Autotrophic Biological Denitrification with Elemental Sulfur or Hydrogen for Complete Removal of Nitrate-Nitrogen from a Septic System Wastewater

A Final Report Submitted to

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Submitted by

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Abstract
Influx of nitrogen from anthropogenic sources is primarily responsible for coastal
eutrophication globally and in the Waquoit Bay NERR – the latter being the area of study
of this project. The predominant source of anthropogenic nitrogen in the WBNERR bay
is the effluent from septic tank systems, which serve more than 85% of the homes in this
region. Conventional septic systems remove at best about 23% of the nitrogen in the
influent wastewater; thus, there is a great need to introduce technologies that can be
applied to onsite wastewater treatment that can achieve higher percentage of nitrogen
removal. Some Innovative/Alternative (I/A) technologies have recently been evaluated
that can remove almost 66% of the influent nitrogen. Conventional heterotrophic
denitrification using an external electron donor can produce better results but suffers from
the limitations of (i) using a toxic/inhibitory chemical such as methanol, and (ii)
producing large amounts of biological sludge that has to handled/disposed of. We
propose a technology - Autotrophic Biological Denitrification - that has the potential to
achieve almost complete nitrogen removal and yet does not suffer from the limitations of
heterotrophic denitrification enumerated above. We propose using elemental sulfur or
hydrogen as the electron donor. The main objectives of this project included, (i)
investigate autotrophic biological denitrification of wastewater using H$_2$ and S$^0$ as
electron donors, (ii) evaluate the use of various sources of alkalinity to improve stability
of S$^0$ oxidizing systems, (iii) gain experience with operation of field-scale S$^0$ oxidizing
systems, (iv) evaluate the effects of supplemental organic carbon addition and dissolved
oxygen in the wastewater on process efficiency, and (v) evaluate the effect of Empty Bed
Contact Time (EBCT) and initial nitrate-nitrogen concentration on process performance.
We conducted enrichment studies and lab-scale bioreactor tests at UMass Dartmouth and
Amherst campuses. We also conducted field-scale tests at the Massachusetts Alternative
Septic System Test Center in Sandwich, MA to achieve the objectives referred above. A
summary of the major research findings can be listed as: (a) high denitrification rates
could be achieved in a sulfur oxidizing bioreactor system treating nitrified wastewater
with a hydraulic residence time of eight hours and sufficient pH buffering, (b) crushed
oyster shell is the most suitable solid-phase buffer in sulfur-oxidizing denitrification
systems, (c) periodic backwashing is needed in sulfur-oxidizing systems to dislodge
excess microorganisms from the packed-bed (sulfur and buffer) bioreactor, (d) pH and
alkalinity can act as process-control variables, (e) mixotrophic conditions did not enhance
the denitrification efficiency for the bioreactor system, and (f) the presence of dissolved
oxygen in the influent did not inhibit denitrification performance. The proposed
technology has the potential for immediate commercial application and can be a potent
tool for federal, state, and local water quality administrators.

Keywords: autotrophic, denitrification, alkalinity buffer, eutrophication, electron donor,
nitrate-nitrogen, nitrite-nitrogen, hydrogenotrophic, biofilm, pH.
Introduction

The influx of excessive amounts of nitrogen from anthropogenic sources is primarily responsible for water quality degradation (especially eutrophication) and habitat loss in near-coastal waters throughout the world (Cederwall and Elmgren, 1980; Jaworski, 1981; Rosenberg, 1985; Nixon et al., 1986; Kelly and Levin, 1986). In the Northeastern and Mid-Atlantic section of the US, eutrophication is a major concern in the Cape Cod, Narragansett Bay, and the Chesapeake Bay areas (Bachman, 1984; Lee and Olsen, 1985; Valiela and Costa, 1988). While in theory either Phosphorus or Nitrogen could be the rate-limiting species controlling eutrophication, it is generally agreed upon that coastal marine systems are often N-limited (Rhyther and Dunstan, 1971; Goldman et al., 1973; Valiela, 1984). This means that given adequate sunlight, if the input flux of nitrogen increases, some algae take up the nitrogen and grow rapidly, causing undesirable ecological effects due to eutrophication, including (i) water quality degradation due to undesirable growth of algae and the accompanying problems of turbidity and dissolved oxygen (DO) depletion and hypoxia, (ii) decimation of commercial harvests of finfish and shellfish, (iii) the presence of nuisance and sometimes toxic algal mats and blooms and (iv) inability to use the waterways for recreational purposes. Primary sources of nitrogen to coastal and estuarine ecosystems include effluents from onsite wastewater treatment systems, wastewater treatment plants, animal wastes, atmospheric deposition, combined sewer overflows (CSO) and runoff from agricultural, residential and recreational areas that are heavily fertilized (NOAA, 1999; Rhyther and Dunstan, 1971; Goldman et al., 1973; Valiela, 1984).

