds to a nitrification rate of  $0.35$  mg L<sup>-1</sup> h<sup>-1</sup> (based on mean 11.8:1 molar ratio of nitrified NH<sub>4</sub><sup>+</sup>-N to C fixed by *Nitrosomonas*; [36]), about half of the rate of  $0.606$  mg L<sup>-1</sup> h<sup>-1</sup> measured by mass balance [26]. During the 6 h <sup>14</sup>C incubations, N incorporation into biomass by the autotrophic community (based on a C/N mass ratio of 4.3 for bacterial biomass; [36]) was small,  $0.011$  mg N L<sup>-1</sup> h<sup>-1</sup> or only 2.4% of the net N mineralization rate. The uptake of N by nitrifiers was similar to the macrophyte N uptake rate of  $0.01$  mg N L<sup>-1</sup> h<sup>-1</sup>.

### 3.2. Carbon availability and denitrification

POC additions (as straw) stimulated denitrification rates (acetylene inhibition assay) in laboratory sewage

incubations from  $<0.005$  mg N L<sup>-1</sup> h<sup>-1</sup> in unamended controls to up to  $0.229 \pm 0.023$  mg N L<sup>-1</sup> h<sup>-1</sup> in samples amended with  $1.4$  g POC L<sup>-1</sup> (Fig. 3a). In addition, the magnitude of the effect of C amendments was directly related to the initial NO<sub>x</sub><sup>-</sup>-N concentration of the wastewater, indicating simultaneous limitation of denitrification in these samples by both OC and NO<sub>x</sub><sup>-</sup> availability. At the lowest of the four initial NO<sub>x</sub><sup>-</sup>-N concentrations ( $0.13$  mg L<sup>-1</sup>), no denitrification was detected despite POC addition. In aerobic treatments, there was a lag between POC addition and the onset of denitrification while in anaerobic treatments, Denitrification was observed in the first 2 h. The lag in denitrification rate was defined as the amount of time required for the denitrification rate in aerobic treatments to equal the initial denitrification rate (first 2 h) of the corresponding anaerobic treatment. Lag time decreased by 1.1 h for each  $1$  g L<sup>-1</sup> increase in biomass POC additions up to  $3.5$  g POC L<sup>-1</sup> (Fig. 3b).

In situ monitoring of the aquatic tanks, denitrification rates were directly related to septage POC loading rates ( $r^2 = 0.63$ ) suggesting the importance of actively respiring organic particles in promoting denitrification (Fig. 4). A similar stimulation of denitrification was seen in the full-scale biomass POC amendment experiment in which  $2.5$  g POC (as plant litter) L<sup>-1</sup> was suspended in mesh bags within three of the aquatic tanks (T7–T9) of one of the parallel tank trains under normal facility operations. Over the 7 week experiment, NO<sub>x</sub><sup>-</sup>-N concentrations were consistently about 50% lower in the amended tanks,  $6.4 \pm 1.9$  mg N L<sup>-1</sup>, compared to the corresponding tanks of the parallel control train,  $12.7 \pm 2.7$  mg L<sup>-1</sup>. In contrast, the influent NO<sub>x</sub><sup>-</sup>-N levels to these tanks in both trains were equal, as determined by measurements of the upgradient tanks, T5 and T6 (Fig. 5). The biomass POC amendment resulted in no

