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Balancing the Nitrogen Cycle: A Biogeochemical Evaluation of

Ecotechnology for Nitrogen Removal from Concentrated Wastewater

by

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A MASTER'S DEGREE PROJECT SUBMITTED TO THE FACULTY OF ENVIRONMENTAL DESIGN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENVIRONMENTAL DESIGN

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The undersigned certify that they have read, and recommend to the Faculty of Environmental Design for acceptance, a Master's Degree Project entitled "Balancing the Nitrogen Cycle: A Biogeochemical Evaluation of Ecotechnology for Nitrogen Removal from Concentrated Wastewater" submitted by M. Robert Hamersley in partial fulfillment of the requirements for the degree of Master of Environmental design.

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Abstract

BALANCING THE NITROGEN CYCLE: A BIOGEOCHEMICAL EVALUATION OF ECOTECHNOLOGY FOR NITROGEN REMOVAL FROM CONCENTRATED WASTEWATER

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January, 1996

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A Master's Degree Project submitted to the Faculty of Environmental Design in partial fulfillment of the requirements for the degree of Master Of Environmental Design (Environmental Science)

Excess nitrogen from wastewater results in eutrophication of coastal waters and contamination of groundwater. In rural areas, septic tanks are the major method of onsite wastewater treatment and disposal. Septage is the waste solids and water which must be periodically removed from septic tanks. Septage is a major source of nitrogen loads to the environment, accounting for an amount equal to groundwater discharge from septic systems. Existing treatment processes provide only secondary treatment, have high chemical requirements (> 2900 g/m³ septage), and produce large quantities of waste solids (> 1.45 g/g influent TSS). The Solar Aquatic System (SAS) is a hybrid ecotechnological wastewater treatment technology which incorporates aeration and activated solids recycling into a design combining aquatic and constructed wetlands treatment components. A septage treating SAS in coastal Massachusetts produced an effluent of 113 mg/l COD, 16 mg/l TSS, 0.56 mg/l NH₄-N, 6.1 mg/l TN, and 1.6 mg/l TP. Chemical use averaged 33 g lime/m³ septage, and 0.71 g waste solids/g influent TSS were produced (including plant biomass). Nitrogen mineralization, nitrification and

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denitrification occurred simultaneously throughout the treatment process. 40.7 % of influent nitrogen was denitrified. Preliminary treatment was by biological oxidation with activated solids, followed by primary clarification. During preliminary treatment, nitrogen removal was limited by nitrification, which was controlled by the availability of oxygen and nitrifying bacteria. Primary sedimentation produced recalcitrant organic solids (half-life = 16.7 days) and a more labile supernatant (half-life = 5.0 days). Further oxidation and ammonia removal occurred in planted aerated tanks (enclosed in a greenhouse), followed by secondary clarification. Nitrogen removal in the aquatic treatment tanks was limited by denitrification, which occurred in anaerobic microsites within organic particles resulting from the inefficiency of the 1' clarifier. The addition of particulate organic carbon as plant biomass in mesh bags to the tanks resulted in a 50 % reduction in NO_x concentration. Final polishing and denitrification occurred in a constructed wetland. Denitrification was limited by carbon availability, but soluble carbon addition was not required after a year's accumulation of below-ground biomass. The wetland was the largest component of the SAS, but its size could be reduced by lowering NOx levels in the tanks with wetland-derived biomass amendments. The SAS appears to have potential as a method of treating septage in small communities, but a full economic analysis based on operating facilities needs to be made.

Key Words: biogeochemistry, Massachusetts, constructed wetlands, denitrification, ecological engineering, eutrophication, nitrification, nitrogen, septage, wastewater

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1.0 INTRODUCTION: BALANCING THE NITROGEN CYCLE

Nitrogen (N) is an essential nutrient and component of all life, composing 12 % of all biomass. Nitrogen is necessary for the production of proteins, enzymes and DNA, as well as other essential components of the cell. The earth's atmosphere contains about 10,000 times as much nitrogen as the biosphere, but in a form unavailable to life (Schlesinger, 1991). Before the industrial era, the rate at which nitrogen-fixing bacteria converted atmospheric nitrogen (N₂) into organic nitrogen limited its availability to the biosphere. Biological nitrogen fixation totals about 120 billion kg each year. Lightning fixation accounts for another 10 billion kg N/yr (Schlesinger, 1991; Vitousek, 1994). Preindustrial nitrogen fixation was balanced by an approximately equal quantity converted back to nitrogen gas by denitrifying bacteria.

Over the last century, humans have increasingly supplemented natural nitrogen fixation with industrial processes to supply fertilizer and industrial needs. Industrial nitrogen fixation accounts for 105 billion kg N/yr, increasing nitrogen flux from the atmosphere to 180 % of pre-industrial levels (Vitousek, 1994). Most of this increase has occurred in the last 30 years. The global effects of this growing anthropogenic input of nitrogen into the environment are not fully known, but localized effects are becoming better understood.

Seventy-five percent of industrially fixed nitrogen is used as fertilizer for food production (Vitousek, 1994). This organic nitrogen makes its way to dinner tables, and eventually down the drain or toilet. Along with all the other contaminants of wastewater, much of this nitrogen finds its way to rivers, lakes, groundwater, and coastal waters. There, it accumulates, since natural cycling and denitrification rates are insufficient to return it to the atmosphere. Excess nitrogen in natural waters leads to changes in biodiversity and ecological structure. These can result in the destruction of aesthetic value and loss of commercial fisheries, as well as disrupt ecological communities whose origins date back thousands of years (Vitousek, 1994). Excess human fixation of nitrogen has created a problem, and humans must intervene to create a solution. Part of this solution is to recycle fixed nitrogen in order to reduce the need for industrial fixation. Where reuse is still impracticable or uneconomic, the short term solution is to return it to the atmosphere as nitrogen gas.

Most wastewater nitrogen removal processes use physicochemical or biological processes to convert wastewater nitrogen back into a gas, balancing the nitrogen cycle. Biological nitrogen removal, based on mass culturing of denitrifying bacteria, has grown in use in North America, from two or three facilities in the early 1980's to nearly 300 today (Reardon, 1995). These processes have proved to be effective, but can be expensive to construct and operate, and require highly trained personnel. The economies of scale make them impractical for many small wastewater flows (Gray, 1989; Halling-Sørensen and Jørgensen, 1993).

In the last decade, a parallel technology for wastewater treatment and biological nitrogen removal has developed. Ecotechnological (or ecologically engineered) treatment systems use complex ecological associations of plants, invertebrates, and bacteria to facilitate nitrogen removal from wastewater. They are most suited to treating smaller flows, and require less expertise to operate (Crites and Mingee, 1987; Mitch and Jørgensen, 1989; Reed and Brown, 1992; Green and Upton, 1994). Constructed wetlands are the most common application, numbering more than 200 in the U.S. alone (Reed and Brown, 1992; 1995).

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The purpose of this project is to experimentally examine the biogeochemical processes underlying nitrogen removal in an operating ecotechnological wastewater treatment facility, to propose, implement, and evaluate improvements to the treatment process, and to make recommendations for future research directions. I studied nitrogen transformations in a septage treating Solar Aquatic System[™] by biogeochemical analysis of the flows of nitrogen entering and leaving the system, as well as the flows between the various components of the system. I determined the rates of important nitrogen transformations, and the factors controlling those rates. Based on this understanding, I was able to create a conceptual model of the functioning of the system, which I used to

propose improvements to the system. These proposals were aimed at increasing the degree of nitrogen removal in the system, while maintaining or reducing costs. Although a complete economic analysis was beyond the scope of this project, I have estimated the relative economic costs and benefits of proposed changes wherever possible. I evaluated the performance of the system by comparing it to existing septage treatment systems, and to effluent discharge limits set by state regulators. I also implemented and evaluated a process improvement designed to increase the rate of nitrogen removal from the system. Finally, I made recommendations for additional research required to increase understanding of the mechanisms of ecotechnological wastewater treatment, with a view to providing the technical knowledge required to increase its cost effectiveness.

In the first chapter of this report, I summarize the background knowledge necessary to understand ecotechnological treatment processes, and the rationale behind nitrogen removal from wastewater. I examine the various alternative technologies available, including the hybrid ecotechnological systems that I researched in detail. In the second chapter, I present the results and conclusions of my experimental work on as a technical paper. Finally, in Chapter 3, I summarize the conclusions and recommendations resulting from this project.

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1.1 THE BIOGEOCHEMICAL CYCLES OF NITROGEN

Eighty per cent of the earth's atmosphere is composed of nitrogen gas (N_2) . In this form, nitrogen is relatively inert, and unavailable to most lifeforms. Other forms of nitrogen are also present in the atmosphere, notably nitrous oxide $(N_2O) - a$ 'greenhouse gas' -- but in much smaller quantities. Nitrogen flows from the atmosphere into the biosphere and back again, through many chemical forms, in reactions that are catalyzed mostly by bacteria. Atmospheric nitrogen gas is fixed to ammonia (NH_3) , and in that form it can be incorporated into organic matter (Figure 1.1). Organic matter can be degraded to produce ammonia again. Ammonia is converted to nitrate (NO_3^-) by a process called nitrification, and nitrate can be converted to nitrogen gas by denitrification, completing the cycle.

Nitrogen fixation

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Biologically available nitrogen enters terrestrial and aquatic systems mainly through the process of biological nitrogen fixation (Figure 1.1). Certain bacteria have the capability of changing atmospheric nitrogen into biologically available ammonia, which can then be converted into biomass.

 $N_2 + 3H_2 \longrightarrow 2NH_3$ (1.1)

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Figure 1.1 - The nitrogen cycle (simplified).

Many nitrogen-fixing bacteria exist in associations with plants. In terrestrial environments, the symbiotic bacterium *Rhizobium*, associated with plant roots, is the largest contributor to nitrogen fixation. In aquatic habitats, the cyanobacteria are the most important (Atlas and Bartha, 1981). Nitrogen fixation is slow relative to other nitrogen reactions, and is suppressed by the presence of ammonia and nitrate (Capone, 1983). Many organisms can assimilate ammonium, using it to synthesize their tissues.

Nitrogen mineralization

When organic matter (containing particulate organic nitrogen or PON) is consumed by heterotrophs (organisms that get their energy from digesting organic molecules), nitrogen compounds are released as soluble amino acids, nucleotides or other nitrogen-containing organic compounds (dissolved organic nitrogen or DON). Solid organic compounds can be directly ingested and degraded by animals; bacteria and fungi secrete extracellular enzymes which degrade solids to dissolved forms which can then be absorbed. Further digestion by decomposing bacteria results in the production of soluble ammonia (NH₃) or ammonium (NH₄⁺) ions. For example the simple nitrogen-containing compound urea is broken down intracellularly by urease:

$$NH_2CONH_2 + H_20 \longrightarrow 2NH_3 + CO_2$$
(1.2)

Since ammonia is a non-organic form of nitrogen, its release is referred to as mineralization.

Nitrification

Nitrification is the transformation of ammonia into nitrite (NO_2) and then to nitrate (NO_3) by chemosynthetic bacteria (which get energy from chemical sources) of the genera *Nitrosomonas* and *Nitrobacter*, among others.

$$NH_4^+ + 1.5 O_2 \longrightarrow NO_2^- + H_2O + 2H^+$$
 (Nitrosomonas) (1.3)

$$NO_2 + 0.5 O_2 \longrightarrow NO_3$$
 (Nitrobacter) (1.4)

This process generates only a small amount of energy for the bacteria; consequently they are slow growing. Nitrification requires oxygen, and so can only take place in aerobic environments. Nitrite and nitrate may be utilized directly for growth by plants and algae, but animals must absorb the nitrogen they require from more complex compounds such as proteins. Their wastes or their bodies are decomposed in turn, mineralizing ammonium that enters the cycle again. Most of the nitrogen in the biosphere is recycled in this way, the flows to and from the atmosphere being small in comparison (Focht and Chang, 1975; Söderlund and Svensson, 1976; Atlas and Bartha, 1981; Kaplan, 1983).

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Nitrification is controlled by a variety of environmental parameters besides oxygen availability. Nitrification is very sensitive to temperature. The optimal temperature ranges from 25 - 35°C; at 15°C, nitrification rates are approximately 50 % of maximum (De Leval and Remacle, 1976, Kaplan, 1983; Henriksen and Kemp, 1988). Nitrification is also very sensitive to pH, with an optimum at 7.5, falling off very rapidly both above and below that level (De Leval and Remacle, 1976). Nitrification is enhanced by the availability of surfaces for the attachment of bacteria. Most nitrifying activity occurs on the surfaces of suspended particles or other substrates (Kaplan, 1983; Henriksen and Kemp, 1988)

Denitrification

Under anaerobic conditions, some bacteria, such as *Pseudomonas*, use nitrate or nitrite as the terminal electron receptor for respiration instead of oxygen. This process results in the release of gaseous N_2O and N_2 , completing the nitrogen cycle to the atmosphere (Focht and Chang, 1975; Söderlund and Svensson, 1976; Atlas and Bartha, 1981; Hattori, 1983). Denitrification requires an organic carbon source:

$$(CH_2O)_6 + 4NO_3 \longrightarrow 6CO_2 + 6H_2O + 2N_2$$
 (1.5)

While other biologically mediated reactions of nitrogen are possible, these are usually the most important in the global nitrogen cycle.

1.2 EUTROPHICATION IN COASTAL AND ESTUARINE ECOSYSTEMS

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Eutrophication is an ecological term that means literally, 'well nourished'. It refers specifically to the changes that occur in a lake as incoming sediments rich in organic matter gradually fill it, changing its ecological and biogeochemical characteristics (Wetzel, 1975). However, the term 'eutrophication' is commonly used to indicate changes that occur in any body of water receiving higher than usual fluxes of nutrients or organic matter. 'Cultural eutrophication' refers to such changes resulting from human activities such as wastewater disposal.

1.2.1 Limiting Nutrients

1.1.1.2.1

Much scientific research on eutrophication has focused on the identification of 'limiting nutrients'. A nutrient is limiting when its availability relative to that of other nutrients is lower than the proportions required for primary production. An addition of that nutrient brings about a proportional increase in the biological productivity of the system. The addition of other nutrients has no effect, because they are already present in overabundance (Odum, 1971). The impetus for this research has come partly from the desire of managers and regulators to identify and control the limiting nutrient. The belief is that the effect of reducing the limiting nutrient will be greater than that gained by reducing other nutrients (Hecky and Kilham, 1988).

In freshwater systems, decades of research have clearly established that phosphorus is usually the limiting nutrient (Vollenweider, 1976). In marine and estuarine systems, however, the evidence has been less clear. Early work (Ryther and Dunstan, 1971; Caperon and Meyer, 1972; Eppley *et al.*, 1979; McCarthy and Goldman, 1979) seemed to indicate that nitrogen was usually limiting in these environments, but in the last decade, the generalization has been questioned (Nixon and Pilson, 1983; Smith, 1984; Hecky and Kilham, 1988; Fourqurean *et al.*, 1992). It appears that different coastal regions may have different responses to the addition of nitrogen or phosphorus. In particular, saltmarsh, seagrass and seaweed growth are nitrogen limited in temperate waters (Valiela and Teal, 1974; Thayer *et al.*, 1984; Fujita *et al.*, 1989). Regulators usually impose nitrogen as well as phosphorus concentration limits on wastewater effluents to coastal zones.

1.2.2 The Process of Eutrophication

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The symptoms of eutrophication vary with the characteristics of each body of water. Generally, eutrophication is associated with decreasing dissolved oxygen concentrations in the water and higher dissolved nutrient concentrations. These conditions result in changes in species composition, decreased biodiversity, and increased productivity, especially of phytoplankton (algae) (National Academy of Sciences, 1969; Wetzel, 1975).

Large amounts of organic matter from poorly treated wastewater can lead directly to eutrophication. When organic matter (and the nutrients that accompany it) is decomposed

by bacteria or animals, there is a net consumption of oxygen. Large amounts of incoming organic matter (e.g. from sewage dumping) create an oxygen deficit (Lynch and Poole, 1979). Large amounts of organic matter also increase the turbidity of the water, reducing the ability of photosythesizers to produce oxygen (Day *et al.*, 1989).

When the productivity of aquatic systems is limited by the availability of nutrients (rather than by temperature or light, for example), excess nutrients can result in increased biomass production. Bacterial growth consumes oxygen, and while algae produce oxygen during photosynthesis, in the dark they respire, consuming oxygen. In addition, as their cells die and decompose, they place another net oxygen burden on the aquatic system. Thus, excess nutrients eventually have the same effect on a body of water as excess organic matter (Day *et al.*, 1989; Howes and Goehringer, 1994a).