Waquoit Bay - the area of the NEER site associated with this project - is an enclosed estuary located on the south shoreline of Cape Cod, Massachusetts, in the towns of Falmouth and Mashpee. It encompasses 3000 acres of open waters, wetlands and uplands. Its watershed comprises nearly 65 km² extending roughly 10 km north from the head to the bay. The bay on average is relatively shallow with a mean depth of 1m. Major freshwater sources include the Quashnet River to the east and the Childs River to the west. The Waquoit Bay region was part of the Wampanoag tribal lands when European settlers arrived in the early 1600s on what is now Cape Cod (Gallagher, 1983). For more than 200 years the Waquoit watershed was used primarily for hunting, farming mainly potatoes and strawberries, and maritime industries such as fishing, whaling, and shipbuilding. Since the late 1800s, with the introduction of public transportation, the natural beauty of the area has attracted more people. Today, more than 8,000 people live within the Waquoit watershed. The population swells in the summer months as the area has become an extremely well known for tourists. The once-rural surroundings have become increasingly suburbanized with the development of bedroom and retirement communities. According to the U.S. Census Bureau, the population of Barnstable County, within which the Waquoit Bay watershed is located, increased by 13.9% between 1990 and 1999. By comparison, the increase in population of the entire state of Massachusetts was 2.6% during the same period (U.S. Census Bureau, 2000).

As the stress on the Waquoit Bay watershed increases due to the booming growth rate of the population, the challenge to the environment has concomitantly become a major concern. One of the major concerns is the contribution of nutrients and contaminants to the bay. In terms of nutrients, the biggest concern is the presence of nitrogen in the
wastewater that ends up in the bay (Weiskel and Howes, 1991; Canter and Knox, 1985; Costa et al., 1999). More than 85% of homes in this region use Title 5 systems (aka septic tank and leach field system) for wastewater treatment and disposal (USEPA, 2002). The effluent from the leaching field is rich in nitrogen and easily percolates through the permeable sandy soil to reach the groundwater. The nitrogen in the groundwater travels to coastal waters and stimulates eutrophication. Table 1 (Bowen and Valiela, 2001) clearly shows that while there are other sources of nitrogen, the contribution of Title 5 systems to the nitrogen load in the estuary is the highest.

### Table 1

Relative contributions of each of the major sources of nitrogen to the Waquoit Bay estuary in 1990 (from Bowen and Valiela, 2001).

<table>
<thead>
<tr>
<th>Source of nitrogen</th>
<th>1990 Nitrogen Load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^3$ Kg/year %</td>
</tr>
<tr>
<td>To the watershed</td>
<td></td>
</tr>
<tr>
<td>Atmospheric deposition</td>
<td>95.5 59</td>
</tr>
<tr>
<td>Wastewater disposal</td>
<td>35.7 22</td>
</tr>
<tr>
<td>Fertilizer use</td>
<td>30.5 19</td>
</tr>
<tr>
<td></td>
<td>161.7 100</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>To the estuary</td>
<td></td>
</tr>
<tr>
<td>Atmospheric deposition</td>
<td>9.1 38</td>
</tr>
<tr>
<td>Wastewater disposal</td>
<td>10.5 43</td>
</tr>
<tr>
<td>Fertilizer use</td>
<td>4.7 19</td>
</tr>
<tr>
<td></td>
<td>24.3 100</td>
</tr>
</tbody>
</table>

From Table 1, it is clear that control of nitrogen from the discharge of Title 5 systems is extremely critical to the success of any strategy to mitigate eutrophication in the Waquoit Bay area. In fact, simulation models (USEPA, 2002) have shown that if nitrogen is completely removed from the discharge of all Title 5 systems in the Waquoit Bay watershed, the nitrogen loading would decrease from values extant in 1990 to those in the early 1940s; a significant reduction. Thus, exploration of innovative/alternative (I/A) technologies (to conventional Title 5 systems) to achieve nitrogen removal is a major research area.