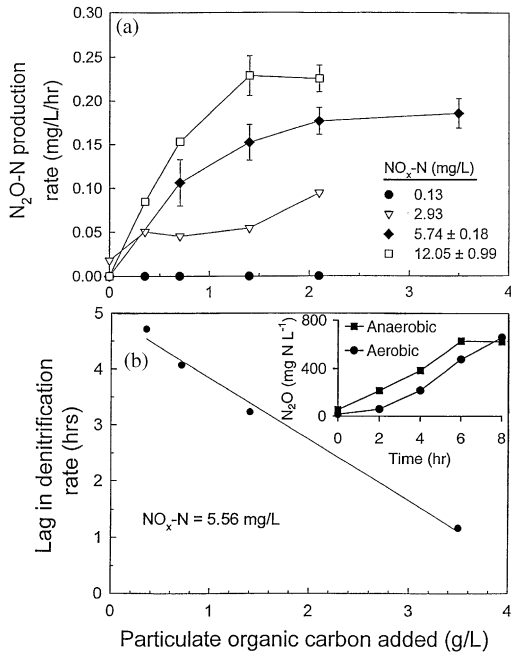


Fig. 3. (a) Stimulation of denitrification rate in sewage (measured by N<sub>2</sub>O production under acetylene block) in laboratory bottle experiments at 4 initial NO<sub>x</sub><sup>-</sup>-N concentrations by addition of POC as wheat straw. Each point of the lower two NO<sub>x</sub><sup>-</sup>-N levels represents two measurements on duplicate bottles; the upper two NO<sub>x</sub><sup>-</sup>-N levels are quadruplicates. Error bars represent standard errors. (b) Effect of POC addition as straw on the lag in the stimulation of denitrification resulting from initial aerobic conditions during laboratory sewage incubations. Lag time is the time required for denitrification rates in aerobic samples to equal the initial denitrification rates in the anaerobic samples and reflects time required to develop anaerobic conditions within the straw matrix through organic matter respiration. Solid line is linear regression. Inset shows timecourse of N<sub>2</sub>O production for 3.6 g POCL<sup>-1</sup>. Note N<sub>2</sub>O production lag in aerobic treatment.

significant differences in NH<sub>4</sub><sup>+</sup> or TSS levels between pairs of amended and control tanks (2-tailed *t*-test; *p* > 0.10, *n* = 8). The average rate of C loss from the added litter during the experiment was determined to be 0.15 mg CL<sup>-1</sup> wastewater h<sup>-1</sup>. Rates of C loss from straw were twice as high (1.4% d<sup>-1</sup>) as those from cattails (0.73% d<sup>-1</sup>). Most of the reduction in NO<sub>x</sub><sup>-</sup>-N levels occurred in tank T7, the first tank which received biomass POC additions. The NO<sub>x</sub><sup>-</sup>-N decrease represents an increase in the denitrification rate from 0.26 to 0.58 mg NL<sup>-1</sup> h<sup>-1</sup> resulting from POC addition. This rate of denitrification in the amended tanks, which contained a total of 2.7 g CL<sup>-1</sup>, is similar to that during primary treatment, 0.75 mg L<sup>-1</sup> h<sup>-1</sup>, at a similar septage POC level of 2.3 g CL<sup>-1</sup> (Table 2).

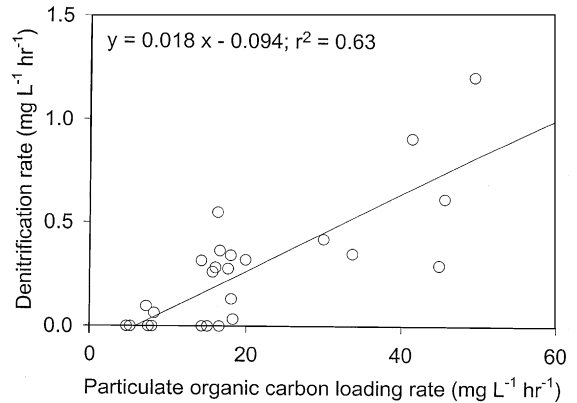


Fig. 4. Relationship of septage POC loading rate to denitrification in the aquatic treatment tanks. POC loading data derived from measurements of POC concentrations in individual tank adjusted with simultaneous flow rate measurements. Denitrification data determined from difference in total nitrogen between two sequential tanks.