In a slightly eutrophic system, serious depletions of oxygen are likely to be localized in time or space (D'Elia *et al.*, 1979; Howes and Goehringer, 1994a). Acute oxygen depletions can result from variations in the quality or quantity of sewage effluent dumped into the water combined with natural factors. Oxygen concentrations are naturally at their lowest at dawn, after a night of respiratory activity by bacteria and animals and dark respiration by photosynthetic organisms. Added nutrients are less likely to cause oxygen deficits in the winter, when algal and bacterial growth are slow and limited by light availability or temperature rather than by nutrients (Nixon and Pilson, 1983; Hecky and Kilham, 1988). Harmful oxygen depletion is also likely to be limited in spatial distribution, occurring around sewage outfalls and areas with restricted tidal flushing.

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Changes in species composition and biodiversity can result from low oxygen levels and increased nutrient availability, as well as from the disturbance due to fluctuating levels of oxygen and nutrients. Unstable environments promote the success of opportunistic organisms, which reproduce quickly when conditions are right, exploiting a flush of nutrients, or a period of high or low oxygen levels (Howes and Goehringer, 1994a; 1994b). Increases in productivity, especially that of phytoplankton and floating plants may be related to an increase in nutrient availability. Long-term changes in the aquatic environment result in the replacement of species by ones more adapted to the new environmental conditions.

Eutrophic coastal waters such as estuaries and coastal salt ponds frequently develop blooms or massive growths of algae during the summer months (Furnas *et al.*, 1976; Howes and Goehringer, 1994a), or diatom blooms during the winter and spring. Blooms increase the turbidity of the water, affecting submerged aquatic vegetation (see below). The thick, scummy mats that they form are esthetically objectionable, and may create nuisance odors when they decompose. The large quantity of organic matter generated in blooms eventually results in a decomposition-related oxygen deficit.

Eutrophication is frequently accompanied by a shift in algal communities away from algae that provide good food for animals (and therefore do not accumulate as blooms), and decompose rapidly. They are replaced by algae that tend to accumulate in high population densities, do not provide a good food source for higher animals, and decompose slowly, creating a long-term oxygen burden (Ryther and Officer, 1981). Some algae associated with eutrophication, such as *Prymnesium parvum*, may actually be toxic to marine life.

The decline or disappearance of submerged aquatic vegetation is another effect of eutrophication in coastal or estuarine regions. This effect has been documented in systems ranging from river estuaries in Chesapeake Bay (Orth and Moore, 1983; see also Cambridge and McComb, 1984) to large marine areas like the Baltic Sea (Kautsky *et al.*, 1986). Increased phytoplankton populations resulting from eutrophication lead to lower light penetration, reducing the maximum depth of water in which submerged vegetation can grow. Increased levels of nutrients in the water can also stimulate the growth of epiphytes (plants that grow on other plants) on leaf surfaces, reducing the amount of light available for plant growth (Borum, 1985; Sand-Jensen and Søndergaard, 1981).

Dinoflagellate blooms resulting from nutrient enrichment can cause massive mortality of animal life. For example, a bloom of *Gymnodinium breve* in Tampa Bay, Florida caused fish and invertebrate deaths, destroying 77 % of benthic invertebrate species and 97 % of the individuals (Darnell and Soniat, 1981). The shift in algal species associated with

eutrophication can result in a decrease in edible species, with a harmful result on animal species. In two embayments of the south shore of Long Island, New York, eutrophication resulted in a shift from an algal community composed of a diversity of diatom species to one dominated by a small coccoid green alga, *Nannochloris atomus*. This alga could not be eaten by oysters, resulting in the crash of the oyster industry in the area (Ryther and Officer, 1981). The decline of submerged aquatic vegetation due to eutrophication also reduces the habitat available for numerous animal species, including shellfish (Costa, 1988).

Low oxygen levels in extremely eutrophic estuaries can cause periodic fish kills, which strike sensitive species such as salmon, resulting in a shift toward species such as catfish which can tolerate low oxygen levels. Chronically low oxygen levels result in the death of nearly all fish life such as occurred in the Thames Estuary in England in the 1950's, or in New York Harbor at the turn of the century. In these areas, shellfish, crustacean, and finfish populations were virtually eliminated (Gross, 1976). A decline in oxygen concentration causes a shift from aerobic to anaerobic bacteria. Some anaerobic bacteria use sulfur rather than oxygen for respiration. This process results in the production of hydrogen sulfide gas and an unpleasant rotten egg smell (Howes and Goehringer, 1994a).

Eutrophication can have a number of undesirable economic effects. Excess nutrients can result in the growth of algae such as *Synedra*, which impart an objectionable taste to

drinking water supplies (Edmondson, 1969). Eutrophication can cause massive growths of floating plants such as water milfoil (*Myriophyllum spicatum* L.), water hyacinth (*Eichornia crassipes* [Mart] Solms), or macroalgae (seaweed), which can impede navigation (Mulligan, 1969). The greater productivity of eutrophic systems may result in an increase in the abundance of insects such as mosquitoes and gnats, interfering with recreation (Edmonson, 1969). Eutrophication can have an adverse effect on commercial fisheries and can lead to the closure of shellfishing beds (Costa, 1988).

1.2.3 Anthropogenic Sources of Excess Nitrogen

Anthropogenic sources may be classified into two categories: point sources and non-point sources. Point sources to coastal areas include rivers and wastewater treatment outfalls. Non-point sources of nitrogen to coastal waters include precipitation on the water surface and groundwater flows. Nitrogen in precipitation originates from industrial fluxes into the atmosphere from both within and outside of the watershed. Septic tanks add nitrogen to groundwater, as does leachate from fertilizer use in agriculture and landscaping (Biggar and Corey, 1981; Howes and Goehringer, 1994b). Groundwater nitrogen concentrations are ameliorated by plant uptake and denitrification before reaching coastal waters (Weibel, 1969; Smith *et al.*, 1991).

1.3 CASE STUDY: WESTERN CAPE COD

In the coastal aquatic environments of western Cape Cod, Massachusetts, USA, rapid residential development has resulted in increased nitrogen loadings to coastal salt ponds, embayments and estuaries. Most residences in the area use septic tanks for onsite wastewater disposal, and inadequate treatment combined with high residential density has raised the levels of nitrogen compounds in groundwater.

Eutrophication effects are greatest in coastal salt ponds, which tend to accumulate dissolved nutrients because of their low exchange rates with ocean water. High groundwater nutrient loading stimulates the growth of algae and phytoplankton, causing unsightly algal blooms (Howes and Goehringer, 1994a; 1994b). Algal growth in turn depletes the oxygen in the pond, placing stress on animal life in the water, and in advanced stages, causes fish kills. Low oxygen conditions in the summer result in the offensive 'rotten egg' smell associated with eutrophic waters. High nutrient loadings in these salt pond ecosystems and other coastal systems also contribute to the increased growth of epiphytes on the surfaces of eelgrass (*Zostera marina* L.) beds. The epiphytes eventually kill the eelgrass, which provide habitat for commercially important fish and shellfish (Howes and Goehringer, 1994a). Eelgrass cover has declined to 0.3 % of available habitat

in the heavily industrial New Bedford area and to 60 % in less developed areas, resulting in concomitant declines in commercial oyster harvests (Costa, 1988).

Buzzard's Bay, with a surface area of 550 square kilometres, divides the western portion of Cape Cod from the mainland. The buildable area in the Buzzard's Bay watershed is at present approximately 55 % occupied; therefore, the potential exists to nearly double the amount of nitrogen being flushed into the bay. While the bay itself is well flushed by tidal action, the numerous coves, embayments, and coastal ponds that line it are at risk of further eutrophication. These waters are also of greatest importance both biologically and economically, since many marine animal species depend on coastal wetland areas for at least part of their lifecycle (Howes and Goehringer, 1994b). Coastal wetlands also support most of the commercial and recreational shellfishing industries, as well as other recreational activities.

The total nitrogen flux into the Buzzard's Bay is 2508 Mt/year. Wastewater accounts for approximately 73.8 % of this (Table 1.1). Septic treatment of wastewater is responsible for loadings of about half of those from municipal sewage treatment facilities. Precipitation accounts for 19.4 %, and fertilizer for 6.9 %. Although eutrophication in these waters has not nearly approached the advanced stage of areas like Chesapeake Bay or the Hudson estuary, state and federal regulations are in force to control nitrogen

| • | Table | e 1.1 | - Nitrog | jen bud | iget for | Buzzard's | Bay, | Massachuse | lts |
|---|-------|-------|----------|---------|----------|-------------|-------|------------|-----|
| (| (adap | oted | from He | owes a | nd Goe | hringer, 19 | 94b). | | |

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| Source | Nitrogen Load Mt/year | % of Total |
|-----------------------------|--------------------------|------------|
| Precipitation | | |
| On Bay Waters | 433 | 17.3 |
| Runoff - Developed Surfaces | 37 | 1.5 |
| Groundwater (background) | 16 | 0.6 |
| Wastewater Treatment | | |
| Municipal Facilities | 1210 | 48.2 |
| Septic Tank Drainage Field | 320 | 12.8 |
| Septage (estimated) | 320? | 12.8 |
| Fertilizer | 172 | 6.9 |
| Total | 2508 | 100.0 |

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emissions to coastal zones. Buzzard's Bay and other coastal Cape waters are protected under the Massachusetts Ocean Sanctuaries Act, which constrains off-shore development and prohibits new or increased wastewater discharges into the ocean (Slater *et al.*, 1987; Howes and Goehringer, 1994b).

Another problem associated with nitrogen in the ground water is contamination of drinking water. Nitrogen discharges into groundwater are predominately converted to nitrates (NO₃⁻), which can cause a potentially fatal disease (methemoglobinemia) in infants (Halling-Sørensen and Jørgensen, 1993). The groundwater under Cape Cod has been designated as a sole source aquifer by the U.S. Environmental Protection Agency (EPA), placing it under regulations that severely restrict nitrogen discharges.

1.4 NITROGEN REMOVAL FROM WASTEWATER

Attempts to halt or reverse the progression of eutrophication in coastal areas have met with mixed short term success (Gross, 1976). Organic sediments laid down during years of nutrient inputs to waters continue to decompose for years, releasing nutrients and reducing dissolved oxygen levels. Restoration methods used in lakes may not work as well in coastal waters due to their larger water volumes. Techniques include physicochemical alteration, flushing or dilution, and various methods that provide symptomatic relief (National Academy of Sciences, 1969). The short term approach to dealing with eutrophication in severely affected coastal bodies of water may involve restoration. In the long term, solutions must be found to keep nitrogen out of natural waters.

Since wastewater is often the largest source of anthropogenic nitrogen inputs to coastal waters (Howes and Goehringer, 1994b), nitrogen removal from wastewater is becoming a common regulatory requirement for cities and municipalities (Reardon, 1995). A range of technologies, from physicochemical to biological, exist to reduce nitrogen levels in wastewater.

1.4.1 Physicochemical Techniques

Nitrogen compounds in wastewater may be removed by sedimentation. Since small particles and dissolved compounds do not settle well or at all, chemicals are often added which cause them to aggregate. Some form of sedimentation of organic solids, with or without the addition of chemicals, is an important step in nearly all wastewater treatment (Gray, 1989; Horan, 1990; Halling-Sørensen and Jørgensen, 1993). Some soluble compounds can be removed by chemical precipitation. Removing ammonium from wastewater by precipitation with magnesium salts (the only known process) is difficult because phosphate is required and most wastewaters do not contain the right nitrogen:phosphate ratio for this reaction to proceed (Halling-Sørensen and Jørgensen, 1993). Organic nitrogen compounds can be precipitated with compounds such as ligno-

sulfonic acid, ferric chloride, and calcium hydroxide. Chemical precipitation is costly, and has the economic and environmental disadvantage of producing large amounts of chemical wastes, with their attendant disposal problems.

'Stripping' is the process of volatilizing ammonia (NH₃) by raising the pH of the wastewater (Atlas and Bartha, 1981) or by strong aeration (Halling-Sørensen and Jørgensen, 1993). Adding a strong base, such as sodium hydroxide (NaOH) to the water causes all the ammonium ions (NH₄⁺) to be converted into ammonia, which comes out of aqueous solution as a gas. However, ammonia gas is toxic, and is likely to precipitate back into the watershed, causing further environmental problems.

Another technique is 'breakpoint chlorination' (Atlas and Bartha, 1981). The addition of chlorine (Cl_2) at a proportion of 8 - 10 times the nitrogen concentration, converts ammonium to molecular nitrogen (N_2) (Halling-Sørensen and Jørgensen, 1993). While this process, unlike stripping, does not result in atmospheric pollution, it does contaminate the water with a large amount of chlorine. Besides being toxic itself, even small amounts of chorine in water can react with organic constituents to form carcinogenic trihalomethanes (El-Dib and Ali, 1995). Although these physicochemical techniques have their appropriate area of application, biological removal of nitrogen has gained acceptance in the past decade as an economical and environmentally benign alternative (Horan, 1990; Reardon, 1995).

1.4.2 Biological Nitrogen Removal

Biological nitrogen removal (BNR) takes advantage of the ability of certain microorganisms to oxidize ammonium to nitrate, and of other bacteria to reduce nitrate to nitrogen gas (N_2), the primary component of our atmosphere. Some techniques also accumulate dissolved nitrogen into microbial biomass, so that it can be removed and recycled or disposed of as solids. Most BNR techniques rely on ecologically simple microbial systems, although the action of invertebrates such as nematodes and rotifers aids treatment by breaking down organic particles. The most common techniques require the support of complex technological systems to create the appropriate conditions or to increase rates (Gray, 1989; Halling-Sørensen and Jørgensen, 1993).

Conventional BNR technologies are divided into two classes: suspended and attachedgrowth processes. In suspended growth processes, the nitrifying and denitrifying bacteria grow on the surfaces of organic particles suspended in the wastewater. The most common application of suspended growth BNR is the activated sludge process (Gray, 1989; Horan, 1990; Halling-Sørensen and Jørgensen, 1993). Wastewater is treated in an aerated basin, during which bacteria (including nitrifiers) grow on organic particles present in the wastewater, causing them to clump together. Next, these particles are separated from the wastewater by sedimentation. These solids are microbially very active, and are referred to as activate sludge. A regulated proportion of activated sludge is returned to the first basin to increase the number of bacteria available to treat waste. Activated sludge treatment can be expensive for small installations, requires highly trained personnel, and has high energy costs. However, it is the most widely used process to treat large flows (Serfling and Mendola, 1979; Gilette, 1988).

In attached growth BNR, bacteria grow as a biofilm on a mechanical substrate, which is fixed or rotates through the water (Gray, 1989; Horan, 1990; Halling-Sørensen and Jørgensen, 1993). Many variations on this theme are in use. In a trickling filter, wastewater flows over a bed of gravel, and in a rotating biological contactor, a rotating series of discs covered with biofilm are partly immersed in the water. Attached growth BNR involves the production of less suspended solids, and relies on high-surface area substrates to ensure adequate oxygen for bacterial growth.

1.4.3 Ecotechnology for Nitrogen Removal from Wastewater

Conventional BNR focuses on creating conditions conducive to the growth of nitrifying and denitrifying bacteria. In contrast, ecotechnological nutrient removal (ENR) systems use ecologically complex associations of plants, animals, and microorganisms to simulate natural conditions that promote nitrogen mineralization, nitrification, and denitrification. During the past decade, ecotechnological systems have come into use all over the world (Alexander and Wood, 1987; Santos *et al.*, 1987; Brix and Schierup, 1989; Bhamidimarri *et al.*, 1991; Gumbricht, 1992; Zhenbin *et al.*, 1993). These systems may be classified as

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ecotechnological or ecologically engineered systems, but should be considered a subclass of BNR. Ecotechnological systems have evolved as two major forms. Aquatic treatment systems are shallow ponds of wastewater, typically with a cover of floating plants, such as water hyacinth (*Eichornia crassipes*) (Reddy and De Busk, 1987; Orth and Sapkota, 1988; Kumar and Garde, 1990). Constructed wetlands are typically 0.3-0.6 m deep beds of gravel, planted with emergent wetland plants such as cattail (*Typha latifolia* L.) or reed canary grass (*Phragmites australis* [Cav.] Trin.) (Reed and Brown, 1992; 1995; Green and Upton, 1994).

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The most widely used ecotechnological wastewater treatment is the stabilization pond or lagoon, although it is not usually considered as such. This method of treatment has been in use for centuries, but only recently have design specifications for successful treatment been established (Gray, 1989). Pond treatment can be anaerobic, or if aerobic (facultative ponds), relies on oxygen production by algae to supply heterotrophic respiration. The low availability of oxygen in these systems means that longer retention times are required for treatment; it also means that nitrification is inhibited (Horan, 1990). While ponds are widely used for wastewater treatment, their use for nitrogen removal is limited by low oxygen availability.