Nitrogen in wastewater, animal wastes and fertilizers is typically in the form of ammonia and organic nitrogen. Common aerobic soil bacteria convert ammonia and organic nitrogen to nitrate ($\text{NO}_3^-$) in soil, through the process of nitrification. Nitrate is a highly mobile and persistent molecule and almost all of the nitrogen transported to bays and estuaries is in this form. Therefore, the issue of nitrogen control is essentially about controlling and containing the nitrification process and then converting the nitrate formed to an innocuous form. In coastal areas, the biggest contributor of nitrogen is the
discharge from decentralized, sub-surface wastewater treatment systems (known as Title 5 systems in Massachusetts). Thus, exploration of innovative/alternative (I/A) technologies (to a conventional Title 5 system) to achieve nitrogen removal through reduction of NO$_3^-$ is a major research area. Since N$_2$ gas is the most benign form of nitrogen, the preferred treatment process is the reduction of NO$_3^-$ to N$_2$ gas through biological denitrification.

Biological denitrification is carried out by facultative bacteria that are capable of using nitrate as a terminal electron acceptor for respiration under anoxic conditions. Dissimilatory nitrate reduction is coupled with oxidation of an electron donor, generating energy for cell synthesis and maintenance. Reduction of nitrate to nitrogen gas proceeds in a four-step process:

$$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$$ (1)

As a group, denitrifying bacteria are genetically diverse and metabolically versatile. Heterotrophic biological denitrification is commonly coupled with nitrification for removing total nitrogen from domestic and industrial wastewater. **Heterotrophic denitrifying bacteria require an organic carbon source for energy and cell synthesis.** An internal organic carbon source can be provided by recirculating nitrified wastewater to an anoxic zone in the bioreactor; however total N removal is limited in these systems. Methanol is usually favored as an external electron donor due to its lower cost and sludge production compared with other organic carbon sources. However, methanol is difficult to handle, deliver and store and residual methanol in the effluent may pose a toxicity problem. Thus, a biological denitrification process that can overcome the limitations of heterotrophic denitrification and is passive such that it can be integrated or retrofitted to existing Title 5 systems would be an ideal treatment scheme to control coastal eutrophication. **Autotrophic denitrification using elemental sulfur (S$^0$) or hydrogen gas (H$_2$) as the electron donor has the potential to meet all the criteria for a technology that can be applied to subsurface treatment systems.**

**Sulfur Oxidizing Denitrification**

A number of common soil bacteria, such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*, are able to use reduced sulfur compounds as electron donors and respire on nitrate in the absence of oxygen. A stoichiometric equation for autotrophic denitrification using sulfur as an electron donor is (Batchelor and Lawrence, 1978):

$$55\text{S}^0 + 20\text{CO}_2 + 50\text{NO}_3^- + 38\text{H}_2\text{O} + 4\text{NH}_4^+ \rightarrow 4\text{C}_3\text{H}_7\text{O}_2\text{N} + 55\text{SO}_4^{2-} + 25\text{N}_2 + 64\text{H}^+$$ (2)

Based on this equation, for each gram of NO$_3^-$-N removed approximately 0.64 g cells and 2.5 g of SO$_4^{2-}$ are generated and 4.5 g alkalinity (as CaCO$_3$) are consumed. A number of researchers have used reduced sulfur compounds for biological denitrification of domestic wastewater (Kuai and Verstraete, 1999), industrial wastewater (Nugroho et al., 2002), nitrified landfill leachate (Kim and Bae, 2000; Oh et al., 2001) and drinking water (Furumai et al., 1996; Soares, 2002; Kimura et al., 2002). Several early studies focused
on thiosulfate as an electron donor (Claus and Kutzner, 1985; Furumai et al., 1996). However, the use of elemental sulfur granules in packed bed bioreactors eliminates the need for expensive feed control systems. In addition elemental sulfur is less expensive, easier to store and handle and produces less effluent SO$_4^{2-}$ than thiosulfate. Advantages of autotrophic sulfur oxidizing denitrification for wastewater treatment include: (1) high nitrate removal efficiencies, (2) elemental sulfur, which is a by-product of oil processing, is less expensive than ethanol or methanol, (3) little or no external carbon source is required, minimizing the possibility of carry-over of excess organic carbon into the effluent, (4) sulfur oxidizing denitrification can take place under aerobic conditions (Yamamoto-Ikemoto et al., 2000), although more biomass is produced, this eliminates the need to deoxygenate the influent wastewater, (5) due to lower biomass yields for autotrophic bacteria, less sludge is produced and (6) autotrophic sulfur oxidizing denitrifying bacteria produce less N$_2$O (a greenhouse gas) than heterotrophic denitrifying bacteria (Park et al., 2002).