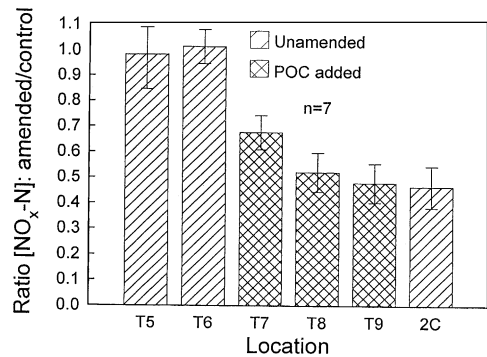


Fig. 5. Ratio of NO<sub>x</sub><sup>-</sup>-N concentration in parallel aquatic treatment tank trains amended with POC as cattails and wheat straw versus control. The two trains show the same NO<sub>x</sub><sup>-</sup>-N levels in the tanks upstream of the biomass POC additions (T5, T6) but NO<sub>x</sub><sup>-</sup>-N was reduced by an average of 50% after amendment. Error bars represent standard errors.

87% of the NO<sub>x</sub><sup>-</sup>-N flowing into the anaerobic gravel wetland bed was denitrified, at a rate of 0.18 mg NL<sup>-1</sup> h<sup>-1</sup>. Evidence suggests that the OC at this stage was highly refractory and was not oxidized [26]. However, 0.24 mg CL<sup>-1</sup> h<sup>-1</sup> were required to support the metabolic requirements of the observed denitrification. This OC was supplied by additions of soluble OC (acetate). Denitrification in the gravel wetland was then limited by the supply of NO<sub>x</sub><sup>-</sup>-N alone, as indicated by the linear dependence of the denitrification rate on NO<sub>x</sub><sup>-</sup>-N loading rates (denitrification = 0.87 NO<sub>x</sub><sup>-</sup>-N loading; r<sup>2</sup> = 0.99; Fig. 6).

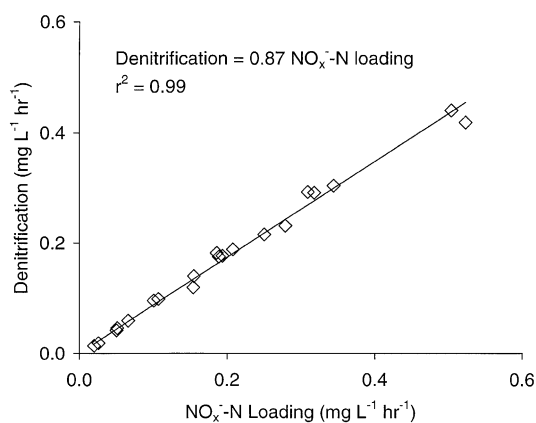


Fig. 6. Denitrification rate in the gravel wetland as a function of  $\text{NO}_x^-$ -N loading rates over a 6 month period. OC was added as acetate. Denitrification was calculated from the disappearance of  $\text{NO}_x^-$ -N, under the conservative assumption that nitrification was inhibited in the anaerobic wetland [26].  $\text{NO}_x^-$ -N loading was determined using concurrent flow rate measurements.

#### 4. Discussion

Denitrification within the aquatic tanks of the septage-treating wetland is limited by the availability of labile POC. However, it appears that biodegradable organic matter is less important as a substrate for nitrate reduction in these aerated tanks, than as a mechanism for establishing the low-oxygen niches needed for this microbial pathway. The aquatic tanks contrast with the POC-rich primary treatment unit, where the rate of  $\text{NO}_x^-$ -N supply (via nitrification) limits N removal by denitrification. Likewise, in the gravel wetland, denitrification appears to be limited by  $\text{NO}_x^-$ -N (Fig. 6), since conditions are anaerobic and sufficient labile C was added to supply the metabolic needs of denitrifiers.

In the aquatic tanks,  $\text{NO}_x^-$ -N accumulates along the treatment train as a result of nitrification in excess of denitrification [26], consistent with C limitation of denitrification. However, the  $0.35 \text{ mg CL}^{-1} \text{ h}^{-1}$  (based on 6:4 (molar) C:N stoichiometry for denitrification; [3]) required to support the observed denitrification rate was only 12% of the observed respiration rate of  $2.90 \text{ mg CL}^{-1} \text{ h}^{-1}$ . Further evidence that denitrification was not limited by C substrate availability is provided by the plant litter amendments. Although the amendments doubled the denitrification rate, they increased the C respiration rate in the tanks by only  $0.15 \text{ mg CL}^{-1} \text{ h}^{-1}$ , only 35% of the  $0.43 \text{ mg CL}^{-1} \text{ h}^{-1}$  that would have been required to support the additional denitrification.