ENR systems require less technical expertise to operate, are more suitable for small wastewater flows, and have lower operating costs than conventional BNR (Tchobanoglous

et al., 1980; Crites and Mingee, 1987; Conley et al., 1991; Green and Upton, 1994). In the UK, constructed wetlands are cheaper than the usual alternative, sand filters, for populations below 2,000 (Green and Upton, 1994). Although they require large land areas, and so generally are suitable where land is inexpensive, land is usually discounted in cost analysis, because its value is not expected to depreciate.

The role of plants

In a society so aware of environmental issues, the *perception* that something is environmentally 'friendly' can become as important as the actuality. The use of plants in ecotechnological wastewater treatment has certainly contributed to the concept's popularity (Van der Ryn, 1978; Gilette, 1988; Meadows, 1988; Todd, 1988), making it quite literally a 'green' technology. The claims have not always been well supported, but recent work is clarifying the role of plants in ENR.

There are a number of mechanisms by which plants are thought to facilitate nitrogen processing in ENR. Their relative contributions to nutrient removal from wastewater depend on climate and each facility's design. Early research on the role of plants in nitrogen removal focused on plant growth and uptake of nutrients from wastewater into plant tissues. Plants also may transport oxygen into the water through their roots, promoting nitrification. Plant root systems can provide microenvironments that support nitrifying and denitrifying bacteria. Decaying plants are sources of organic carbon

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particles, which in aerobic and/or carbon-limited wastewaters provide food and anaerobic microsites necessary for denitrification.

The amount of nitrogen that plants are capable of removing from wastewater by direct uptake depends on their growth rate. Water hyacinth is most frequently used because of its very high growth rate, up to 154 t dry weight/ha/year (Wolverton and McDonald, 1979). The ability of water hyacinth to grow and spread in eutrophic and polluted waters makes it a nuisance plant in many tropical areas of the world (Wolverton and McDonald, 1979; Reddy and Sutton, 1984). These same qualities make it suitable for ENR. Nitrogen removal through plant uptake is highest in tropical areas, which receive high solar radiation and have higher ambient temperatures. Water hyacinth grows best at water temperatures between 25^o and 30^oC, does not grow in water below approximately 10^oC, and is killed by temperatures of 0^oC or lower (Reddy and Sutton, 1984). Nitrogen removal by water hyacinth growth reaches 5 - 57 mg N/m²/hr (Reddy, 1983; Reddy and Sutton, 1984; Reddy and De Busk, 1985).

The benefits of plant nitrogen uptake are limited by the cost of harvesting and disposing of plant biomass. While plant uptake of nitrogen occurs in any plant-containing system, nutrient removal only occurs if plant biomass is harvested. Without harvesting, plant biomass eventually reaches a steady state, and new growth is balanced by senescence and decay of old plant material, which leaches its nitrogen back into the wastewater.

Harvesting is also required to maximize plant growth rates (Reddy and Sutton, 1984). Disposal requires costly composting and drying procedures (Tchobanoglous *et al.*, 1989). Emergent vegetation in constructed wetlands grows more slowly and in practice is not harvested for nutrient removal. However, some portion of nitrogen is accumulated in below-ground plant parts (White and Howes, 1994).

The greatest potential for ENR is its use in sustainable nutrient recycling through the use of harvested biomass. Plant biomass can be converted into animal feed, fertilizer, or methane (Serfling and Mendola, 1979; Reddy and Sutton, 1984; Reddy and Smith, 1987). ENR facilities can also be used to grow ornamental plants for commercial markets (Meadows, 1988). However, despite two decades of research, resource recovery is not yet cost effective, and disposal is usually the cheapest option (Kawai *et al.*, 1987; Tchobanoglous *et al.*, 1989). Nevertheless, a number of trends may change this situation in the future. Continuing research may lower costs of resource recovery. Environmental systems of accounting may raise the cost of fuels and fertilizers whose production or use is environmentally harmful, making resource recovery more economical.

In wastewaters that are very concentrated in organic material, carbon respiration competes with nitrification for oxygen (Gray, 1989). Nitrification of wastewater usually requires an outside oxygen source. Supplying this oxygen demand can be an expensive process. In conventional BNR, aeration is supplied mechanically. In a facultative pond, oxygen for nitrification is supplied by algae, but rates are slow (Horan, 1990). Oxygen can also be transported through the stems of some aquatic plants to the water surrounding their roots (Armstrong, 1964; Reddy *et al.*, 1989; Brix, 1993). However, plant oxygen transport rates may be small relative to the rate of oxygen diffusion from the atmosphere (Howes and Teal, 1993). Estimates and measures of plant oxygen transport vary widely, from 0.02 to 12 g/m²/day (Brix, 1993; Howes and Teal, 1993). In wetland soil columns, plants were found to facilitate sequential nitrification and denitrification, but it is unclear how much this mechanism contributes to ENR (Reddy *et al.*, 1989).

Despite the claims (Brix, 1987), the oxygen supplied by plants does not in practice seem to contribute significantly to nitrification in ENR (Watson *et al.*, 1990; Reed and Brown, 1995). Unaerated constructed wetlands typically have very low dissolved oxygen concentrations (0.5 mg/l), too low to support significant nitrification (Gray, 1989; Reed and Brown, 1995). Unaerated aquatic systems often require long retention times for complete nitrification (Dinges and Doersam, 1987).

Plant roots also act as a substrate for the formation of bacterial biofilms, although the importance of this to ENR is not known (Gersberg *et al.*, 1986; Kumar and Garde, 1989; Teal and Peterson, 1993). The availability of attachment sites for nitrifying bacteria can limit nitrification rates (Kaplan, 1983). In suspended growth BNR, settled sewage solids are reintroduced as a substrate, while in attached growth BNR, a fixed, high surface-area

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mechanical support is used. The surface area of a plant's root system is difficult to measure, but can be 40 - 200 times the plant's areal 'footprint' (Kinsinger *et al.*, 1991). Thus, the addition of plants to a pond could potentially increase the fixed biofilm area by 40 - 200 times. However, a survey of the literature revealed no measurements of nitrifier activity associated with plant roots in ecotechnological systems. In natural systems, nitrifiers are found associated with plant roots (Matulewich and Finstein, 1978), but the relative contribution of plants to total nitrifier populations in ecotechnological wastewater treatment systems still needs to be quantified (but see Table 2.5).

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Denitrification requires a source of organic carbon to proceed (Hattori, 1983; Seitzinger, 1994). In most treatment systems, organic material removal occurs simultaneously with nitrification, since both processes require oxygen. This results in a wastewater that is high in nitrate but low in organic carbon. To compensate, soluble organic carbon may be added directly as acetate, methanol, or food-processing wastes to support denitrification (Isaacs and Henze, 1995; Halling-Sørensen and Jørgensen, 1993). Acetate and methanol use increases costs, while the availability of other organic carbon sources may be limited. Another method is to use the carbon present in untreated sewage (Gersberg *et al.*, 1984). In pre-denitrification, nitrate-rich treated water is recycled back to an earlier, high organic carbon-containing stage of the treatment where it can be denitrified (Rusten *et al.*, 1995). Pre-denitrification, however, necessitates doubling the size of treatment components to handle the increased flow. In post-denitrification, a small amount of untreated wastewater

is added to the nitrate-rich water to feed denitrifiers (Rusten *et al.*, 1995). The danger of post-denitrification is that of reintroducing fecal bacteria near the end of the treatment with possible contamination of the effluent. Plants can also be used as a source of organic carbon. Plant material has been successfully used to increase denitrification in constructed wetlands (Gersberg *et al.*, 1984; Weisner *et al.*, 1994), eliminating the need for introduced carbon sources. The plants can simply be allowed to decay in place, supplying organic carbon to the wastewater.

Despite decades of research, the role of plants in ENR is not yet clear. Other possible roles for plants in ENR have been less well researched. Some roles may not contribute to nitrogen removal, but may have other benefits. Plant root systems may shield nitrifiers from shock loadings and toxins, resulting in faster recovery times. The reservoir of bacteria associated with plant roots may reduce variations in the effluent which result from variable influent (Orth and Sapkota, 1988). Plant root systems also provide environments that support a variety of animal life. A more complex fauna with multiple trophic levels may aid in the decomposition of solids, resulting in lower waste solids production, and decreased costs for solids treatment and disposal (Gray, 1989; Horan, 1990). Plant covers may reduce aerosol formation, inhibiting odors and reducing the risk of airborne pathogens.

Solar Aquatics

The Solar Aquatics SystemTM (SAS) is an ecotechnological wastewater treatment system that uses both aquatic and constructed wetlands components combined with aeration and activated solids recycling for nutrient removal. The rationale behind SAS design is to increase ecological stability and complexity, and it is believed that this environment is conducive to dependable nitrogen removal (Teal and Peterson, 1991; 1993; Teal *et al.*, 1994). The same concept has been advocated by others (Van der Ryn, 1978; Cooper, 1993), but only a few instances where ecological complexity is part of design can be found in practice (Serfling and Mendola, 1979; Dinges and Doersam, 1987; Ogden, 1993).

SAS have been used to treat sewage, septage, boat waste, and food processing waste (Meadows, 1988; Todd, 1988; Reed, 1990; 1992; Dillon, 1990; Hedbor, 1990; Marinielli, 1990; Peterson, 1991; Spencer, 1990, 1992; Teal and Peterson, 1991; 1993; Gover, 1993; Teal *et al.*, 1994). There are now 10 such systems in operation from Baja, Mexico to Nova Scotia, Canada. Most systems treat either high-strength organic waste or act as a nutrient removal step for the effluent of conventional wastewater treatment systems. The combination of ENR with conventional BNR concepts requires four times less land area than typical constructed wetlands while producing an effluent three or more times lower in total nitrogen (Gersberg *et al.*, 1984; Brix and Schierup, 1989; Tanner, 1995; Table 2.2,

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this paper). The disadvantages of this system compared with constructed wetlands are higher operation costs resulting from aeration and pumping.

Ecotechnological nutrient removal has the potential for improving wastewater treatment efficiency by reducing costs and producing a high quality effluent. However, the benefits of ENR are only likely to be realized with appropriate application. In the near future, wastewater treatment planning is likely to continue to rely on conventional BNR to treat the city wastewater. ENR will play a greater role in on-site and rural wastewater treatment. Hybrid systems like the SAS may also find a niche treating very concentrated wastes, and in small communities with high land prices. • ·

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2.0 NITROGEN REMOVAL IN AN ECOLOGICALLY ENGINEERED SEPTAGE TREATMENT SYSTEM

2.1 INTRODUCTION

Contamination of natural waters by sewage-derived nitrogen (N) is an increasing public and regulatory concern. Coastal watersheds are most sensitive to nitrogen pollution (Ryther and Dunstan, 1971; McCarthy and Goldman, 1979; *cf.* Nixon and Pilson, 1983; Smith, 1984; Hecky and Kilham, 1988). Increased nitrogen loads to natural waters can cause eutrophication, resulting in nuisance conditions and harm to fisheries (Officer *et al.*, 1984; Day *et al.*, 1989). Sewage-derived nitrogen is discharged to coastal waters by surface flow from point sources (wastewater treatment facilities), and by groundwater flows resulting from land application, rapid infiltration basins, sewage lagoon leachate, and septic drainage fields (Weiskel and Howes, 1991; Howes and Goerhinger, 1994b).

Septic tanks are used for on-site disposal of wastewater in about 25 % of U.S. households (USEPA, 1987) as well as some commercial facilities. In rural areas, their distribution may rise to 100 %. In septic treatment, wastewater undergoes anaerobic biodegradation and sedimentation in an underground tank, and the clarified wastewater flows to a subsurface leaching field (Willhelm *et al.*, 1994). The mean residence time of wastewater

entering a septic tank varies with ratio of the flow rate to the tank size, but ranges from 0.5 to 8.3 days (Canter and Knox, 1985; Willhelm *et al.*, 1994). Solids generally accumulate in at a rate of 40 - 73 *l*/person/year (Philip *et al.*, 1993) Since the septic tank functions as a clarifier, periodic removal of accumulated solids through pumping is required. Upon removal, the mixture of accumulated solids and liquid is termed septage. The mean residence time of solids in a septic tank ranges from one to > 5 years, depending on the frequency of septage removal (Philip *et al.*, 1993). The more biologically labile solids are degraded, resulting in the accumulation of recalcitrant solids. Septage contains a very high concentration of organic matter (total volatile solids 8,170 - 27,600 mg/l), and biodegradation of these solids results in high levels of dissolved nutrients (Canter and Knox, 1985).

Septic disposal of wastewater is an important contributor of nitrogen to coastal waters (Giblin and Gaines, 1990; Weiskel and Howes, 1991). Septic leachate adds 320 MT N/yr to coastal ecosystems of Buzzards Bay, Massachusetts, USA, 26 % of the total municipal wastewater loading (Howes and Goerhinger, 1994b). Nitrogen loads from septage disposal are not well studied, but may contribute an amount equal to septic leachate (Weiskel and Howes, 1991).

Septage differs from sewage in many ways, requiring different approaches to management and treatment. Septage production is highly seasonal, so septage treatment facilities must operate seasonally or have adequate storage to enable year-around operation. Unlike sewage, which is continuously collected from a relatively densely populated region, septage is collected periodically by tankers from less densely populated areas (1 - 103 septic systems/km²) (Canter and Knox, 1985). Given the dispersed nature of the source, large, centralized septage treatment facilities are less practical than smaller, local facilities due to transport costs and the interposition of jurisdictional boundaries. Septage is many times more concentrated than sewage, both in its dissolved and solid constituents (Table 2.1). The high organic and nitrogenous strength of septage requires different treatment processes than are used for sewage (Kersten, 1994). A number of treatment approaches have been used, ranging from those which treat septage as a sludge, to those which treat it as a wastewater.

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Until recently, Massachusetts coastal communities discharged septage into unlined lagoons, which allowed solids to settle while the liquid evaporated or percolated into the groundwater. This practice resulted in plumes of nitrate-enriched groundwater, which forced the closure of municipal and private wells and pose an ecological threat to ponds and coastal waters. Septage lagoons in Massachusetts were phased out in 1990. Increasingly strict state and federal nutrient discharge regulations have created a need for

better septage treatment processes which can serve the seasonally fluctuating needs of regional communities.

Septage can also be co-treated with sewage in municipal facilities, but the proportion must be carefully regulated to avoid process upsets (Kersten, 1994). Considering that septage may be 30 - 70 times as concentrated in solids and biological oxygen demand (BOD) and 3 - 6 times more concentrated in ammonium (NH_4^+) than sewage, the capacity of small municipal sewage facilities to accept septage may be quite limited (Canter and Knox, 1985; Kersten, 1994). In most rural areas, municipal facilities do not have the capacity to co-treat regional septage production.

Septage may also be treated as a sludge, for example by precipitation and dewatering with a filter press. Dewatering produces an alkaline (pH = 12.4) filtrate very high in NH₄⁺ but low in available phosphate (PO₄³⁻) and solids (Slater *et al.*, 1987; Kersten, 1994). The filtrate is difficult to treat biologically, because of the high pH and the limitation of bacterial growth by low PO₄³⁻. In a Cape Cod septage treatment facility (Orleans, MA), septage was precipitated with lime and ferric chloride (250 g/kg TSS), followed by dewatering in a filter press. The filtrate was pH neutralized with HCl, and H₃PO₄ was added as a nutrient source. The resulting inorganic precipitate (2.4 g/l septage) was removed by sedimentation, and the water was treated in a rotating biological contactor (RBC), followed by secondary sedimentation (Slater *et al.*, 1987). This process supported very little nitrogen removal or nitrification; total nitrogen levels in the effluent were 52.6 mg/l, mostly as NH_4^+ -N (DeSimone and Howes, 1995). Total primary waste solids produced with this technique exceeded 16.9 g/l septage (1.45g/g influent TSS), excluding secondary solids (Slater *et al.*, 1987).

A new septage treatment process is the Solar Aquatic SystemTM (SAS) (Ecological Engineering Associates [EEA], Marion, Mass.) -- an ecotechnological process which treats septage and other high-strength organic wastewaters to Advanced Water Treatment (AWT) standards. The SAS is a hybrid wastewater treatment technology that incorporates aeration and activated solids recycling into a design combining aquatic and constructed wetlands components (Teal and Peterson, 1991; 1993; Teal *et al.*, 1994). A septage-treating SAS treating 10.6 m³/d. operated from 1988 to 1992 in Harwich, Mass., during which the process gained conditional approval from the state Department of Environmental Protection (DEP) after an independent evaluation (Wright-Pierce, 1993). The evaluation demonstrated that the SAS produced a consistent, tertiary treated effluent, even under stress loadings of 1.5 - 2 times design flow. However, the factors which control nitrogen removal in these systems are still not well understood.