A salient feature of sulfur oxidizing denitrification is the alkalinity requirement. Since the denitrification rate is severely inhibited below pH 5.5 (Liu and Koenig, 2002), sufficient alkalinity must be provided. In addition, nitrite (NO$_2^-$) has been shown to accumulate at low pH (Furumai et al., 1996). One approach to overcome the alkalinity requirements for sulfur-oxidizing denitrification is the sulfur: limestone autotrophic denitrification (SLAD) process. The SLAD process uses fixed bed upflow anaerobic reactors packed with elemental sulfur and limestone granules as an alkalinity source (Zhang and Lampe, 1999; Koenig and Liu, 2002). Complete denitrification has been observed in SLAD systems, treating nitrified landfill leachate with NO$_3^-$-N concentrations up to 700 mg/L (Kim and Bae, 2000). Studies of the use of SLAD for domestic wastewater are more limited. Kuai and Verstraete (1999) achieved 75-86% total N removal from nitrified domestic wastewater. Both single household and multiple household wastewaters were tested. Effluent total N concentrations were stable at below 0-2 mg/L despite wide fluctuations in influent concentrations. Pathogen and suspended solids removal was observed, however, no P removal was observed. Advantages of the system included small footprint (2-3 hour HRT) and low operational costs. Backwashing was required every 6-8 weeks due to accumulation of biomass and precipitated CaSO$_4$. Solids accumulation and short-circuiting has also been observed in systems treating contaminated drinking water and other wastewaters (Lee et al., 2001; Flere and Zhang, 1999).

Although limestone has been studied in the SLAD process, other agricultural liming materials include (Tisdale and Nelson, 1975) slaked lime (Ca(OH)$_2$), unslaked lime (CaO), limestone (CaCO$_3$), crushed oyster shell (CaCO$_3$), dolomite (CaMg(CO$_3$)$_2$), marl (earth and CaCO$_3$) and slags (CaSiO$_3$). The reaction of all liming materials involves the neutralization of H$^+$ ions by OH$^-$ or SiO$_2$(OH)$_2^{2-}$ ions furnished by the buffer. Differences in neutralizing capacity and speed of reaction of these materials depend on the molecular composition, purity and particle size.
Hydrogenotrophic Denitrification

A number of common soil bacteria are able to use H₂ as an electron donor and inorganic carbon for biosynthesis. Some of these species can respire on NO₃⁻ in the absence of oxygen. A stoichiometric equation for autotrophic denitrification using H₂ as an electron donor is (Ergas and Reuss, 2001):

\[
\text{H}_2 + 0.35 \text{NO}_3^- + 0.35 \text{H}^+ + 0.052 \text{CO}_2 \rightarrow 0.17 \text{N}_2 + 1.1 \text{H}_2\text{O} + 0.010 \text{C}_5\text{H}_7\text{O}_2\text{N} \quad (3)
\]

Based on this equation, for each gram of NO₃⁻-N removed, approximately 0.24 g cells, are generated, which is considerably lower than the 0.6 to 0.9 g cells/g NO₃⁻-N typically reported for heterotrophic systems. A number of studies (Gross and Treutler, 1986; Kurt et al., 1987; Dries et al., 1988; Smith and Duff, 1988; Liessens et al., 1992) have shown that hydrogenotrophic bacteria can denitrify contaminated drinking water to acceptable levels; however, little research has been conducted on hydrogenotrophic denitrification of wastewater. Advantages of hydrogenotrophic denitrification over heterotrophic denitrification include: 1) low sludge production, 2) elimination of carryover of added organic carbon to the effluent, 3) the relatively low solubility of H₂ makes it easy to remove it from the effluent by air stripping and 4) the low cost of H₂. A major disadvantage of using H₂ for denitrification is the low solubility of H₂ which makes it difficult to supply enough H₂ to the biomass for denitrification. To overcome this limitation, hollow fiber membrane bioreactors (HFMB) were used to supply H₂ to a denitrifying biofilm growing on the microporous hydrophobic hollow fiber membranes. The membranes increase the mass transfer rate of H₂ to the biomass and serve as a support for the denitrifying population.

This project investigated the use of both S⁰ and H₂ in autotrophic denitrification systems. The target audience and beneficiaries of the project results would be coastal managers and small-flow originators (residences, small businesses, nursing homes, restaurants, etc.).