Aside from  $\text{NO}_3^-$  and C substrate, denitrification also requires anaerobic conditions. In heterotrophic–auto-

trophic biofilms, respiration produces sufficiently low-oxygen levels ( $<0.1 \text{ mg DO L}^{-1}$ ) only 100–150 mm below the biofilm surface even in oxygen-saturated solutions [6,7]. Thus, bioavailable POC acts not only as a C substrate for denitrifiers, but also depletes DO levels within particles via aerobic respiration, supporting denitrification within aerobic wastewaters [37,38]. The observed increase in denitrification in the biomass POC-amended aquatic tanks was therefore likely due to this respiration-driven creation of anaerobic microsites within the plant litter where denitrification was fueled both by OC from the degrading plant litter and by OC and  $\text{NO}_x^-$ -N from the wastewater stream.

Additional support for this mechanism came from laboratory experiments on the response of denitrification rates to varying levels of  $\text{NO}_x^-$  and biomass POC, under aerobic versus anaerobic conditions (Fig. 3). Samples which had been made anaerobic had denitrification rates initially higher than aerobic samples (Fig. 3b inset). In addition, the time required for the denitrification rates in aerobic samples to reach the same levels as the initial rates in the anaerobic samples (lag time) decreased with increasing POC addition (Fig. 3b). This lag results from the time required for C respiration to create anaerobic microsites in particles and in the water. Higher POC additions resulted in higher respiration rates, and the faster creation of anaerobic microsite volume to support denitrification.

In aerobic septage with high oxygen demand, the role of POC in providing anaerobic microsites may be more important than its role as a source of OC substrate for denitrifier growth. However, the availability of  $\text{NO}_x^-$  can also limit denitrification, as observed in the laboratory experiments where the maximum denitrification rate achievable at the highest POC addition level was related to the  $\text{NO}_x^-$  concentration (Fig. 3a). Low levels of  $\text{NO}_x^-$  which inhibit denitrification were found during primary treatment, and occasionally, throughout the first half of the aquatic treatment tank series (Table 2). However, in general,  $\text{NO}_x^-$  levels after tank T2 were not limiting to denitrification [26] and so were amenable to biomass POC supplementation. Consistent with our expectations from process monitoring and laboratory incubations, the addition of POC as plant litter directly to the aquatic tanks in the septage-treating wetland resulted in a 50% reduction in  $\text{NO}_x^-$ -N and a near doubling of the denitrification rate (Fig. 5). Plant litter has been used in other studies to promote denitrification [12–14]. In the relatively anaerobic environment of a constructed wetland, the addition of soluble OC was found to result in a greater reduction of  $\text{NO}_3^-$  than did additions of plant litter [12]. In contrast, in the present study, where the aquatic tanks were aerated, plant litter is probably more effective, since it provides both C substrate and anaerobic microsites for denitrification, and is not washed out by the septage flow.

During primary treatment, the high respiration rate ( $3.86 \text{ mg CL}^{-1} \text{ h}^{-1}$ ), low DO ( $2.14 \text{ mg L}^{-1}$ ), and high OC concentrations ( $2270 \text{ mg L}^{-1}$ ) suggest that labile OC and anaerobic microsites were plentiful. However,  $\text{NO}_x^-$ -N availability was low ( $1.1 \text{ mg L}^{-1}$ ; Table 2), while  $\text{NH}_4^+$  concentrations were high ( $20.6 \text{ mg L}^{-1}$ ), indicating that the supply of  $\text{NO}_x^-$  via nitrification limited denitrification in this environment [26]. Denitrification capacity was great enough to denitrify the nitrate-rich ( $14.9 \text{ mg L}^{-1}$ ) secondary recycle flow in addition to the nitrate generated in situ through nitrification, in further support of nitrate limitation of denitrification during primary treatment. In contrast, the water flowing through the gravel wetland was high in  $\text{NO}_x^-$ -N, low in suspended organic matter, and anaerobic [26], suggesting OC substrate limitation of denitrification. This limitation was relieved by the addition of soluble OC, switching conditions in the gravel wetland toward nitrate limitation of denitrification (Fig. 6).