Progress in developing rational design criteria for ecotechnological wastewater treatment systems such as constructed wetlands has been limited by the lack of experimentally verified understanding of treatment mechanisms (Reed, 1991). Ecological engineering has progressed from the input/output approach to rational design based on improved hydraulic engineering and analogies with natural wetlands processes (Nichols, 1983; Falkner and Richardson, 1989; Johnston, 1993).

Septage enters treatment with its total nitrogen distributed between particulate organic nitrogen (PON), dissolved organic nitrogen (DON), ammonium (NH_4^+) and oxidized nitrogen (NO_2^- and NO_3^- , referred to hereafter as NO_x). During biological treatment, degradation of organic nitrogen results in NH_4^+ production (nitrogen mineralization) (Figure 1.1). Nitrifying bacteria oxidize NH_4^+ to NO_x in the presence of oxygen. In the absence of oxygen, denitrifying bacteria reduce NO_x to nitrogen gas (N_2), the form which composes nearly 80 % of the earth's atmosphere (Section 1.1). Nitrogen removal by denitrification has no eutrophication potential and forms no toxic compounds.

A number of factors limit the rate at which denitrifying bacteria reduce NO_x . Aside from anaerobic conditions, denitrifying bacteria require a source of organic carbon. Since nitrification and denitrification require different oxygen conditions, nitrification reactors are often placed upstream of denitrification reactors. Oxidation of NH_4^+ is accompanied by organic carbon oxidation, so that nitrified effluents are low in available carbon, inhibiting denitrification. Carbon can be added as simple organic molecules like methanol (Gersberg, 1984), food processing waste, (Halling-Sørensen and Jørgensen, 1993) or untreated sewage (Gersberg, 1984). Denitrification can also be stimulated by

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recirculating nitrified wastewater to the beginning of the treatment process (Runsten, *et al.*, 1994b), but component size must be increased proportionately to the added flow. Many different reactions of nitrogen can occur simultaneously; in biological nutrient removal, conditions are manipulated to maximize nitrogen transformation in the direction of denitrification and N_2 production.

I have applied methods and analysis derived from biogeochemical studies of wetlands and aquatic systems to investigate *in situ* nutrient removal processes in an operating ecologically engineered septage treatment system. I investigated the factors controlling nitrogen removal by monitoring nitrogen transformations throughout an operating SAS, in experimental modifications of process streams, and in laboratory-scale experiments. From these results, I developed a nitrogen budget, which suggests process improvements for increasing nitrogen removal efficiency in the Solar Aquatic septage treatment system.

2.2 METHODS

System description

Research was conducted at the Marion Solar Aquatic Research Facility in Marion, Massachusetts, a demonstration-scale SAS (Figure 2.1) which treated an average of 2,200 *l* septage/d. The facility, designed based on an earlier, larger facility, has been operating since 1993 (Teal and Peterson, 1991; 1993; Teal *et al.*, 1994). Raw septage from



Figure 2.1 - Flow diagram of Marion Solar Aquatic septage treatment facility. SD - screening and degritting. ET - equalization tank. PC - preliminary conditioning tank. 1C and 2C - primary and secondary clarifiers. T1 .. T9 - aquatic treatment tanks 1 - 9 with floating plants. SFW - subsurface flow constructed wetlands. Tanks and wetlands are enclosed in a greenhouse. domestic and commercial sources is trucked to the site, screened and degritted, and stored in a 19,000 *l* aerated equalization tank (ET) which allows blending of septage loads and preliminary biological oxidization. The characteristics of the incoming septage are highly variable (Table 2.1). Influent chemical oxygen demand (COD) averaged 11,700 mg/*l* (range 1,950 - 22,300) total suspended solids (TSS) averaged 7,460 mg/*l* (2,520 -23,600), NH₄⁺-N 32.8 mg/*l* (1.6 - 128), total nitrogen (TN) averaged 483 mg/*l* (160 -1,540) - 92 % (444 mg/*l*) of which was PON, and TP 51.5 mg/*l* (16.5 - 88). Influent NO_x was low, at 1.1 mg/*l*.

A grinder pump fills an aerated 3,800 *l* preliminary conditioning (PC) tank with septage from the ET, where it is mixed 3:1 with an activated solids suspension from the secondary clarifier in the greenhouse portion of facility (see below). The PC tank is occasionally bioaugumented with bacteria. Septage is then pumped into a 3,000 *l* primary clarifier (1C), for solids sedimentation. Waste solids are pumped to an aerated holding tank for stabilization and disposal, and the supernatant (primary treated septage) is pumped to the greenhouse. The pumping rate of primary treated septage into the greenhouse was 4,230 *l*/d., although the flow occasionally stopped due to variations in septage delivery, resulting in a mean daily flow rate of 3,000 *l*/d. during the period of study.

| Table 2.1 - Flow-weighted mean characteristics of screened and |
|---|
| degritted septage at Marion SAS over 6 month period with ranges |
| (n = 22). Screened and degritted sewage from Providence, RI |
| shown for comparison ($n = 64$). |

| ma/l | Septage | Min. | Max. | Sewage |
|--------------------|---------|------|-------|--------|
| COD | 11700 | 1950 | 22300 | 323 |
| TSS | 7460 | 2520 | 23600 | 165 |
| NH ⁺ -N | 32.8 | 1.6 | 128 | 10.1 |
| NO. | 1.1 | 0.5 | 2.1 | - |
| PON | 444 | 150 | 1400 | - |
| TN | 483 | 160 | 1540 | - |
| TP | 51.5 | 16.5 | 88 | 3.8 |

Within the greenhouse, septage is gravity fed into the first tank of each of two series of 9 aerated aquatic treatment tanks (0.75 m dia. x 1.5 m high, 620 *l*; Total vol. 11,200 *l*). The tanks are planted with a mixture of floating plants including willows (*Salix nigra*. Marshall), pennywort (*Hydrocotyle umbellata* L.), primula (*Primula veris* L.), and mint (*Mentha arvensis* L.) (total surface area 11.6 m²). Translucent tank walls allow the development of an algal-bacterial mat. The tanks also support large populations of zooplankton, nematodes, and snails. From the tanks, wastewater flows to an unaerated secondary clarifier (2C) (620 *l*). Solids and 30 % of the wastewater flow are recycled to the PC. The secondary treated supernatant from the clarifiers flows into 22.3 m² of 0.6 m deep subsurface flow gravel bed wetlands (SFW) -- planted with cattails (*Typha latifolia* L.), sedges (*Cyperus spp*. L.), and primula. Final solids polishing and denitrification occur in the wetlands. The final SFW effluent is disposed of in the town's existing sewage treatment lagoons.

System performance

Overall system performance was determined using data reported to state regulators (DEP) by EEA. These data included influent, waste solid, and effluent volumes, characteristics of the influent, waste solids, 1C and 2C supernatants, and effluent during 6 month's operation (05/94 - 12/94). Organic N (ON) was estimated from total solids (TS) data based on nitrogen content analysis (5.95 % \pm 0.04 N, n = 174). 2C recycle flow rates were measured by calibrating the timed recycle pump.

Experimental replicate tanks

I studied nitrogen transformations *in situ* in the aquatic treatment tanks under normal flow conditions. Earlier testing had shown that N concentrations in the tanks were highly variable. To increase replication, flow from tank T2 in each of the two treatment trains was redirected to the experimental system (Figure 2.2), after which it was pumped back into tank T5. Each experimental system consisted of 2 parallel sub-trains of two tanks (186 *l*) in series. The 8 replicate tanks were planted and aerated like the main tanks. The distribution of water to the sub-trains was periodically measured with graduated flasks and a stopwatch, and calibrated by adjusting the level of the splitter box. Tanks T3 and T4 were selected for study because of their high rates of nitrogen transformations.

Intensive monitoring

To study the controls on nitrogen transformations within the aquatic treatment tanks, nitrogen species were intensively monitored under both flow and batch conditions. Both trains were sampled 8 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 (see below). Temperature, dissolved oxygen (DO), and flow rate were measured concurrently. Dissolved oxygen was measured using an YSI oxygen meter. Aeration averaged 1 m³/hr per tank, and was adjusted to maintain DO > 5.0 mg/l in all tanks. Chloride tracer studies showed that flow in both the main and the replicate tanks





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conformed to the tanks-in-series model (Ruzicka and Hansen, 1988); mixing in the tanks was effectively 73 % of the total tank volume. Rates of nitrogen transformations were determined from the difference in concentration of N species between a tank and the previous one, divided by the tank's hydraulic retention time (determined by its concurrently measured flow rate and effective mixed volume). Nitrification rates were determined by the sum of the changes in PON, DON, and NH_4^+ ; denitrification rates were determined by mass balance from the changes in PON, DON, NH_4^+ , and NO_x .

The tanks were also monitored under batch conditions, where all transformations and losses result from *in situ* processes and sampling errors resulting from variation in septage characteristics and flow rate are eliminated. During the incubation, septage flow through the greenhouse was temporarily stopped, and tank T2 and the replicate tanks were isolated to prevent water exchange between adjacent tanks. Water samples were taken every two hours for 12 hours, and placed on ice for transport to the laboratory.

Denitrification and carbon availability

Organic carbon particles should promote denitrification in aerated environments by providing anaerobic microsites for denitrification. The effect of the addition of organic carbon particles on denitrification rates was determined using conditioned wheat straw (3-5 days in the aquatic treatment tanks to promote bacterial growth). Duplicate samples received either no straw, varying levels of straw, autoclaved straw, and/or were made

anaerobic by sparging with N₂. Denitrification rates were measured in laboratory incubations using the acetylene block method (Balderston *et al.*, 1976; Knowles, 1982). Sewage samples (75 ml) from aquatic treatment tanks 1, 8, and 13 at the Field's Point SAS, Providence, RI (Gover, 1993) were incubated for 10 hours in sealed bottles (160 ml) injected with 9 ml acetylene. Sewage was used because of the unavailability of septage. NO_x concentrations ranged from 0.13 to 13.05 mg/l, similar to those found in septage. N₂0 evolution in the headspace was measured at 2 hour intervals using a ShimadzuTM ECD gas chromatograph.

Additional organic carbon particle addition experiments were conducted at the Marion Septage Treatment SAS. Wheat straw and dried cattails from the SFW were immersed in aquatic treatment tanks T7, T8, and T9 for 7 weeks. A mean of 5.1 kg dry wt. of biomass/tank (8.2 g litter/l or 2.5 g POC/l) were placed in 9 mesh bags (0.5 cm mesh) and suspended in the tanks. The second tank series was left as a control. Earlier monitoring indicated that there was no significant difference in NO_x levels between pairs of tanks in the two series after tank 4. Water samples were collected 8 times from both trains during the experiment. The bags were then removed, reweighed, and analyzed for carbon and nitrogen content to determine the rate of organic carbon degradation in the plant material.

Carbon fixation

Carbon fixation under light and dark conditions was determined by carbon-14 (¹⁴C) incorporation. Dark fixation measures chemosynthetic activity (mainly nitrification), while the extra fixation under light is due to photosynthetic activity. These measures gave an estimate of exogenous carbon availability as well as a check on nitrification rates determined by mass balance. Three biological oxygen demand (BOD) bottles (300 ml) (two clear and one dark) were filled with septage and suspended inside and against the clear-sided walls of each of tanks T2, T5, and T9. Each bottle was injected with 0.25 ml of 8.5 μ Ci ¹⁴C as NaH¹⁴CO₃ and allowed to incubate near the wall inside a tank for 6 hours in full sunlight. After incubation, samples were analyzed for ¹⁴C. The samples were homogenized and an aliquot was filtered (GFF). Specific activity was determined from 0.200 μ of the filtrate fixed with 0.200 μ hyamine hydroxide and 0.400 μ DI. The filters were fixed in 0.5 ml glacial acetic acid, held over night and dried. Aquasol IITM scintillation fluid (10 ml) was added to both the solid and the liquid samples, and the vials held in the dark for 4 days to reduce chemoluminescence before their activity was determined on a United Technologies Packard Minaxib Tri-Carb 4000TM series liquid scintillation counter. Dissolved CO_2 levels in the tanks were determined by headspace equilibration and infrared analysis (Beckman Model 15ATM).

Carbon lability

Aerobic biodegradation rates of septage organic matter were measured to determine carbon availability for denitrification and the relative lability of organic matter at different treatment stages. I conducted long term (ca 5 week) incubations of samples of raw septage, water from tanks T1 and T9, waste solids from the Marion SAS, and raw sewage (Field's Point, Providence, RI). Dilutions (4.5 - 60 m*l* wastewater/*l* water, depending on the organic carbon concentration) of each sample were placed in 4 BOD bottles (300 m*l*) and incubated at 20 °C. DO readings were taken approximately every two days for 5 weeks. Samples were reaerated to saturation whenever DO fell below 4.0 mg/*l*. The decay of carbon was modeled by the following equations:

$$R_1 = R_0^{(-kt)} \tag{2.1}$$

$$C_{t} = C_{0} + (R_{0}/-k)e^{(-kt)} + (R_{0}/k)$$
(2.2)

in which R_0 is the initial rate of C respiration (m-moles/l/hr, based on equimolar aerobic carbon respiration stochiometry of 32 g O₂/12 g C), R_t is the carbon respiration rate at time t, C_0 is the initial carbon concentration (m-moles/l), C_t is the carbon concentration at

time t (hr), and k is the decay constant (hr⁻¹). Half-lives $(t_{1/2})$ can then be calculated by the formula:

$$t_{\lambda_2} = \ln(2)/k$$
 (2.3)

Analytical methods

Two water samples were taken from each tank. Immediately upon reaching the laboratory, the first sample was filtered through a 0.22 μm MilliporeTM filter and the filtrate used for determinations of NH₄⁺-N, NO_x-N, and total dissolved N (TDN). Biologically inactive filtered samples were held for analysis at 4 °C until the other measurements could be made. NH₄⁺-N was analyzed immediately (to prevent NH₃ volatilization) colorimetrically by the indophenol method (Scheiner, 1976). NO_x-N was analyzed with azo dye after cadmium reduction using a Lachat AutoanalyzerTM (Bendschneider and Robinson, 1952; Wood *et al.*, 1967). TDN determination was made by persulfate digestion to nitrate followed by analysis on the Lachat AutoanalyzerTM (D'Elia, 1977). Dissolved organic N was calculated by subtracting NH₄⁺-N and NO_x-N from TDN. The second sample was vacuum filtered through precombusted WhatmanTM GFF filters to collect solids for TSS and particulate organic carbon (POC) and PON determinations on a Perkin-Elmer Model 2400TM CHN elemental analyzer. All reported errors are ± standard error.

2.3 RESULTS

System performance

System and greenhouse performance was determined using the EEA data. Marion SAS effluent flow-weighted means fell toward the lower end of their ranges (Table 2.2). Total nitrogen was reduced to 6.1 mg/l, 47 % of which was DON. The remaining effluent nitrogen was made up of 28 % NO_x, 16 % PON, and 9 % NH₄⁺. The effluent characteristics of a septage treating RBC at Orleans, Cape Cod are shown for comparison (DeSimone and Howes, 1995).

Flow weighted N measurements of Marion SAS inputs and outputs over 6 months (174 d.) were used to construct a N mass balance (Figure 2.3). Treatment resulted in nearly complete removal of influent N with only 1.0 % of the 187 kg of influent N discharged in the effluent stream. Uptake of N into plant biomass accounted for only 0.5 % of N inputs. 57.5 % of the N was removed by sedimentation of a 3.6 % solids sludge, 98 % as PON. The SAS produced 5.9 g waste solids/*l* septage treated, including plant biomass, or 0.71 g waste solids/g influent TSS. Denitrification (41.3 %) was assessed from the missing N in the mass balance. Significant volatilization of ammonia was ruled out, since at the mean pH of 7.2 and temperature of 20 °C, NH₃ accounts for only 0.35 % of

| mg/I | SAS Effluent | | Orleans | |
|---------------------|--------------|------|---------|------|
| | Mean | Min | Max | Mean |
| COD | 113 | 1.6 | 254 | - |
| TSS | 16 | 1 | 45 | 54 |
| NH₄ ⁺ -N | 0.56 | 0.05 | 1.98 | 28.2 |
| NO _v | 1.7 | 0.5 | 6.2 | 10.9 |
| TN | 6.1 | 4.4 | 11.8 | 52.6 |
| TP * | 1.5 | 0.2 | 1.6 | 0.7 |

Table 2.2 - Flow-weighted mean effluent characteristics of Marion SAS over 6 month period with ranges (n = 22), and means of a

* Ferrochloride used at Orleans to precipitate PO₄³⁻

nearby septage-treating RBC in Orleans, Mass.





the NH_4^+/NH_3 pool. The N balance for the Marion SAS was similar to that reported for another septage treating SAS in the region (Teal *et al.*, 1994).