Objectives

The overall goal of the project was to investigate the applicability of autotrophic biological denitrification using either S⁰ or H₂ as an electron donor. Specific objectives included:

1. Evaluate the feasibility of biological denitrification in decentralized wastewater treatment systems using elemental sulfur or hydrogen as the electron donor.
2. Evaluate the use of various solid-phase alkalinity sources to improve the stability of sulfur-oxidizing denitrification systems.
3. Evaluate the effect of addition of small amounts of domestic wastewater on denitrification rates, cell yields and system stability in autotrophic denitrification systems (both H₂ and S⁰).
4. Evaluate the effect of dissolved oxygen on denitrification rates and cell yields in autotrophic denitrification systems (both H₂ and S⁰).
5. Evaluate the effect of Empty Bed Contact Time (EBCT) and influent nitrate-nitrogen concentration on process performance.

**Methods**

*Bench-Scale Studies with Sulfur Oxidizing Denitrification*

Two cylindrical reactors for autotrophic denitrification were constructed from acrylic tubing with a volume of 1 liter. Each column reactor had an inside diameter of 61 mm and a height of 343 mm. Each column had 4 sampling ports separated every 76 mm along the length of the column. At the bottom of each column were the influent and backwash influent ports. An effluent port was located on the top of the column. See Figure 1 for details. The columns were seeded with sludge from the Lakeville School, Dartmouth, MA (denitrification using methanol) and fed with a synthetic wastewater containing between 40 and 100 mg NO₃⁻ – N/L using an adjustable peristaltic pump. Concentrations of NO₃⁻ – N, PO₄³⁻ – P, SO₄²⁻ as well as alkalinity and pH in the influent and the effluent were normally monitored every 48-72 hours. Both columns were initially packed with sulfur and marble chips in layers at a 3:1 ratio (by volume). After 43 days of operation, one column was packed with a mixture of sulfur granules, marble chips and crushed limestone and the second column was packed with a mixture of sulfur granules, marble chips and crushed oyster shell. The column packed with sulfur/marble/limestone was operated for 83 days, while the column packed with sulfur/marble/oyster shell was operated for 97 days.
**Figure 1:** Bench-Scale Autotrophic Denitrification Columns with S$^0$ Oxidation

*Titration tests on solid-phase buffer*

Kinetics of dissolution of the two solid-phase buffers (marble chips and crushed oyster shells) and their buffering rates were investigated in laboratory-scale tests. Two identical stirred reactors, each containing a buffer (with a mass such that it could buffer the addition of an acid for many cycles) and a synthetic solution, were employed. An external source of H$^+$ was added to each reactor in a cycle. The equivalents of H$^+$ added corresponded to the alkalinity that would be destroyed on a stoichiometric basis in one 24-hour time period (after normalization of volumes) by a wastewater entering the field-scale bioreactors at a flowrate of 330 gpd and containing 40 mg/L NO$_3^-$ - N. The pH profile as a function of time after addition of H$^+$ was created. When the pH value stabilized, another cycle of H$^+$ was added; a total of 5 cycles of H$^+$ addition were conducted.

*Bench-Scale Studies with Hydrogenotrophic Denitrification*

(a) Enrichment of Hydrogenotrophic Denitrifying Bacteria

Denitrifying bacteria were cultured from wastewater obtained from the Berkshire mall (Pittsfield, MA) and Belchertown, MA wastewater treatment plants. Batch cultures were set up in 1000 mL Erlenmeyer flask, sealed to ensure anoxic
conditions, covered with aluminum foil and placed on a shaker table at 150 rpm. Each flask contained 250 mL of the supernatant from the sludge and 250 mL of synthetic water. Groundwater from a nearby farm in Amherst, MA was used to prepare synthetic water (0.416 µg/L NO$_2^-$ - N and 0.088 mg/L NO$_3^-$ - N). The synthetic water contained 100 mg/L NO$_3^-$ - N, 0.5 g/L NaHCO$_3$, and 1 mL of each of nutrient stock solutions shown in Table 2. The flasks were flushed with hydrogen gas to remove oxygen present in the headspace. Excess hydrogen was collected in a 1 L tedler bag (SKC) attached to the flask headspace. To maintain a pH range of 7 to 8, carbon dioxide gas was sparged through the media when required.

**TABLE 2. Composition of Nutrient Stock Solution**

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Chemical Formula</th>
<th>Concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate Buffer</td>
<td>KH$_2$PO$_4$</td>
<td>8.5 g/L</td>
</tr>
<tr>
<td></td>
<td>K$_2$HPO$_4$</td>
<td>21.75 g/L</td>
</tr>
<tr>
<td></td>
<td>Na$_2$HPO$_4$.7H$_2$O</td>
<td>33.4 g/L</td>
</tr>
<tr>
<td>Other Nutrients</td>
<td>MgSO$_4$.7H$_2$O</td>
<td>22.5 g/L</td>
</tr>
<tr>
<td></td>
<td>FeCl$_3$.6H$_2$O</td>
<td>0.25 g/L</td>
</tr>
<tr>
<td></td>
<td>CaCl$_2$</td>
<td>27.5 g/L</td>
</tr>
</tbody>
</table>

*The nutrient solutions were prepared in Super Q water.