The results of the C degradation incubations indicate that not only the mass, but the lability of POC can limit denitrification. Recalcitrant organic material inhibits denitrification rates in three ways: by reducing the availability of  $\text{NH}_4^+$  for nitrification and therefore the availability of  $\text{NO}_3^-$ , by providing less C substrate, and by reducing the formation of anaerobic microsites to support denitrification. Oxidation and sedimentation during septage treatment affects POC both by reducing its overall quantity and by changing its lability. After primary biological treatment in the septage-treating wetland, primary sedimentation separates septage solids into two fractions: easily settleable waste solids of low biodegradability ( $t_{1/2} = 16.7 \text{ d}$ ), and labile solids ( $t_{1/2} = 5 \text{ d}$ ; Table 3) which remain in the supernatant. It is this highly bioavailable fraction which is pumped into the aquatic tanks for further treatment. The bioactive nature of the clarified waste stream is best illustrated by noting that although its C respiration rate was 75% of that of raw septage ( $2.9$  compared to  $3.86 \text{ mg CL}^{-1} \text{ h}^{-1}$ ), the OC concentration of the clarified wastewater ( $360 \text{ C mg L}^{-1}$ ) was only 16% of that found during primary treatment ( $2270 \text{ mg CL}^{-1}$ ). Thus the C-specific respiration rate during primary treatment was only  $1.7 \text{ mg C g}^{-1} \text{ OC h}^{-1}$  compared to  $14.8 \text{ mg C g}^{-1} \text{ OC h}^{-1}$  in the aquatic tanks. Diel variations in the age and quality of the incoming waste stream resulted in variations in the lability of OC (unpublished obs.), and may account for some of the variability in the relationship of septage POC loading to denitrification observed in the aquatic tanks (Fig. 4).

Another measure of OC lability is the proportion of influent C oxidized over the course of its passage through the treatment component, which might be called the functionally labile C. During the 6.7 d

retention time of primary treatment, 26% of influent C was oxidized, while during the 4.8 d passage through the aquatic tanks, 67% of the influent C was degraded (Fig. 7). This larger proportion of organic matter degraded in the aquatic tanks resulted from the higher lability of OC ( $t_{1/2} = 5.0 \text{ d}$  versus  $8.7 \text{ d}$ ), and possibly the higher DO concentrations ( $7.2 \text{ mg L}^{-1}$  versus  $2.1 \text{ mg L}^{-1}$ ) [26] and the presence of large numbers of invertebrates associated with wetland plants (pers. obs.). While in each subsystem, roughly the same proportion of influent C was discharged to the next treatment step, the proportion oxidized versus that settled as solids was reversed, with approximately 3 times as much OC settled than was oxidized during primary treatment, while in the aquatic tanks, 3 times as much was oxidized than was settled (Fig. 7).

It appears that the active microbial community within the aquatic tanks supports the treatment process primarily through organic degradation and N transformations, while autotrophic fixation of C (through photosynthesis or nitrification) contributes an insignificant amount ( $<1\%$ ) in comparison to the OC degradation rate. Similarly, plants were periodically removed from the aquatic tanks, and aboveground biomass was harvested annually from the wetland, removing much of the C fixed by macrophytes. However, leaching losses from senescing leaves and roots, root exudates, or mechanical damage to the plants must have resulted in some addition of fixed C to the system [14,39,40]. Nonetheless, since total OC production by the plants was only  $\sim 2\%$  of the OC degradation rate, any leaching losses must have been insignificant. Although macrophyte-produced OC probably enhanced denitrification in both the aquatic tanks and in the gravel wetland, OC availability still limited denitrification in the aquatic tanks, as demonstrated by the full-scale biomass POC addition experiments.