Greenhouse performance

Within the SAS greenhouse, 93.8 % of entering N was removed. Increased N loading to the greenhouse, through increased flow or influent concentration, resulted in increased N removal over the measured loading range of 4.5 - 15.7 g N/m³/d. (Figure 2.4a). There was a trend toward an increased N removal efficiency of 0.46 % for every 1 g/m³/d. increase in loading ($r^2 = 0.29$) (Figure 2.4b). Mean N removal for a different septage-treating SAS (Harwich, Mass.) are shown for comparison (Wright-Pierce, 1993).

Aquatic treatment tanks

Nitrogen transformations within the aquatic treatment tanks were evaluated during intensive monitoring of the regular and experimental replicate tanks. TDN rose over the course of treatment, as biodegradation of solids continually released dissolved N (Figure 2.5). However, dissolved organic N (DON) changed little, averaging 4.94 ± 0.12 mg/l; with most of the change in TDN due to the dissolved inorganic fraction. NH₄⁺-N decreased over the course of treatment, and by tank T6 was below 1 mg/l. In contrast, NO_x increased most rapidly from tanks 2 to 6, and then more slowly until the secondary clarifier (2C) located after tank T9. After passage through the tanks, 75 % of the TDN was in the form of NO_x-N, available for removal by denitrification in the wetlands (SFW).



Figure 2.4 - Response of greenhouse components to N loading during 6 months operation with flow-weighted means. Mean of another septage treating SAS shown for comparison.



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Figure 2.5 - Dissolved nitrogen species in influent, tanks T2 - T9, 2C supernatant, and final effluent during intensive monitoring. Mean DON in tanks = 4.94 ± 0.12 .

Within the tanks, the mean N mineralization rate was $0.451 \pm 0.042 \text{ mg/l/hr}$, lower than the mean nitrification rate of $0.606 \pm 0.059 \text{ mg/l/hr}$ (Figure 2.6). The result of lower NH₄⁺ production than consumption was depletion of the NH₄⁺ pool during treatment (Figure 2.5). However, when NH₄⁺-N concentrations were less than 3.0 mg/l (white diamonds, Figure 2.6), nitrification ($0.461 \pm 0.042 \text{ mg/l/hr}$) and mineralization ($0.541 \pm$.075 mg/l/hr) were more comparable. In these cases, nitrification was likely limited by the supply of NH₄⁺ from N mineralization, the NH₄⁺ pool being already depleted.

These results are supported by the batch studies, where NH_4^+ -N concentration increased nitrification rates gradually above 1 mg/l NH_4^+ -N, and decreased nitrification rapidly below 1 mg/l NH_4^+ -N (Figure 2.7). Constructing a Lineweaver-Burke plot from the data (Figure 2.7 inset) indicates that substrate limitation of nitrification rates follows Michaelis-Menten enzyme kinetics with a maximum nitrification rate (V_{max}) under these conditions of 0.65 mg/l/hr, and a Michaelis constant (k_m) of 0.14 mg/l ($r^2 = 0.84$). The regression equation is:

$$r^{-1} = ms^{-1} + c$$
 (2.4)

where r is the nitrification rate, s is the substrate (NH₄⁺-N) concentration, c and m are regression constants. V_{max} and k_m are calculated by:

 $V_{max} = c^{-1}$ (2.5)

 $k_m = -c/m \tag{2.6}$



Figure 2.6 - Relationship between N mineralization and nitrification rates in the experimental replicate tanks. Rates at NH4-N < 3.0 mg/L shown by white diamonds. Dashed line indicates 1:1 ratio of nitrification and denitrification rates.



Figure 2.7 - Limitation of nitrification rates by NH4--N under batch conditions. Inset shows Lineweaver-Burke plot demonstrating substrate limited enzyme kinetics. Vmax = maximum nitrification rate.

Nitrification rates (0.606 ± 0.059) in the tanks were always higher than denitrification rates (0.260 ± 0.048 mg/l/hr) (Figure 2.8), consistent with the observed accumulation of NO_x during treatment in the tanks (Figure 2.5). Although the response of the denitrification rate to particulate organic carbon (POC) loading was highly variable ($r^2 = 0.56$) (Figure 2.9), denitrification rates tended to increase at higher POC loading rates.

Constraints and

Subsurface flow wetlands

Treatment in the SFW resulted in linear removal of 5 contaminants (Table 2.3). Regressions of influent against removal demonstrated linear removal for TSS, COD, TP, NO_x , and TN. Regression analysis suggested that there was a minimum concentration past which no further treatment was possible for TN and COD, but not for the other parameters. Approximately 4.3 mg/l of effluent TN is composed of refractory organic compounds, while about 80 mg/l COD is also refractory. 80 mg/l COD corresponds to that calculated from the COD due to refractory TN plus the estimated COD of organic carbon calculated using a C/N ratio of 6.4.

Controls on denitrification: organic carbon and anaerobic microsites

Organic carbon particle additions of up to 3.5 mg carbon/l to sewage in laboratory experiments caused an increase in denitrification rates as determined by N₂O production under acetylene (Figure 2.10). The effect of carbon addition was increased by the initial

N.7.


Figure 2.8 - Relationship between nitrification and denitrification rates in the experimental replicate tanks. Dashed line indicates 1:1 ratio of mineralization and nitrification rates.



13.56

Figure 2.9 - Relationship between particulate organic carbon loading (as septage solids) and denitrification rate in the experimental replicate tanks.

| | Loading g/m ³ /d. | Ra | ng | e | % Removal | r ² | Refractory mg/l |
|-----|---------------------------------|------|----|-----|-----------|----------------|--------------------|
| COD | 33 | 6.0 | - | 69 | 73 | 0.89 | 80 |
| TSS | 7.9 | 1.3 | - | 18 | 79 | 0.95 | - |
| NOx | 2.0 | 0.2 | - | 5.7 | 91 | 0.99 | - |
| TN | 3.2 | 0.8 | - | 8.4 | 89 | 0.99 | 4.3 |
| TP | 0.47 | 0.19 | - | 1.1 | 74 | 0.72 | - |

| Table 2.3 | - Remova | I of contam | inants in | the subsu | Inface flow |
|------------|----------|--------------|------------|------------|-------------|
| wetland, b | based on | linear regre | ssion of l | loading vs | . removal. |
| n = 21. | | _ | | - | |



Figure 2.10 - Limitation of denitrification rate in sewage (measured by N20 production under acetylene block) by organic carbon particles added as straw. Curves represent 4 different initial NOx-N concentrations.

 NO_x concentration of the wastewater. No increase was observed at an initial NO_x -N of 0.13 mg/l.

There was an initial temporal lag in the increase of the denitrification rates in initially aerobic compared to anaerobic treatments. The lag in denitrification rate was defined as the amount of time required for the denitrification rate of aerobic treatments to equal the initial denitrification rate of anaerobic treatments. Lag time decreased by 1.1 hours for each 1 g/l increase in organic carbon particle concentration up to 3.5 g/l (Figure 2.11).

Similarly, adding 2.5 g POC (as plant litter)// in mesh bags directly to aquatic treatment tanks T7, T8, and T9 resulted in a 50 % reduction of the NO_x concentration from 12.7 \pm 2.7 mg/l in the control to 6.4 \pm 1.9 mg/l by the secondary clarifier (Figure 2.12). Most of the reduction in NO_x levels occurred in tank T7, the first tank which received POC additions. The NO_x decrease represents an increase in denitrification of approximately 0.32 mg N/l/hr due to POC addition. Approximately 0.15 mg carbon/l/hr was lost from the bags, less than that required to support the increased denitrification. There were no significant differences in NH₄⁺ or TSS levels between pairs of carbon amended and control tanks.

Exogenous carbon inputs into the tanks were small relative to the carbon mineralization rate of 2.89 mg/l/hr. Total mean carbon fixation was 0.039 ± 0.005 mg/l/hr, measured by



1.262

Figure 2.11 - Relationship of organic carbon particle addition as straw to the lag in denitrification rate resulting from initial aerobic conditions in sewage. Lag time is the time until denitrification rates in aerobic samples equaled the initial denitrification rates in the anaerobic samples.



Figure 2.12 - Reduction of NOx-N by organic carbon particle addition as straw to tanks 7 - 9 in the Marion SAS. Second tank series used as control. Error bars represent standard errors.

-11.50 (12.50)

ALENSARY III

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¹⁴C incorporation. Photosynthetic carbon fixation averaged $0.009 \pm 0.001 \text{ mg/l/hr}$ across all tanks. Non-photosynthetic carbon fixation was greater, averaging 0.030 ± 0.004 mg/l/hr. Using a C/N mass ratio of 4.3 for living matter results in approximately 0.009 mg N/l/hr incorporated into biomass as a result of autotrophy.

The resistance of organic matter in septage to degradation changed during treatment. The fractionation of septage during primary sedimentation resulted in the settling of recalcitrant solids with a carbon half-life of 16.7 d., leaving a relatively labile supernatant ($t_{1/2} = 5.0$ d.) (Table 2.4). During treatment in the tanks, the more labile carbon fraction was degraded and the half-life of the organic carbon remaining by tank T9 was 7.3 d. Similarly, the organic carbon in raw septage had a longer half-life ($t_{1/2} = 8.4$ d.) than raw sewage ($t_{1/2} = 6.9$ d.). A longer half-life indicates that the organic matter in septage is less labile than sewage, probably related to its long detention time in the septic tank.

Facility nitrogen budget

Combining measurements of flow volumes between major subunits of the Marion facility with N input/output data and *in situ* rate measurements, N transformations in each major subunit were quantified in a detailed N budget for the facility (Figure 2.13). SFW denitrification was determined from NO_x removal and assuming no nitrification (since DO in the SFW averaged only 0.69 ± 0.06 mg/l and NH₄⁺ entering the SFW was low [1.15]

Table 2.4 - Decomposition of biologically active organic material in wastewater during aerobic degradation calculated from equation 2.2. k = decay constant; half-life $(t_{1/2}) = ln(2)/k$. Septage samples from Marion SAS, sewage from Providence, RI (n = 4).

| Source | k (d ⁻¹) | t _{1/2} (d) | r ² |
|--------------|----------------------|----------------------|----------------|
| Septage | | | |
| Influent | 0.082 | 8.4 | 0.92 |
| Waste Solids | 0.041 | 16.7 | 0.84 |
| Tank 1 | 0.138 | 5.0 | 0.91 |
| Tank 9 | 0.095 | 7.3 | 0.93 |
| Sewage | | | |
| Influent | 0.100 | 6.9 | 0.94 |



Figure 2.13 - Nitrogen balance in the Marion SAS. Box sizes show relative area of components. ET - equalization tank. PC - preliminary conditioning tank. 1C and 2C - primary and secondary clarifiers. Tanks - aquatic treatment tanks 1 through 9. SFW - subsurface flow constructed wetlands.

mg/l]). Removal by plant uptake in the greenhouse was determined from the mean N content of harvested biomass and the total harvest weight.

During preliminary treatment, denitrification was the second largest sink for N (34.8 % of the total N input of 187 kg in 174 days), after waste solids removal (57.2 %) (Figure 2.13). 12.3 % of the total influent N entered the greenhouse. Approximately 3.6 % was denitrified in the aquatic treatment tanks. 4.3 % was recycled to the PC as activated secondary solids and NO_x-rich water (NO_x = 17.9 mg/l, 32 % of recycled N). 4.1 % flowed into the SFW (62 % of which was NO_x). 2.3 % was denitrified in the SFW, and 0.5 % was retained in the marsh as solids, leaving 1.0 % which remained in the effluent, mostly as DON. Plant uptake of N played an insignificant role in N removal in both the aquatic treatment tanks and the SFW, totaling 0.5 %.

2.4 DISCUSSION

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N mineralization, nitrification and denitrification occurred simultaneously and significantly in all subunits except the SFW, where low organic N, NH_4^+ , and DO made denitrification the dominant process (Figure 2.5, 2.13). However, the factors controlling their relative importance differed among the 3 SAS treatment subunits.

2.4.1 Controls on Nitrogen Transformations During Preliminary Treatment Preliminary treatment consists of three steps. In the equalization tank, raw influent is equalized and aerated to promote degradation and bacterial growth. In the preliminary conditioning tank, it is aerated and blended with activated secondary solids from the greenhouse which are high in nitrifiers and heterotrophs (Table 2.5). The final step in preliminary treatment is sedimentation (1C). These steps result in the biodegradation of organic matter and N removal, as well as simultaneous adsorption of organics and flocculation of solids with low chemical requirements (33 g lime/m³ septage) (Figure 2.13).

The high loss of N due to denitrification during preliminary treatment (34.8 %) at the Marion SAS is similar to that reported for another septage treating SAS (21.8 %) (Wright-Pierce, 1993). This loss is equivalent to a mean denitrification rate of 0.78 mg/l/hr during preliminary treatment (specific rate = 2.6 mg N/g TSS/d.), which compares well with *in situ* measurements (0.47 mg/l/hr) at another SAS using the acetylene block method (Teal *et al.*, 1994). Biological oxidation of septage during preliminary conditioning resulted in denitrification rates comparable to those obtained for sewage in an activated sludge facility (2.3 - 3.1 mg N/g MLVSS/d.) (Drury *et al.*, 1995).

Although the ET and PC are aerated, they remain relatively anaerobic (0.22 ± 0.08 and $2.14 \pm 0.89 \text{ mg/l O}_2$, respectively), and the availability of organic carbon particles is high (7100 mg/l TSS). These are favorable conditions for denitrification, but the low levels of NO_x (1.1 mg/l) indicate that denitrification is probably limited by the nitrification rate. Nitrification in the ET and PC is likely limited by the low availability of oxygen and nitrifiers. The secondary solids recycled into the PC provide a source of nitrifying bacteria (3.6 x 10⁵ MPN/mg TSS compared with 1.11 x 10⁴/mg TSS coming from the ET), but nitrifier abundance is still low relative to the levels eventually attained in the aquatic treatment tanks (Table 2.5) (White *et al.*, 1996). Some NO_x enters preliminary treatment in the recycle flow from the greenhouse. 1.3 % of the total influent N is recycled back into the equalization tanks as NO_x, where it is denitrified.

57.2 % of the influent N (98 % is PON) leaves the facility in the form of settled solids (Figure 2.3) compared with 86 % estimated for dewatering by lime and ferric chloride precipitation followed by filter pressing (Slater *et al.*, 1987). Sedimentation resulted in the production of 0.16 *l* waste solids/*l* septage treated. The total waste solids production of the facility was 0.81 g/g influent TSS, compared with 1.45 g/g TSS for primary septage treatment by chemical precipitation (Slater *et al.*, 1987).

Sedimentation of solids from wastewater removes N, but adds to solids disposal costs, and solids still represent a potential source of N loading to natural waters. However, septage

53.

Table 2.5 - Most probable number (MPN) of nitrifying bacteria in water from various sources in the SAS and on plant roots. Total MPN of nitrifiers per aquatic treatment tank shown to indicate relative contribution of plants (adapted from White *et al.*, 1996).

| | Nitrifying Bacteria | | |
|----------------|-----------------------|------------------------|--|
| Source | MPN/I | MPN/tank | |
| ET | 8.0 x 10 ⁷ | | |
| PC | 1.4 x 10 ⁸ | | |
| 1C supernatant | 2.0 x 10 ⁸ | | |
| Tanks (mean) | 3.7 x 10 ⁸ | 2.3 x 10 ¹¹ | |
| 2C solids | 5.0 x 10 ⁸ | | |
| | MPN/g (wet) | | |
| Plant roots | 1.9 x 10 ⁷ | 2.5 x 10 ¹⁰ | |

after preliminary treatment in the SAS can be thought of as containing two types of solids: easily settleable material of low biodegradability ($t_{1/2} = 16.7$ d.), and material which tends to remain in suspension and is quite labile, ($t_{1/2} = 5$ d.). SAS waste solids are relatively stable, difficult to degrade further, making further stabilization or disposal easier. Since primary clarification is a simple way of separating the more labile from the more recalcitrant solids, it is an appropriate first step in treating septage. Collense,

Preliminary treatment in the SAS removed 92 % of influent N, and denitrified 34.8 %. However, there are a number of possibilities for improvements in treatment. The factors that control denitrification in this treatment unit could be manipulated to increase denitrification. Although it is not likely that preliminary treatment alone could treat septage to tertiary standards, it may be possible to increase the proportion of N denitrified at this stage.