(b) Hydrogenotrophic HFMB Design and Construction

The membrane module was constructed from a 4 cm diameter, 56 cm length, 750 ml glass cylinder with anodized aluminum end plates and Viton O-rings. Laminated hollow fiber membranes with a thin dense polyurethane layer sandwiched between two microporous polyolefin layers were used for H$_2$ delivery to the biofilm (Mitsubishi MHF200TL). The hollow fibers were manufactured into bundles by Porous Media Inc. (St. Paul MN). Membrane fibers were potted in a flow-through configuration. Each bundle contained 200 fibers with an active length of 50 cm. The inner diameter was 200 µm, the outer diameter was 284 µm, the porosity was 42% and the total membrane surface area was 892 cm$^2$. Synthetic water was supplied to the membrane module from a glass reservoir using a Masterflex C/L 77120-40 peristaltic pump. The glass reservoir was sparged with N$_2$ gas when filled to maintain anoxic conditions. The bioreactor contents were recirculated using a Masterflex (Vernon Hills IL) 7553-70 peristaltic pump. A Parker (Jacksonville Al) pressure-regulating valve was used at the liquid outlet to maintain the liquid pressure above the bubble point. H$_2$ and CO$_2$ were supplied to the lumen of the fibers from gas cylinders (Merriam Graves). A gas flow meter (Aaborg 0-10 mL/min), needle valve, and solenoid valve (Powers) controlled the gas composition and flow rate.
(c) Confocal Microscopy
Biofilms attached on the hollow fiber membranes were observed using an LSM-510 Meta Confocal System equipped with X10 multi-immersion lenses (Zeiss, Jena, Germany). Two fluorescent probes were used to investigate specific chemical compounds. Lectin-FITC from *Canavalia ensiformis* (Sigma-Aldrich) was used to stain $\alpha$-D-Mannose and $\alpha$-D-Glucose of polysaccharides in EPS. SyproOrange (Invitrogen, Molecular Probes, Eugene, OR) was used to stain proteins in extracellular polymeric substances (EPS).

Lectin staining was conducted at the concentration of 100 $\mu$g lectin/cm$^2$ membrane surface area as described in Lawrence et al (2003). The stained membrane was incubated for 20 minutes at room temperature in humidity chamber, and carefully rinsed with phosphate buffer solution. The SyproOrange stock solution was prepared by 5000 times dilution with 7.5% (v/v) acetic acid solution (Invitrogen, Molecular Probes, Eugene, OR), and then 1 cm$^2$ of the membrane was stained with 100 $\mu$l of the stock solution. The membrane was incubated at room temperature in dark chamber for 40 minutes, and carefully rinsed with 7.5% (v/v) acetic acid.