Waste solids production by the septage-treating wetland was  $5.6 \text{ g L}^{-1}$  septage treated, or  $0.81 \text{ g solids g}^{-1}$  influent TSS, containing 61.5% of the total influent C [26]. In contrast, a nearby conventional septage-treating facility utilizing chemical precipitation produced  $1.3 \text{ g solids g}^{-1}$  influent TSS, amounting to  $\sim 99\%$  of the influent POC [41]. This near-total removal of POC at the outset of treatment (for landfill disposal) reduced the amount of OC and N available for removal by oxidation and denitrification as well as the POC essential to support denitrification. Lower solids production in the septage-treating wetland resulted both from the lack of chemical precipitants (which are wasted in conventional treatment along with the settled septage solids) and the greater biological oxidation of OC during treatment. Biological degradation of OC has the further benefit of leaving relatively recalcitrant ( $t_{1/2} = 16.7 \text{ d}$ ) organic material as waste solids, with less potential for environmental harm on disposal.

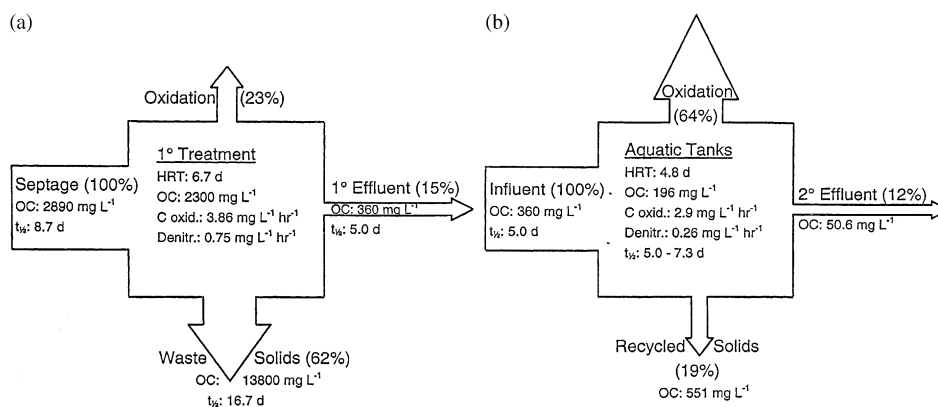


Fig. 7. Organic carbon (OC) balance of (a) primary treatment and (b) aquatic treatment tanks. Width of arrows are proportional to sizes of sources and sinks. The concentration and half-life of OC are shown for the influent, effluent and solids flows for each treatment system, while the boxes show chemical characteristics averaged over the entire treatment system. Abbreviations: HRT—hydraulic retention time; OC—organic carbon; C oxid.—carbon oxidation rate; Denitr.—denitrification rate;  $t_{1/2}$ —carbon half-life.

Our results emphasize the importance of POC supply in controlling denitrification in this septage-treating engineered wetland. The lability of influent POC controls its availability to support denitrification, and is a result of its age and history. Although labile POC provides a substrate for the growth of denitrifying bacteria, in the aerobic conditions found in this septage treating wetland, its primary role is through the creation of anaerobic microsites necessary to support denitrification.

## 5. Conclusions

In the aerated aquatic treatment tanks of the septage-treating wetland, although more than enough C is available to support the metabolic needs of denitrification, POC controls nitrate removal by creating anaerobic microsites where denitrification can occur.

Addition of wetland macrophyte biomass to the aquatic treatment tanks at a concentration of 2.5 g C L<sup>-1</sup> caused a doubling of the denitrification rate to 0.58 mg L<sup>-1</sup> h<sup>-1</sup>. Biomass amendments stimulated denitrification by creating additional anaerobic microsites in the aerobic bulk liquid of the aquatic tanks.

During primary treatment, the rate of nitrification, rather than OC availability limits denitrification, while in the gravel wetland, OC is required as a substrate for denitrification, rather than to create anaerobic conditions.

The capability of septage POC to support denitrification, whether as a substrate or by consuming O<sub>2</sub>, is related to both its concentration and lability. Treatment of septage in the facility changed the half-life of OC by differential degradation and sedimentation of labile and

recalcitrant organic compounds. The waste solids generated by the facility were twice as recalcitrant ( $t_{1/2} = 16.7$  d) as the septage influent, while the clarified primary effluent which was treated in the wetland was the most labile ( $t_{1/2} = 5.0$  d).