Septage comes from a variety of sources, and is delivered to a septage treatment facility in tanker loads. At Marion, the mean size of a load was ca 5,000 *l*, but ranged from 246 - 15,700 *l*. Each load comes from a different source, and its composition varies according to its origin and the degree of treatment it received in the septic tank (Table 2.1). Unless there is a large equalization tank to decrease the variability between septage loads, the treatment units of the facility must be able to cope with the variation in the incoming wastewater flow. A larger equalization tank would increase retention times for

preliminary treatment, and might lead to an increase in N removal. DO levels in the ET were low (0.22 ± 0.08) ; higher DO levels would support increased nitrification, resulting in an increase in N removal.

Extending the retention time of the preliminary conditioning (PC) tank by increasing its size would allow more organic material oxidation, reducing waste solids production. More organic N would mineralize to NH_4^+ , and then be nitrified and denitrified for greater TN removal in this stage. However, longer retention times increase capital and aeration costs. There is also a diminishing return from longer retention times, since the more labile solids are degraded first, leaving a more resistant material which benefits less from continued treatment.

Increasing the recycle rate would result in the denitrification of recycled NO_x, but would have other costs in the larger equalization tanks and treatment tanks required to support the additional flow without lowering retention time. An increase in the recycle rate would probably not have an effect on the influx of nitrifiers into the PC tank, since nitrifiers are associated with solids, and all settlable solids are presently recycled. However, recycling some of the primary waste solids would probably add more nitrifiers and might increase nitrification in the PC tank, increasing denitrification. Although DO in the PC tank was high enough to support nitrification ($2.14 \pm 0.89 \text{ mg}/l \text{ O}_2$), DO levels were highly variable, resulting in inconsistencies in treatment. A more stable DO level in the PC tank may result in more consistently high nitrification rates.

2.4.2 Controls on Nitrogen Transformations in Aquatic Treatment Tanks

Preliminary treatment in the SAS removed 92 % of the influent N. However, further treatment is required to nitrify ammonia, and remove nutrients to tertiary standards. 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 (Figure 2.4). Since N removal efficiency tended to rise at higher N loading rates, there is the possibility that the system might still operate effectively even at N loadings above the maximum observed of 15.7 g/m³/d. This overcapacity in the present facility provides a buffer against the high variability of the influent. A larger ET would result in a better mixed and more consistent influent to the PC and might allow the facility to be operated at higher loadings.

Mineralization

The rate of organic matter decomposition in the aquatic treatment tanks was highly variable, despite constant aeration and temperatures (Figure 2.6). Decomposition rates are also controlled by organic matter concentrations, which were highly variable in the SAS (Table 2.1). Concentration alone was not a good predictor of mineralization rate. The additional variability probably resulted from variations in the biodegradability of the organic matter.

Nitrification

Nitrification is dependent on the metabolic activities of nitrifying bacteria present in biofilms in the tank environment. Biofilms are found on the inside walls of the tanks, on plant roots, and on suspended organic particles in the wastewater. These biofilms are mixtures of heterotrophic organisms (which get energy from organic carbon degradation) and autotrophic organisms, including nitrifiers (which get energy from the transformation of NH_4^+ to NO_x^-).

Dark ¹⁴C incorporation can be used as a check on measurements of nitrification. 0.030 mg/l/hr non-photosynthetic carbon fixation is equivalent to 0.330 mg NH₄⁺-N nitrified to $NO_x/l/hr$, about half of the measured rate of nitrification (0.606 mg/l/hr). However, the incubations took place in unaerated sealed BOD bottles which would have inhibited nitrification, lowering the rate estimated by this method.

Changes in the size of the NH_4^+ pool during treatment result from the imbalance of N mineralization and nitrification rates. At the beginning of the tank series, mineralization was higher than nitrification approximately 20 % of the time (Figure 2.6), causing NH_4^+ levels to rise. As treatment continued, the more labile solids were degraded, slowing mineralization rates and reducing respiratory oxygen demand. These changes shift the

balance of mineralization and nitrification, resulting in a decrease in NH_4^+ concentrations as treatment progressed further.

Nitrification is limited by several factors. Low concentrations of NH_4^+ (< 1.0 mg/l) inhibit nitrification rates (Figure 2.7) but these levels are usually not found until the second half of the tank treatment stream (Figure 2.5). Both heterotrophs and nitrifiers use and compete for oxygen. In the high organic-loaded environment of the aquatic treatment tanks, the oxygen requirements of heterotrophs are high, and competition for oxygen places a limit on nitrification rates.

The ratio of NH_4^+ -N/DO can be used to determine whether NH_4^+ or DO limits nitrification (Zhang *et al.*, 1995). Based on theoretical considerations, the critical value of the ratio above which DO is limiting has been calculated to be 0.34; one experimental determination in solutions with an organic carbon load (glucose) of 20 mg/l yielded a value of 0.774 (Zhang *et al.*, 1995). From the equation in Figure 2.7, the critical value in the replicate tanks was estimated to be 0.19 (based on the NH_4^+ -N concentration at which nitrification was 90 % of V_{max} -- an estimate of the onset of NH_4^+ limitation -- and concurrent DO measurements). The critical value in the SAS was possibly lower than those reported in the literature because of the additional oxygen demand of organic carbon respiration in the highly organic carbon-loaded environment of the aquatic treatment tanks. In the tanks, the ratio varies from a mean of 1.11 in tank 2 to 0.12 in tank 7, indicating that

DO rather than NH_4^+ probably limits nitrification until the final tanks when NH_4^+ concentrations are already very low (Figure 2.5). Stepped aeration could conserve costs while increasing nitrification rates. In addition, aeration above that required to support nitrification is likely to inhibit denitrification.

The availability of nitrifying bacteria can also limit nitrification. Nitrifying bacteria are found in biofilms on suspended organic particles, plant roots, and on the algal-bacterial mat on the tank walls (White *et al.*, 1996) (Table 2.5). The concentration of nitrifying bacteria puts a ceiling on the maximum rate of nitrification (under NH_4^+ saturation). Nitrifier concentration can be increased by increasing the ratio of plant roots to water volume, either by raising plant densities on the water surface or by increasing the ratio of the water surface area to tank depth by changing the tank proportions. Mixing and hydraulic conductivity must be preserved. Since the maximum desired nitrifier concentration varies with the NH_4^+ concentration, the proportions of the each tank in the series could be adjusted to maximize nitrification at the typical NH_4^+ levels found over the course of treatment in the aquatic treatment tanks.

In tanks of the size and proportions of aquatic treatment tanks typically used in septage treating SAS, the proportion of the total nitrifiers present on suspended organic particles outweighs the contribution of plants by more than 10 times, accounting for 91 % of the total (Table 2.5). Plant roots do not contribute significantly to the total nitrifier

concentration in the highly particle-loaded environment of septage. In order to significantly increase total nitrifier concentrations by increasing plant densities, tanks would have to be shallower, increasing facility land use. Plants are likely to have the greatest effect in wastewaters with low concentrations of suspended organic particles and high concentrations of NH_4^+ . However, plants may still play a role in providing a haven for bacteria against washout or poisoning by toxic compounds.

Denitrification

3.6 % of the influent N is lost by denitrification in the tanks, compared with 34.8 % during preliminary treatment (Figure 2.13). Nevertheless, denitrification in the tanks still represents about one third of the total N flowing into the greenhouse. Most of the 4.3 % that is recycled from 2C to preliminary treatment is in the form of settled solids; however about 1 % of the total N is recycled as NO_x and is denitrified in the PC and 1C. 4.1 % of the total influent N flows to the SFW; most of this is NO_x -N.

In the aquatic treatment tanks, denitrification limits N removal (Figure 2.8). Although I found that particulate organic carbon loading was a controlling factor of denitrification rates (Figure 2.9), carbon availability averaged 2.5 mg/l/hr, while a maximum of only 0.33 mg/l/hr would be required to support the observed mean denitrification rate of 0.260 \pm 0.048 mg/l/hr. Therefore carbon availability alone was not responsible for the observed effect on denitrification. Denitrification requires anaerobic conditions; in the aerobic

environment of the tanks, oxygen consumption by bacteria in biofilms limits the depth to which oxygen can diffuse, creating anaerobic microsites within the biofilm. The availability of these sites is dependent on both the carbon respiration rate and total volume of available anaerobic biofilm (Zhang *et al.*, 1995). The interiors of the films become anoxic (< 0.1 mg/l DO) from 100 to 150 μ m below the surface of the biofilm (Zhang *et al.*, 1995). Thus my results indicate that carbon acts not only as a food for denitrifiers, but also drives O₂ levels down in particles as it is respired, creating anaerobic microsites.

Support for this hypothesis was found in laboratory experiments on the response of denitrification rates to varying levels of nitrate and carbon availability as well as anaerobic conditions (Figure 2.10). The addition of plant litter to sewage samples with different initial NO_x concentrations showed that denitrification rates were limited by the availability of particulate organic matter when NO_x concentrations were not limiting. NO_x concentrations averaged above 5 mg/l after tank 2 (Figure 2.5), --- above the denitrification-limiting concentration -- (Figure 2.10) resulting in zero order reaction kinetics.

Samples which had been made anaerobic had initial denitrification rates higher than initially aerobic samples (Figure 2.11). 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. The lag time results from the time required for carbon respiration to create anaerobic microsites in organic carbon particles and in the water. Higher POC additions resulted in higher respiration rates, and the faster onset of anaerobic conditions favorable for denitrification. This experiment gave further evidence that denitrification within the aquatic treatment tanks was limited by the availability of both organic carbon and anaerobic microsites . California and

The addition of organic carbon particles as plant litter directly to the aquatic treatment tanks in the Marion facility also resulted in a 50 % reduction in NO_x indicating an increase in the denitrification rate (Figure 2.12). Plant litter has been used in other studies to promote denitrification (Avnimelech *et al.*, 1993; Gersberg *et al.*, 1984). In the relatively anaerobic environment of a constructed wetland, soluble organic carbon resulted in a greater loss of NO_x than plant litter (Gersberg *et al.*, 1984). In the aerated environment of the aquatic treatment tanks, particulate carbon is probably more effective, since it provides the required anaerobic microsites. Leaching rates of carbon from plant litter (0.15 mg C/l/hr) were insufficient to account for the increase in denitrification (0.32 mg C/l/hr), assuming a stochiometric ratio of 6:4 for denitrification (Focht and Chang, 1975). This means that the most important function of the litter bags was to create anaerobic microsites, and that the carbon requirements of denitrifiers were supplemented by dissolved organic carbon present in the wastewater.

These experiments indicate a potential for increasing denitrification in the aquatic treatment tanks and lowering the NO_x load to the SFW without increasing organic carbon load. One method would be to regulate the efficiency of preliminary treatment and sedimentation. If the clarifier is very efficient — removing most of the particulate solids from the waste stream -- denitrification will be decreased, since it depends on a source of particles and organic carbon. On the other hand, a preliminary treatment which allows too many particles to flow into the greenhouse would increase NH₄⁺ production, and organic matter would compete with nitrification for available oxygen, slowing nitrification. Another method is to use the plant litter generated by the facility (28 g dry wt/m³ septage/year) as a source of both organic carbon and of anaerobic microsites.

2.4.3 Controls on Nitrogen Transformations in the Subsurface Flow Wetland The SFW removed most contaminants both linearly and predictably in the loading ranges tested (Table 2.3). TP removal was probably linked to TSS removal by sedimentation. In the SFW, there are highly anaerobic conditions $(0.69 \pm 0.06 \text{ mg/l DO})$, nitrate levels are high $(15.38 \pm 0.31 \text{ mg/l})$, but there is very little carbon left (36.2 mg/l), and what is left is probably quite recalcitrant, and so unavailable to support denitrification. Therefore, it is likely that denitrification is limited by carbon availability. In 1994, soluble organic carbon (as acetate) was routinely added to the secondary clarifier. That winter, the SFW gravel was left intact without removal and cleaning, since there had been no decline in hydraulic conductivity during the 1994 season. In 1995, adequate denitrification occurred in the SFW without the addition of acetate, presumably because of the contribution of organic material from the previous year's plant litter.

(joggammer)

The performance of the SAS results from simultaneous nitrogen mineralization, nitrification, and denitrification. During preliminary conditioning, nitrification is the ratelimiting process, while in the greenhouse, denitrification limits N removal. Although the SAS treats septage to tertiary standards with minimal chemical addition and with low waste solids production, economic evaluation and comparison with existing septage treatment options is necessary to determine appropriate applications. A number of process improvements are possible, which may further increase the efficiency of the SAS in tertiary treatment of septage.

3.0 CONCLUSIONS AND RECOMMENDATIONS

3.1 CONCLUSIONS: NITROGEN REMOVAL PROCESSES IN THE SAS

- In the SAS, nitrogen mineralization, nitrification, and denitrification occur simultaneously, but in different relative proportions, in each subunit. In a conventional treatment system, flocculation and precipitation at the beginning of the treatment process remove most of the particulate organic matter, so that further nitrogen mineralization is minimal. In the SAS, clarification occurs at the end of each treatment process, resulting in increased mineralization and therefore a decrease in particulate organic waste solids (see section 3.2). Conventional treatment also tends to separate nitrification and denitrification, due to the different oxygen regimes required for optimization of these processes. In the SAS, microenvironments within organic particles enable denitrification to occur simultaneously with nitrification and nitrogen mineralization throughout the treatment process.
- During preliminary treatment, nitrogen removal occurs via a variation of the activated sludge process, and denitrification is limited by the rate of nitrification. Nitrification in turn is controlled by the availability of oxygen and of nitrifying bacteria. The recycle flow from the secondary clarifier supplies a source of nitrifiers to the preliminary conditioning tank, boosting bacterial activity.

 In the aquatic treatment tanks, nitrogen removal occurs by both attached and suspended growth processes. Biofilms are present on suspended particles as well as plant roots and the algal-bacterial mat on tank walls. Biological oxidation of organic material (as seen by the decrease in COD during treatment) accompanies the nearly complete nitrification of ammonia (to 0.56 mg/l). Name -

- Nitrogen removal in the tanks is limited by the rate of denitrification, which is controlled by the availability of anaerobic microsites. Anaerobic microsites are found primarily in suspended organic particles, but are also associated with fixed biofilms. A high concentration of particles in the aquatic treatment tanks results from the 'inefficiency' of the primary clarifier. Conventional flocculation and dewatering removes most of the organic particulates, inhibiting denitrification.
- The role of plants in the aquatic treatment tanks is unclear. Plant root systems do not appear to contribute significantly to total nitrifier populations. Plant uptake of nitrogen is not significant (0.5 % of influent nitrogen). Root systems may provide a habitat for invertebrate communities which may increase the efficiency of solids degradation.
- Nitrogen removal in the wetlands is primarily by denitrification. Organic carbon for denitrification is supplied by decaying plant material.

 The wetlands remove suspended solids by filtration. TSS removal accounts for the reduction in total phosphorus as well as some of the total nitrogen removal observed in the wetlands.

3.2 SOLAR AQUATIC SEPTAGE TREATMENT EFFICIENCY

3.2.1 Evaluation Criteria

No detailed economic analysis of the SAS has yet been made. Comparisons are difficult, since dedicated septage treatment facilities are relatively new, and few treat to tertiary standards. Economic analysis needs to include capital costs amortized over the expected lifetime of the facility, and operating expenses - including maintenance, personnel, and energy consumption, as well as chemical consumption. Finally, the cost of disposing of waste solids must be compared, including treatment costs.

A complete economic analysis was considered to be beyond the scope of this project. The costs of chemicals, disposal of solid waste, and land vary both absolutely and relatively from area to area. However, comparison of the SAS with septage treatment alternatives was possible according to criteria for which information was available.

The efficiency of the Solar Aquatic septage treatment facility was evaluated against four criteria. These are:

 Chemical use. Wastewater treatment facilities use chemicals for a variety of reasons, including flocculation and precipitation, pH control, and disinfection. Chemicals can be costly, may add to solid waste, and/or may have harmful effects on the environment. ł

- 2. Waste solids production. Waste solids disposal adds to operating costs, as well as posing a potential environmental hazard.
- Effluent nitrogen content. Permit levels for nitrogen in wastewater vary from jurisdiction to jurisdiction. Typically, tertiary treatment requires total nitrogen levels of less than 10 mg/l.
 Expectations for ammonia might vary from 0.5 to 2.2 mg/l.
- 4. Land requirements. Land use can be estimated from the degree of removal attained at a given loading rate. Ideally, the land requirements of nitrogen removal should be expressed as nitrogen removed per unit area per unit of time, but comparative data is difficult to find.