Field-Scale Studies with Sulfur Oxidizing Denitrification
Two field-scale units were assembled and placed on the Massachusetts Alternative Septic System Test Center (MASSTC) site at the Massachusetts Military Reserve in Barnstable, MA. The units were located inside an insulated shed to prevent the tanks from freezing during the winter. The two units were identical; a 50-gallon lidded rectangular polyethylene tank designed with spin-weld fittings to ease installation and minimize the possibility of leakage. The tank’s dimensions were 24” X 18” X 30” tall. Influent and effluent lines were constructed from 2” PVC pipe as recommended by MASSTC. The tanks were fitted with sample ports at 1” increments along the height of the reactors so that the progress of the reactions within the tank could be closely monitored. Figure 2 has the details of the tank. In both reactors 50 cm of the height was packed initially with layers of sulfur pellets and marble chips at a 3:1 ratio by volume. Both bioreactors were seeded with sludge from the Lakeville School in Dartmouth, MA and fed with nitrified wastewater from a recirculating sand filter at the Test Center located upstream of the project bioreactors. After 8 months, one of the units was emptied and filled with a fresh batch of sulfur pellets and crushed oyster shell as a solid-phase buffer at the same 3:1 ratio by volume. The new bioreactor was seeded with sludge from the old marble-chip bioreactor that was replaced. Both the reactors were operated under the transient flow conditions as specified in the National Sanitation Foundation (NSF 40) protocol. Both bioreactors were monitored for pH, Total Alkalinity, Nitrate-Nitrogen, Nitrite-Nitrogen, Sulfate, Chemical Oxygen Demand, Biochemical Oxygen Demand, and Total Kjeldahl Nitrogen.
Influent and effluent samples were tested for NO$_3^-$ – N, PO$_4^{3-}$ – P, SO$_4^{2-}$ – S, and NO$_2^-$ – N by ion chromatography (LC20 DIONEX Inc) using a DIONEX Ion Pac AS14A Anion Exchange column. Total Organic Carbon (TOC) was measured with a Shimadzu TOC-5000A Total Organic Carbon Analyzer. Chemical Oxygen Demand (COD), TALK, pH, DO, BOD$_5$ TKN and turbidity were measured according to Standard Methods (APHA, 1995). Gases were analyzed for N$_2$, H$_2$ and CO$_2$ using a HP 5890A gas chromatograph (GC) with thermal conductivity as detector. A Haysep Q 80/100, 1/8 inch stainless steel packed column (Alltech), 6 feet in length, was used with helium as the carrier and reference gas. The GC was operated at high sensitivity (2.2 mV) with a gas flowrate of 35 mL/min. The injection and detection temperatures were set at 120$^\circ$ C. The peaks were analyzed with Spectra Physics SP4290 integrator with an attenuation of 250 and chart speed of 0.25 cm/s. The gases were injected with 100 $\mu$L Hamilton sample lock syringe. Hydrogen and carbon dioxide gases were calibrated with a range of 0-10% in helium and a 2% mixture of each of the above gases were used to experimentally determine the method detection level.
**Results**

*Bench-Scale Studies with Sulfur Oxidizing Denitrification*

Results of the sulfur oxidizing denitrifying column tests are shown in Figures 3 & 4. The following is a summary of the results of these tests:

♦ Although denitrification was observed in the upflow sulfur-marble chip packed-bed reactors from the start of the experiment, an acclimation period with a gradual improvement of denitrification was observed over the first month.

♦ Denitrification rates, effluent pH and alkalinity were significantly higher with the use of crushed oyster shell, rather than limestone or marble chips. Average nitrate removal was 80% in the oyster shell column compared with 53% in the limestone column.

♦ Significant nitrite accumulation (up to 18 mg/L) was observed in the column utilizing limestone, while the oyster shell column had effluent nitrite concentrations below 2 ppm.

♦ Influent DO significantly decreased denitrification in the limestone column but did not appear to inhibit the oyster shell column.

♦ Backwashing did not appear to improve performance in the limestone based column.

♦ TOC levels similar to that of nitrified wastewater effluents did not appear to improve the effluent alkalinity in the oyster shell based column.
Figure 3: Bench-Scale Bioreactor Performance – Limestone and Sulfur
Results of the titration tests on marble chips and crushed oyster shells are shown in Figure 5. It can be noted from Figure 5 that:

- The drop in pH upon addition of $H^+$ is sharper for marble chips than for crushed oyster shells.
- The crushed oyster shell system rebounds from the pH drop faster and to a final pH value closer to the original value compared with the marble chip system.

These two observations clearly demonstrate that crushed oyster shells are better suited to act as a solid-phase buffer compared to marble chips in denitrification systems using sulfur as electron donor.
Denitrifying cultures were obtained from Berkshire Mall wastewater treatment plant (Lanesboro, MA). Synthetic feed water for the cultures was prepared using non-chlorinated groundwater. Groundwater is expected to contain trace elements, such as nickel (Ni$^{2+}$) and selenium (Se$^{2+}$), which are needed for growth of microorganisms. Nickel is essential for growth of hydrogenotrophic bacteria because all hydrogenase (enzymes for growth) contain Ni$^{2+}$ as their cofactors (Madigan et al., 2003). Also, hydrogen-oxidizing bacteria need mineral salts such as Fe$^{2+}$.

Methanol was initially added to the cultures to verify that there was an active denitrifying population present, since the cultures obtained from the wastewater treatment plant that used methanol as an electron donor. On observing denitrification, the conditions were shifted to H$_2$ as the electron donor. Acclimatization to the new conditions occurred over days 15-50 (Figure 6). On day 60, regular sparging of the flasks with CO$_2$ was initiated to control pH, resulting in increased denitrification rates. Stable denitrification was observed in the flasks for approximately 1 year.
Figure 6: Batch denitrification cultures enriched from Berkshire Mall sludge.

A first order denitrification rate with a kinetic coefficient of 0.028 hr\(^{-1}\) was observed in this study (Figure 7). A comparison between published denitrification rates using different substrates are shown in Table 3 (Rezania et al., 2004, Haugen et al., 2002).