Microbial and algal C fixation rates were only 1% of C oxidation rates. The rates of incorporation of dissolved N into organic N by nitrifiers and macrophytes in the aquatic treatment tanks were equivalent, at  $\sim 0.01$  mg L<sup>-1</sup> h<sup>-1</sup>, and were small compared to the denitrification rate of 0.26 mg L<sup>-1</sup> h<sup>-1</sup>.

## Acknowledgements

Financial support for this research was provided by the Island Foundation of Marion, MA, USA and by the Education Department of the Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA. David White helped with both field and laboratory work, and played a role in early discussions. Susan Peterson, Bruce Strong, and Debbie Hamel of Environmental Engineering Associates, Weston, MA operated the Marion, MA septage-treating wetland and with John Teal (WHOI) cooperated in the experimental efforts. Susan Brown-Leger (WHOI) performed the NO<sub>x</sub> and DON determinations. Susan Johnke performed the acetylene block and C fixation assays.

## References

- [1] Ryther JH, Dunstan WM. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 1971;171:1008–13.

- [2] Worm B, Lotze HK, Sommer U. Coastal food web structure carbon storage, and nitrogen retention regulated by consumer pressure and nutrient loading. *Limnol Oceanogr* 2000;45:339–49.
- [3] Focht DD, Chang AC. Nitrification and denitrification processes related to waste water treatment. *Adv Appl Microbiol* 1975;19:153–86.
- [4] Nichols DS. Capacity of natural wetlands to remove nutrients from wastewater. *J Water Pollut Control Fed* 1983;55:495–505.
- [5] Kadlec RH, Knight RL. *Treatment wetlands*. Chelsea, MI: Lewis, 1996. 928pp.
- [6] van Loosdrecht MCM, Tjihuis L, Wijdicks AMS, Heijnen JJ. Population distribution in aerobic biofilms on small suspended particles. *Water Sci Technol* 1995;31:163–71.
- [7] Zhang TC, Fu Y-C, Bishop PL. Competition for substrate and space in biofilms. *Water Environ Res* 1995;67:992–1003.
- [8] Daigger GT, Littleton HT. Characterization of simultaneous nutrient removal in staged, closed-loop bioreactors. *Water Environ Res* 2000;72:330–9.
- [9] Grady Jr CPL, Daigger GT, Lim HC. *Biological wastewater treatment*, 2nd ed. New York: Marcel Dekker, 1999.
- [10] Issacs SH, Henze M. Controlled carbon source addition to an alternating nitrification-denitrification wastewater treatment process including biological P removal. *Water Res* 1995;29:77–89.
- [11] Rusten B, Hem LJ, Ødegaard H. Nitrogen removal from dilute wastewater in cold climate using moving-bed biofilm reactors. *Water Environ Res* 1995;67:65–74.
- [12] Gersberg RM, Elkins BV, Goldman CR. Use of artificial wetlands to remove nitrogen from wastewater. *J Water Pollut Control Fed* 1984;56:152–6.
- [13] Ingersoll TL, Baker LA. Nitrate removal in wetland microcosms. *Water Res* 1998;32:677–85.
- [14] van Oostrom AJ, Russell JM. Denitrification in constructed wastewater wetlands receiving high concentrations of nitrate. *Water Sci Technol* 1994;29:7–14.
- [15] Hunt RJ, Krabbenhoft DP, Anderson MP. Assessing hydrogeochemical heterogeneity in natural and constructed wetlands. *Biogeochemistry* 1997;39:271–93.
- [16] Reed SC, Brown D. Subsurface flow wetlands—A performance evaluation. *Water Environ Res* 1995;67:244–8.
- [17] Burgoon PS, Reddy KR, DeBusk TA. Performance of subsurface flow wetlands with batch-load and continuous-flow conditions. *Water Environ Res* 1995;67:855–62.
- [18] Gale PM, Reddy KR, Graetz DA. Nitrogen removal from reclaimed water applied to constructed and natural wetland microcosms. *Water Environ Res* 1993;65:162–8.