3.2.2 SAS Compared to Conventional Septage Treatment

The SAS uses fewer chemicals than conventional physicochemical treatment. The SAS used a small amount of lime (33 g/m³ septage) for pH control, whereas the conventional system at Orleans used 2900 g/m³ of lime and ferric chloride in its flocculation and dewatering process,, and unreported amounts of hydrochloric and phosphoric acid for pH control (Slater *et al.*, 1987).

- 2. The SAS produces fewer waste solids. 0.71 g waste solids/g influent TSS were produced, compared with 1.45 g solids/g TSS for conventional septage treatment. The lower waste production results from the clarification process used at the SAS, which requires no chemicals, as well as from extended biological oxidation of organic matter.
- 3. The SAS treats septage to tertiary standards, removing 99 % of influent nitrogen, reducing total nitrogen to 6.1 mg/l, and ammonia to 0.56 mg/l. These levels are low compared to those achieved by the conventional facility in Orleans, which are 52.6 and 28.2 mg/l, respectively. Apparently, the rotating biological contactor at this facility is incapable of supporting much nitrification or denitrification.
- 4. The SAS achieves greater N removal in a smaller land area than conventional constructed wetlands. I have estimated land requirements for constructed wetlands rather than for conventional septage treatment because of the availability of data and because land requirements are of special concern in constructed wetlands. The SAS greenhouse achieved 92 % N removal at a mean loading of 4.1 g/m²/d., while reported loadings for constructed wetlands treating sewage vary from 39 % removal at 3.0 g/m²/d. to 84 % at 1.3 g/m²/d.

3.3 RECOMMENDATIONS FOR PROCESS IMPROVEMENTS

Based on my research and on the literature, I recommend the following process improvements for further study and experiment. All recommendations are made with the intention of increasing the removal of nitrogen at minimal cost. Increasing the nitrogen removal rate lowers greenhouse size, land requirements, and other capital costs, as well as operating costs such as power. Recommendations for changes have been made where potentially large increases in removal rates appeared to be possible with minimal expense. However, the practicality of these changes is dependent upon two factors. First, experiments must be made to determine the degree of nitrogen removal increase possible. Secondly, an estimate of the cost savings due to a given removal rate increase must be balanced with the costs of implementing and maintaining the proposed improvement.

My research gives the strongest support to the method of immersing plant biomass in the aquatic treatment tanks to promote denitrification. All waste biomass from the tanks and wetland components should be dried and placed in mesh receptacles in tanks 6 to 9 to promote denitrification. These receptacles should be rigid and open at the top to allow easy loading. Plant biomass is produced in the greenhouse at a rate of 28 g dry wt/m³ septage/year. This biomass must be composted on site before removal. Using this biomass to promote denitrification would eliminate the need for composting space and maintenance while

promoting denitrification in the aquatic treatment tanks. With adequate monitoring, this modification could be made immediately.

Preliminary treatment

- The size of the equalization tank could be increased to reduce the variability in the characteristics of septage flowing into the preliminary conditioning tank. A larger equalization tank would reduce shock loads to the system, allowing it to be operated closer to capacity. It would also allow for year-round operation, despite the seasonality of septage delivery. A larger equalization tank would, however, result in increased capital expenses, as well as increased power consumption to support aeration. The benefits must be weighed against these costs.
- The preliminary conditioning (PC) tank should be aerated at a level which maintains dissolved oxygen (DO) at approximately 1.5 mg/l, as recommended for nitrification (Gray, 1989). Currently, DO in the PC tank fluctuates widely, resulting in inconsistent nitrification.
- A portion of the primary solids should be recycled back to the PC tank in order to maintain high bacterial populations. A small pump with a timer could accomplish this at minimal additional expense.

 Waste solids should be dewatered onsite and the filtrate returned to the equalization tank for treatment. The current system results in the exportation of approximately 16 % of incoming septage volume, which then must be treated elsewhere. Onsite dewatering and filtrate treatment would decrease the cost of solids disposal.

Aquatic treatment tanks

 Aeration in the tanks should be stepped, with aeration greatest at the beginning, and decreasing toward the end of the series. Stepped aeration would allow increased nitrification at the beginning of the process, while reducing costs due to excess aeration after ammonium levels have been reduced.

Subsurface flow wetlands

The size of the subsurface flow wetlands (SFW) should be reduced if sufficient NO_x reduction is attained in the aquatic treatment tanks by the measures recommended above. However, further research on denitrification in the wetlands needs to be done before attempting to reduce their size. The rates of denitrification and solids filtration at various points in the SFW must be determined. Presently the wetlands occupy approximately twice as much areal as the tanks, and decreasing their size could have a major impact on greenhouse size and land requirements.

- Below-ground biomass should not be removed unless it impairs hydraulic conductivity.
 Below-ground biomass contributes organic carbon essential for effective denitrification in the SFW.
- The extended treatment time of septage in septic tanks and in the septage treatment facility means that recalcitrant organics accumulate to a greater degree in the effluent than in sewage effluent. Approximately 60 % of the nitrogen remaining in the effluent appears to be biologically inactive, making very low effluent nitrogen levels (<5 mg/l) very difficult and costly to achieve. The high proportion of biologically inactive organic compounds in septage effluents should be taken into account by regulators when defining permitted levels of nitrogen discharge, since recalcitrant organics pose little threat to the environment.

3.4 WASTEWATER TREATMENT IN CONTEXT

While wastewater nutrient removal ameliorates the immediate problem of eutrophication, it should be viewed in the context of global water management strategies. Present water management practices are wasteful and unsustainable. The average inhabitant of an industrialized country uses approximately 200 *l* of water per day for cleaning, cooking, and flushing human wastes (Gray, 1989). Using water to flush wastes results in the global production of trillions of litres annually of water contaminated with excess organic matter and nutrients, toxic chemicals, and harmful bacteria, resulting in higher costs throughout the cycle

of water use. The diversion of water to meet the needs of densely populated areas causes reductions in the habitats required for fish and other aquatic animals. The treatment of all municipal water to drinking water standards regardless of the ultimate use is unnecessarily costly. At the other end of the pipe, wastewater treatment is costly, as is the environmental damage caused by insufficiently treated wastewater. Losses in recreation value and damage to fisheries due to water pollution have resulted in requirements for increasingly stringent and costly wastewater purification. Yet wastewater production continues to rise. As long as present patterns of water use continue, contaminated wastewater will have to be purified to protect natural waters.

Human effects on the global nitrogen cycle are linked with water management practices. Society spends money to vent valuable nitrogen to the atmosphere during wastewater treatment, while at the same time industrial processes fix more than 100 billion T of nitrogen a year using valuable energy. A sustainable solution to the wastewater problem requires decreasing water use and recycling waste nitrogen as fertilizer in order to decrease our reliance on costly industrial nitrogen fixation.

In this light, improved wastewater purification is perhaps an inferior solution to problems which have their source in an imbalance of the nitrogen cycle and an overuse of water. However, it is a solution which is consistent with current water management policies. Additionally,
wastewater treatment will still be required in a world of nutrient recycling and water conservation.

Ecotechnological systems like the SAS may provide an opportunity to effectively and inexpensively mitigate the effects of wastewater production in small communities. Although more experience with these systems is still required to evaluate capital and operating costs, the available evidence suggest that tertiary treatment of septage is possible at moderate cost and with minimal environmental side effects. Regulators and engineers searching for methods of improving septage treatment should consider ecotechnological systems such as the SAS as an alternative to existing methods.

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4.0 **BIBLIOGRAPHY**

- Alexander, W.V. and Wood, A. (1987) Experimental investigations into the use of emergent plants to treat sewage in South Africa. *Water Sci. Technol.*, **19**, 1751.
- Armstrong, W. (1964) Oxygen diffusion from the roots of some British bog plants. *Nature*, **204**, 801.
- Atlas, R.M. and Bartha, R. (1981) Microbial Ecology: Fundamentals and applications. Addison-Wesley, Reading, MA.
- Avnimelech, Y., Diab, S., and Kochba, M. (1993) Development and evaluation of a biofilter for turbid and nitrogen-rich irrigation water. *Water Res.* 27, 785.
- Balderston, W.L., Sherr, B. and Payne, W.J. (1976) Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. Environ. Microbiol., 31, 504.
- Bendschneider, K. and Robinson, R. (1952) A new spectrophotometric method for the determination of nitrite in sea water. J. Mar. Res., 11, 87.
- Bhamidimarri, R., Shilton, A., Armstrong, I., Jacobson, P., and Scarlet, D. (1991) Constructed wetlands for wastewater treatment: The New Zealand experience. *Water Sci. Technol.*, 19, 247.
- Biggar, J.W. and Corey, R.B. (1969) Agricultural drainage and eutrophication. In: National Academy of Sciences. Eutrophication: Causes, consequences, correctives. Proceedings of the International Symposium on Eutrophication held in Madison, Wisconsin, June 11-15, 1967. National Academy of Sciences, Washington, D.C. p. 404.
- Borum, J. (1985) Development of epiphytic communities on eelgrass (Zostera marina) along a mutrient gradient in a Danish estuary. Marine Biol., 87, 211.
- Brix, H. (1987) Treatment of wastewater in the rhizosphere of wetland plants The root-zone method. *Water Sci. Technol.*, **19**, 107.
- Brix, H. (1993) Macrophyte-mediated oxygen transfer in wetlands: Transport mechanisms and rates. *In*: G.A. Moshiri, *ed*. Constructed Wetlands for Water Quality Improvement. Lewis, Chelsea, MI. p. 391.

- Brix, H. and Schierup, H.-H. (1989) Sewage treatment in constructed reed beds Danish experiences. *Water Sci. Technol.*, **19**, 1665.
- Cambridge, M.L. and McComb, A.J. (1984) The loss of seagrasses in Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. *Aquat. Bot.*, **20**, 229.
- Canter, L.W. and Knox, R.C. (1985) Septic Tank System Effects on Ground Water Quality. Lewis, Chelsea, Mich.
- Caperon, J. and Meyer, J. (1972) Nitrogen-limited growth of marine phytoplankton. *Deep-Sea Res.*, **19**, 601.
- Capone, D.G. (1983) Benthic nitrogen fixation. *In*: E.J. Carpenter and D.G. Capone, *eds*. Nitrogen in the Marine Environment. Academic Press, New York, NY. p. 105.
- Cooper, P.F. (1993) The use of reed bed systems to treat domestic sewage: The European design and operations guidelines for reed bed treatment systems. *In*: G.A. Moshiri, *ed*. Constructed Wetlands for Water Quality Improvement. Lewis, Chelsea, MI. p. 203.
- Costa, J.E. (1988) Eelgrass in Buzzards Bay: Distribution, production and historical changes in abundance. USEPA 503/4-88-002.
- Conley, L.M., Dick, R.I., and Lion, L. W. (1991) An assessment of the root zone method of wastewater treatment. *Res. J. Water Pollut. Control Fed.*, **63**, 239.
- Crites, R.W. and Mingee, T.J. (1987) Economics of aquatic wastewater treatment. In: K.R. Reddy and W.H. Smith, eds. Aquatic Plants for Water Treatment and Resource Recovery. Proceedings of the conference on Research and Applications of Aquatic Plants for Water Treatment and Resource Recovery in Orlando, Florida. June 20-24, 1986. Magnolia, Orlando, Florida. p. 879.
- D'Elia, C.F., Webb, K.L and Wetzel, R.L. (1981) Time varying hydrodynamics and water quality in an estuary. *In*: B.J. Neilson and L.E. Cronin, *eds*. Estuaries and Nutrients. Humana, Clifton, NJ. p. 597.
- D'Elia, C.F., Steudler, P.A. and Corwin, N. (1977) Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.*, **22**, 760.
- Darnell, R.M. and Soniat, T.M. (1981) Nutrient enrichment and estuarine health. *In*: B.J. Neilson and L.E. Cronin, *eds.* Estuaries and Nutrients. Humana, Clifton, NJ, p. 225.

- Day, Jr., J.W., Hall, C.A.A., Kemp, W.M. and Yanez-Arancibia, A. (1989) Estuarine Ecology. John Wiley & Sons, New York, NY.
- De Leval, J. and Remacle, J. (1976) The influence of environmental factors on nitrification. *In*: J.O. Nriagu, *ed*. Environmental Biogeochemistry. Vol. 1. Carbon, Nitrogen, Phosphorus, Sulfur and Selenium Cycles. Ann Arbor Science, Ann Arbour, MI. p. 259.
- DeSimone, L.A. and Howes, B.L. (1995) Hydrogeologic, Water-Quality, and Biogeochemical Data Collected at a Septage-Treatment Facility, Orleans, Cape Cod, Massachusetts, October 1988 Through December 1992. U.S. Geological Survey Open-File Report 95-439. Marlborough, MA.
- Dillon, J. (1990) Natural forces harnessed to treat waste. Rutland Herald & Times Argus, Dec. 2.
- Dinges, R. and Doersam, J. (1987) The Hornsby Bend hyacinth facility in Austin, Texas. Water Sci. Technol., 19, 41.
- Drury, D., Dezham, P., Torres, B., Ooten, K., and Gabb, D. (1995) Novel nitrogen removal. Wat. Environ. Technol. 10 (9), 74.
- Edmondson, W.T. (1969) Eutrophication in America. *In*: National Academy of Sciences. Eutrophication: Causes, consequences, correctives. Proceedings of the International Symposium on Eutrophication held in Madison, Wisconsin, June 11-15, 1967. National Academy of Sciences, Washington, D.C. p. 124.
- El-Dib, M.A. and Ali, R.K. (1995) THMs formation during chlorination of raw Nile River water. Water Res., 29, 375.
- Eppley, R.W., Renger, E.H. and Harrison, W.G. (1979) Nitrate and phytoplankton production in Southern California coastal waters. *Limnol. Oceanogr.*, 24, 483.
- Falkner, S.P. and Richardson, C.J. (1989) Physical and chemical characteristics of freshwater wetland soils. *In*: D.A. Hammer, *ed*. Constructed Wetlands for Wastewater Treatment: Municipal, Industrial, and Agricultural. Proceedings from the First International Conference on Constructed Wetlands for Wastewater Treatment held in Chattanooga, Tennessee on June 13-17, 1988. Lewis, Chelsea, MI. p. 41.
- Focht, D.D. and Chang, A.C. (1975) Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol.*, **19**, 153.

- Fourqurean, J.W., Zeiman, J.C. and Powell, G.V.N. (1992) Phosphorus limitation of primary production in Florida Bay: Evidence from C:N:P ratios of the dominant seagrass *Thalassia testudinum. Limnol. Oceanogr.*, **37**, 162.
- Fujita, R.M., Wheeler, P.A., and Edwards, R.L. (1989) Mar. Ecol. Prog. Ser. 53, 293.
- Gersberg, R.M., Elkins, B.V., and Goldman, C.R. (1984) Use of artificial wetlands to remove nitrogen from wastewater. J. Water Pollut. Control Fed., 56, 152.
- Gersberg, R.M., Elkins, B.V., Lyon, S.R., and Goldman, C.R. (1986) Role of aquatic plants in wastewater treatment by artificial wetlands. *Water Res.*, 20, 363.
- Giblin, A.E. and Gaines, A.G. (1990) Nitrogen inputs to a marine embayment: the importance of groundwater. *Biogeochemistry*, **10**, 309.
- Gillette, B. (1988) Revolution in wastewater treatment. BioCycle., 29(3), 48.
- Gover, N. (1993) Greenhouse technology treats wastewater naturally. Small Flows, 7, 1.
- Gray, N.F. (1989) Biology of Wastewater Treatment. Oxford, New York, NY.
- Green, M.B. and Upton, J. (1994) Constructed reed beds: A cost-effective way to polish wastewater effluents for small communities. *Water Environ. Res.* 66, 188.
- Gross, M.G. (1976) Estuarine cleanup Can it work? in: M. Wiley, ed. Estuarine Processes: Volume I. Uses, Stresses and Adaption to the Estuary. Academic, New York, NY. p. 3.
- Gumbricht, T. (1992) Tertiary wastewater treatment using the root-zone method in temperate climates. *Ecological Engineering.*, 1, 199.
- Guterstam, B. and Todd, J. (1990) Ecological engineering for wastewater treatment and its application in New England and Sweden. *Ambio*, **19**, 173.
- Halling-Sørensen, B. and Jørgensen, S.E. (1993) The Removal of Nitrogen Compounds from Wastewater. Elsevier, Amsterdam.
- Hattori, A. (1983) Denitrification and dissimilatory nitrate reduction. In: E.J. Carpenter and D.G. Capone, eds. Nitrogen in the Marine Environment. Academic, New York, NY. p. 191.