- [19] Kemp MC, George DB. Subsurface flow constructed wetlands treating municipal wastewater for nitrogen transformation and removal. *Water Environ Res* 1997;69:1254–62.
- [20] Canter LW, Knox RC. *Septic tank system effects on ground water quality*. Chelsea, MI: Lewis, 1985.
- [21] Howes BL, Goehringer DD. The ecology of Buzzards Bay: an estuarine profile. National Biological Service Biological Report 31. Washington, DC, 1996. vi+141pp.
- [22] Teal JM, Peterson SB. A solar aquatic system septage treatment plant. *Environ Sci Technol* 1993;27:34–7.
- [23] Teal JM, Peterson SB. The next generation of septage treatment. *Res J Water Pollut Control Fed* 1991;63:84–9.
- [24] Peterson SB, Teal JM. The role of plants in ecologically engineered wastewater treatment systems. *Ecol Eng* 1996;6:137–48.
- [25] Teal JM, Howes BL, Peterson SB, Petersen JE, Armstrong A. Nutrient processing in an artificial wetland engineered for high loading: a septage treatment example. In: Mitsch WJ, editor. *Global wetlands: old world and new*. New York: Elsevier, 1994. p. 421–8.
- [26] Hamersley MR, Howes BL, White DS, Johnke S, Young D, Peterson SB, Teal JM. Nitrogen balance and cycling in an ecologically engineered septage treatment system. *Ecol Eng* 2001;18:61–75.
- [27] DeSimone LA, Howes BL. Hydrogeologic, Water-Quality and Biogeochemical Data Collected at a Septage-Treatment Facility, Orleans, Cape Cod, Massachusetts, October 1988 through December 1992. US Geological Survey Open-File Report 95-439, Marlborough, MA, 1995. 73pp.
- [28] Scheiner D. Determination of ammonia and Kjeldahl nitrogen by indophenol method. *Water Res* 1976;10:31–6.
- [29] Wood E, Armstrong F, Richards F. Determination of nitrate in sea water by cadmium copper reduction to nitrite. *J Mar Biol Assoc UK* 1967;47:23–31.
- [30] D'Elia CF, Stuedler PA, Corwin N. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol Oceanogr* 1977;22:760–74.
- [31] APHA. *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association, 1989.
- [32] Taylor CT, Howes BL. Effect of sampling frequency on measurements of seasonal primary production and oxygen status in nearshore coastal ecosystems. *Mar Ecol Prog Ser* 1994;108:193–203.
- [33] Yoshinari T, Knowles R. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochem Biophys Res Commun* 1976;69:705–10.
- [34] Boussaid F, Martin G, Morvan J, Collin JJ, Landreau A. Denitrification in situ of groundwaters with solid carbon matter. *Environ Technol Lett* 1988;9:803–16.
- [35] Lowengart A, Diab S, Kochba M, Avnimelech Y. Development of a biofilter for turbid and nitrogen-rich irrigation water. A: Organic carbon degradation and nitrogen removal processes. *Bioresource Technol* 1993;44:131–5.
- [36] Fenchel T, Blackburn TH. *Bacteria and mineral cycling*. New York: Academic Press, 1979.
- [37] Janke R. A model of microenvironments in deep-sea sediment: formation and effects on porewater profiles. *Limnol Oceanogr* 1985;30:956–65.
- [38] Jørgensen BB, Revsbech NP. Diffusive boundary layers and the oxygen uptake of sediments and detritus. *Limnol Oceanogr* 1985;30:111–22.

- [39] Pinney ML, Westerhoff PK, Baker L. Transformations in dissolved organic carbon through constructed wetlands. *Water Res* 2000;34:1897–911.
- [40] Rice DL, Tenore KR. Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. *Estuarine Coastal Shelf Sci* 1981;13:681–90.
- [41] Slater AD, Weisman PD, Carlson KW. A regional approach to septage management on Cape Cod. *J N Engl Water Pollut Control Assoc* 1987;21:156–74.
- [42] Ruzicka J, Hansen EH. *Flow injection analysis*, 2nd ed. New York: John Wiley & Sons, 1988. 498pp.