Hecky, R.E. and Kilham, P. (1988) Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.*, 33, 796.

Hedbor, E. (1990) System uses nature to treat waste. Burlington Free Press, Dec. 3.

- Henriksen, K. and Kemp, W.M. (1988) Nitrification in estuarine and coastal marine sediments. In: T.H. Blackburn and J. Sørensen, eds. Nitrogen Cycling in Coastal Marine Environments. SCOPE. John Wiley & Sons, New York, NY. p. 207.
- Horan, N.J. (1990) Biological Wastewater Treatment Systems: Theory and operation. John Wiley & Sons, Chichester, England.
- Howes, B.L. and Teal, J.M. (1994) Oxygen loss from *Spartina alterniflora* and its relationship to salt marsh oxygen balance. *Oecologia*, **97**, 431.
- Howes, B.L. and Goehringer, D.D. (1994a) Falmouth Pond Watchers: Water quality monitoring of Falmouth's coastal ponds. Results from the 1993 season. Woods Hole Oceanographic Institution. Woods Hole, MA.
- Howes, B.L. and Goehringer, D.D. (1994b) The Ecology of Buzzards Bay: An estuarine profile. Report submitted to National Coastal Ecosystems Team, National Wetlands Research Center. Lafayette, Louisiana. Woods Hole Oceanographic Institution. Woods Hole, MA.
- Isaacs, S.J. and Henze, M. (1995) Controlled carbon source addition to an alternating nitrification-denitrification wastewater treatment process including biological P removal. Water Res., 29, 77.
- Johnston, C.A. (1993) Mechanisms of wetland-water quality interaction. *In*: G.A. Moshiri, *ed*. Constructed Wetlands for Water Quality Improvement. Lewis, Chelsea, MI. p. 293.
- Kaplan, W.A. (1983) Nitrification. In: E.J. Carpenter and D.G. Capone, eds. Nitrogen in the Marine Environment. Academic, New York, NY. p. 139.
- Kautsky, N., Kautsky, H., Kautsky, U., and Waern, M. (1986) Decreased depth penetration of *Fucus vesiculosus* (L.) since the 1940's indicates eutrophication of the Baltic Sea. *Mar. Ecol. Prog. Ser.*, 28, 1.

- Kawai, H., Uehara, M.Y., Gomes, J.A., Jahnel, M.C., Rossetto, R., Alem, P., Ribeiro, M.D., Tinel, P.R., and Grieco, V.M. (1987) Pilot-scale experiments in water hyacinth lagoons for wastewater treatment. *Water Sci. Technol.*, 19, 129.
- Kinsinger, W.C., Mankiewicz, P.S., and Mankiewicz, J.A. (1991) A living symbol of participatory ecology: Wastewater, groundwater, and air purification schematics for the René Dubos Bioshelter, planned south transept of the Cathedral of St. John the Divine in New York City. In: C. Etnier, and B. Guterstam, eds. Ecological Engineering for Wastewater Treatment. Proceedings of the International Conference at Stensund Folk College, Sweden. March 24-28, 1991. Bokskogen, Gothenburg, Sweden. p.327.

Knowles, R. (1982) Denitrification. Microbiol. Rev., 46, 43.

- Kumar, P. and Garde, R.J. (1990) Upgrading wastewater treatment by water hyacinth in developing countries. *Water Sci. Technol.*, **22**, 153.
- Kumar, P. and Garde, R.J. (1989) Potentials of water hyacinth for sewage treatment. *Res. J. Water Pollut. Control Fed.*, **61**, 1702.
- Lynch, J.M. and Poole, N.H., *eds.* (1979) Microbial Ecology: A conceptual approach. John Wiley & and Sons, New York, NY.

Marinielli, J. (1990) After the flush: The next generation. Garbage, Jan/Feb.

- Matulewich, V.A. and Finstein, M.S. (1978) Distribution of autotrophic nitrifying bacteria in a polluted river (the Passaic). *Appl. Environ. Microbiol.* **35**, 67.
- McCarthy, J.J. and Goldman, J.C. (1979) Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science.*, **203**, 670.
- McErlean, A.J. and Reed, G. (1981) Indicators and indices of estuarine enrichment. *In*: B.J. Neilson and L.E. Cronin, *eds.* Estuaries and Nutrients. Humana, Clifton, NJ. p. 165.

Meadows, D.H. (1988) The New Alchemist. Harrowsmith, Nov./Dec., 38.

Mitch, W.J. and Jørgensen, S.E., *eds.* (1989) Ecological Engineering: An introduction to ecotechnology. Wiley, New York, NY.

106

- Mulligan, H.F. (1969) Management of aquatic vascular plants and algae. In: National Academy of Sciences. Eutrophication: Causes, consequences, correctives. Proceedings of the International Symposium on Eutrophication held in Madison, Wisconsin, June 11-15, 1967. National Academy of Sciences, Washington, D.C. p. 464.
- National Academy of Sciences. (1969) Eutrophication: Causes, consequences, correctives. Proceedings of the International Symposium on Eutrophication held in Madison, Wisconsin, June 11-15, 1967. National Academy of Sciences, Washington, D.C.
- Nichols, D.S. (1983) Capacity of natural wetlands to remove nutrients from wastewater. J. Water Pollut. Control Fed., 55, 495.
- Nixon, S.W. and Pilson, M.E.Q. (1983) Nitrogen in estuarine and coastal marine ecosystems. *In*: E.J. Carpenter and D.G. Capone, *eds*. Nitrogen in the Marine Environment. Academic, New York, NY. p. 565.
- Officer, C.B., Biggs, R.B., Taft, J.L., Cronin, L.E., Tyler, M.A., and Boynton, W.R. (1984) Chesapeake Bay anoxia: Origin, development, and significance. *Science*, **223**, 22.
- Ogden, M.H. (1993) The Treatment of septage using natural systems. In: G.A. Moshiri, ed. Constructed Wetlands for Water Quality Improvement. Lewis, Chelsea, ML p. 525.
- Orth, R.J. and Moore, K.A. (1983) Chesapeake Bay: An unprecedented decline in submerged aquatic vegetation. *Science*, **222**, 51.
- Orth, H.M. and Sapkota, D.P. (1988) Upgrading a facultative pond by implanting water hyacinth. *Water Res.*, **22**, 1503.
- Peterson, S. (1991) Greenhouse aquaculture treatment. Paper presented at the Conference on Sustainable Wastewater Management. November 21-23. 1991.
- Philip, H., Maunoir, S., Rambaud, A., and Philippi, L.S. (1993) Septic tank sludges: Accumulation rate and biochemical characteristics. *Water Sci. Technol.*, **28**, 57.
- Reardon, R. (1995) Advanced wastewater treatment. Water Environ. Technol., 10 (9), 66.
- Reddy, K.R. (1983) Fate of nitrogen and phosphorus in a waste-water retention reservoir containing aquatic macrophytes. J. Environ. Qual., 12, 137.
- Reddy, K.R. and De Busk, W.F. (1985) Nutrient removal potential of selected aquatic macrophytes. J. Environ. Qual., 4, 459.

- Reddy, K.R. and De Busk, T.A. (1987) State-of-the-art utilization of aquatic plants in water pollution control. *Water Sci. Technol.*, **19**, 61.
- Reddy, K.R., Patrick, Jr., W.H., and Lindau, C.W. (1989) Nitrification-denitrification at the plant root-sediment interface in wetlands. *Limnol. Oceanogr.*, **34**, 1004.
- Reddy, K.R. and Smith, W.H., eds. (1987) Aquatic Plants for Water Treatment and Resource Recovery. Proceedings of the conference on Research and Applications of Aquatic Plants for Water Treatment and Resource Recovery in Orlando, Florida. June 20-24, 1986. Magnolia, Orlando, Florida.
- Reddy, K.R. and Sutton, D.L. (1984) Waterhyacinths for water quality improvement and biomass production. J. Environ. Qual., 13, 1.
- Reed, S.C. (1990) Solar Aquatics for wastewater treatment at Sugarbush, Vermont. Paper presented at the 1990 Water Pollution Control Federation Conference. October 8-15. Washington, D.C.
- Reed, S.C. (1991) Constructed wetlands for wastewater treatment. BioCycle, 32(1), 44
- Reed, S.C. (1992) Solar Aquatics treat resort wastewater: An innovative way to meet ammonia limits. *Water Environ. Technol.*, 7 (7), 62.
- Reed, S.C. and Brown, D. (1995) Subsurface flow wetlands A performance evaluation. Water Environ. Res. 67, 244.
- Reed, S.C. and Brown, D.S. (1992) Constructed wetland design: The first generation. *Water Environ. Res.*, 64, 779.
- Ryther, J.H. and Dunstan, W.M. (1971) Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science*, **171**, 1008.
- Ryther, J.H. and Officer, C.B. (1981) Impact of nutrient enrichment on water uses. In: B.J. Neilson and L.E. Cronin, eds. Estuaries and Nutrients. Humana, Clifton, NJ. p. 247.
- Runsten, B., Hem, L.J., and Ødegaard, H. (1995) Nitrogen removal from dilute wastewater in cold climate using moving-bed biofilm reactors. *Water Environ. Res.* 67, 65.
- Rusten, B., Hem, L.J., and Ødegaard, H. (1995) Nitrogen removal from dilute wastewater in cold climate using moving-bed biofilm reactors. *Water Environ. Res.*, 67, 65.

- Ruzicka, J. and Hansen, E.H. (1988) Flow Injection Analysis. 2nd ed. John Wiley and Sons, New York, NY.
- Sand-Jensen, K. and Søndergaard, M. (1981) Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. *Int. Revue. Ges. Hydrobiol.*, **66**, 529.
- Santos, E.J., Silva, E.H.B.C., Fiuza, J.M., Batista, T.R.O. and Leal, P.P. (1987) A high organic load stabilization pond using water hyacinth - A "Bahia" experience. *Water Sci. Technol.* 19, 25.
- Scheiner, D. (1976) Determination of ammonia and Kjeldahl nitrogen by indophenol method. *Water Res.*, **10**, 31.
- Schlesinger, W.H. (1991) Biogeochemistry: An analysis of global change. Academic, San Diego, CA.
- Seitzinger, S.P. (1994) Linkages between organic matter mineralizaton and denitrification in eight riparian wetlands. *Biogeochemistry.*, 25, 19.
- Serfling, S.A. and Mendola, D. (1979) The Solar Aquacell AWT lagoon system for the city of Hercules, California. *In*: Proceedings of the Water Reuse Symposium, vol. 1. March 25-30. Washington, D.C. p. 671.
- Slater, A.D., Weisman, P.D. and Carlson, K.W. (1987) A regional approach to septage management on Cape Cod. J. New Eng. Wat. Poll. Cont. Ass., 21, 156.
- Smith, S.V. (1984) Phosphorus versus nitrogen limitation in the marine environment. *Limnol. Oceanogr.*, **29**, 1149.
- Smith, R.L., Howes, B.L., and Duff, J.H. (1991) Denitrification in nitrate-contaminated groundwater: Occurrence in steep vertical geochemical gradients. *Geochimica et Cosmochimica Acta*, **55**, 1815.
- Söderhund, R. and Svensson, B.H. (1976) The global nitrogen cycle. In: B.H. Svensson and R. Söderhund, eds. Nitrogen, Phosphorus and Sulphur: Global cycles. SCOPE Report 7. Ecol. Bull., 22, 23.

Spencer, R. (1992) Lower cost way to septage treatment. BioCycle, 33(3), 64.

Spencer, R. (1990) Solar Aquatic treatment of septage. BioCycle, 31(5), 66.

- Tanner, C.C., Clayton, J.S., and Upsdell, M.P. (1995) Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands - II. Removal of nitrogen and phosphorus. *Water Res.*, 29, 27.
- Tchobanogous, G., Colt, J.E., and Crites, R.W. (1980) Energy and resource consumption in land and aquatic treatment systems. *In*: Proceedings of the U.S. Department of Energy, Energy Optimization of Water and Waastewater Management for Municipal and Industrial Applications Conference. December 10 - 13, 1979, New Orleans, LO. P.431.
- Tchobanoglous, G., Maitski, F., Thompson, K., and Chadwick, T.H. (1989) Evolution and performance of city of San Diego pilot-scale aquatic wastewater treatment system using water hyacinths. *Res. J. Water Pollut. Control Fed.*, **61**, 1625.
- Teal, J..M. and Peterson, S.B. (1993) A Solar Aquatic System septage treatment plant. Environ. Sci. Technol., 27, 34.
- Teal, J..M. and Peterson, S.B. (1991) The next generation of septage treatment. *Res. J. Water Pollut. Control Fed.*, 63, 84.
- Teal, J..M., Howes, B.L., Peterson, S.B., Petersen, J.E., and Armstrong, A. (1994) Nutrient processing in an artificial wetland engineered for high loading: A septage treatment example. *In*: W.J. Mitsch, *ed*. Global Wetlands: Old world and new. Elsevier, New York, NY. p. 421.
- Thayer, G.W., Kenworthy, W.J., and Fonesca, M.S. (1984) The ecology of eelgrass meadows of the Atlantic coast: A community profile. U.S. Fish Wild. Serv. Div. Biol.Serv. FWS/OBS-84/02, 147.
- Todd, J. (1988) Solar Aquatic wastewater treatment. BioCycle, 29(2), 38.
- U.S. EPA. (1987) A Statistical Abstract of the Unsewered U.S. Population. EPA 600/5-87/004.
- Valiela, I. and Teal, J.M. (1974) Nutrient liitation in salt marsh vegetation. *In*: R.J. Riemold and W.H. Queen, eds. Ecology of Halophytes. Academic, New York. P. 547.
- Van der Ryn, S. (1978) The Toilet Papers: Designs to recycle human waste and water: Dry toilets, greywater systems, and urban sewage. Capra, Santa Barbara, CA.

- Vitousek, P.M. (1994) Beyond global warming: Ecology and global change. *Ecology*, **75**, 1861.
- Watson, J.T., Choate, K.D., and Steiner, G.R. (1990) Performance of constructed wetland treatment systems at Benton, Hardin, and Pembroke, Kentucky during the early vegetation establishment phase. *In*: Cooper, P.F. and Findlater, B.C., *eds.*, Constructed Wetlands in Water Pollution Control. Pergamon, Oxford. p. 171.
- Weibel, S.R. (1969) Urban drainage as a factor in eutrophication. In: National Academy of Sciences. Eutrophication: Causes, consequences, correctives. Proceedings of the International Symposium on Eutrophication held in Madison, Wisconsin, June 11-15, 1967. National Academy of Sciences, Washington, D.C. p. 383.
- Weisner, S.E.B., Eriksson, P.G., Granéle, W., and Leonardson, L. (1994) Influence of macrophytes on nitrate removal in wetlands. *Ambio.*, **23**, 363.

Wetzel, R.G. (1975) Limnology. W.B. Saunders, Philadelphia, PA.

- White, D.S. and Howes, B.L. (1994) Translocation, remineralization, and turnover of nitrogen in the roots and rhizomes of *Spartina alterniflora* (Gramineae). *Am. J. Bot.* 81, 1225.
- White, D.S, Howes, B.L., and Hamersley, M.R. (1996) The effect of plants on wastewater treatment in an ecologically engineered wetland. *In preparation*.
- Wilhelm, S.R., Schiff, S.L. and Cherry, J.A. (1994) Biogeochemical evolution of domestic waste water in septic systems: 1. Conceptual model. *Ground Water*, **32**, 905.
- Wolverton, B.C. and McDonald, R.C. (1979) The water hyacinth: From prolific pest to potential provider. *Ambio*, **8**, 2.
- Wood, E., Armstrong, F. and Richards, F. (1967) Determination of nitrate in sea water by cadmium copper reduction to nitrite. J. Mar. Bio. Ass. U.K., 47, 23.
- Wright-Pierce (1993) Engineer's Report on Ecological Engineering Associates' Harwich Septage Treatment Pilot Plant Final Report. Prepared for Massachusetts Department of Environmental Protection. Division of Water Pollution Control. Wright-Pierce, Topsham, Maine.
- Zhang, T.C., Fu, Y-C., and Bishop, P.L. (1995) Competition for substrate and space in biofilms. *Water Environ. Res.*, 67, 992.

Zhenbin, W., Yicheng, X., Jiaqi, D., Qijun, K., Facui, Z., and Xitao, C. (1993) Studies on wastewater treatment by means of integrated biological pond system: Design and function of macrophytes. *Water Sci. Technol.*, **19**, 97.

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