Table 3: Denitrification rates using different substrates

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Biomass concentration g/L</th>
<th>Temperature °C</th>
<th>PH</th>
<th>K hr(^{-1})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1.2-3.1</td>
<td>25</td>
<td>6.8</td>
<td>0.018-0.025</td>
<td>Foglar et al., 2005</td>
</tr>
<tr>
<td>Thiosulfate</td>
<td>Not given</td>
<td>33-25</td>
<td>6.5-7.5</td>
<td>0.3 – 0.4</td>
<td>Oh et al., 1989</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.19-6.61</td>
<td>25</td>
<td>7-8</td>
<td>0.006-0.008</td>
<td>Koeing and Liu et al., 2004</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.5</td>
<td>25</td>
<td>7.5-9.5</td>
<td>0.024-0.071</td>
<td>Rezania et al., 2004</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.5</td>
<td>12</td>
<td>7.5-9.5</td>
<td>0.010-0.021</td>
<td>Rezania et al., 2004</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.104</td>
<td>20</td>
<td>7.0</td>
<td>0.028</td>
<td>Current study</td>
</tr>
</tbody>
</table>
Mass Transfer Tests

Abiotic mass transfer tests were performed on both membrane configurations. The solubility of H\textsubscript{2} in water is 1.7 g/m\textsuperscript{3} at 1 atm and 10\textdegree{}C (Sell et al., 1993). To avoid the formation of gas bubbles on the liquid side of the membrane, the saturation concentration of the gas in water should not exceed this value (Sell et al., 1993). During the abiotic operation of the membrane modules, formation of gas bubbles was controlled by adjusting the inlet gas pressure and the liquid side pressure with the pressure control valve.

Abiotic mass transfer tests were carried under pseudo-steady state conditions at recirculation liquid flow rates that yielded three different Reynolds numbers, 18.2, 37.8 and 67.1. The influent gas flow rate was maintained at a Reynolds number of 37.9. The overall mass transfer coefficients were calculated and are shown in Figure 8.

\textbf{Figure 7:} Batch kinetics for Berkshire Mall sludge using hydrogen as an electron donor
Bioreactor Results

Influent and effluent NO$_3$-N concentrations over time for the HFMB are shown in Figure 9. The HFMB was initially operated with a 12.6 hour HRT and a shell side recirculation rate of 286 mL/min. The influent NO$_3$-N concentration was maintained at approximately 50 mg NO$_3$-N /L while H$_2$ was supplied to the lumen of the membrane in excess of the stoichiometric requirements (>10 mL/min). The acclimation time for complete denitrification was approximately 10 days. Effluent nitrite concentrations increased during this period from 3 to 33 mg NO$_2$-N /L but decreased on day 10 to 0.2 mg NO$_2$-N /L, as shown in Figure 10. This transient increase in NO$_2$-N concentration has been observed in other studies (Ergas and Reuss, 2001) and has been attributed to the need for organic carbon for nitrite reduction, which is provided either by decaying cells or soluble organic matter from the densely populated biofilm (Liessens et al., 1992). Between days 4 and 41 effluent NO$_3$-N and NO$_2$-N concentrations were less than 5 and 0.2 mg/L, respectively. The World Health Organization (WHO) has set a safe limit of 11 mg NO$_3$-N /L, although the toxicity of NO$_3$-N is not clearly established (Mateju et al., 1992). U.S. drinking water standards for NO$_2$-N are set at 1 mg/L (EPA, 2002).

One of the tasks of this study was to operate the bioreactor at minimum HRT for economic efficiency. Complete denitrification was observed at a HRT of 12.6 hour between days 4 and 32. Both NO$_3$-N and NO$_2$-N concentrations were within safe drinking water limits (EPA, 2002). On day 33, the HRT was decreased to 10 hours and a small accumulation of NO$_2$-N of 5 mg/L was observed for 2 days. On further reduction of the HRT to 5.7 hours, the effluent NO$_3$-N concentration increased to 26 mg NO$_3$-N /L and a gradual increase in NO$_2$-N concentration was also observed. Since nitrite inhibits the denitrification process (Haugen et al., 2002), the reactor was flushed with nitrate free influent for one day and the HRT was increased to 6.5 hours. An optimum HRT of 8.7 hours was established after day 60.
Between days 66 and 80, an increase in effluent nitrite concentration was observed (Figure 10), accompanied by a decrease in effluent pH to 7.47. In previous studies (Lee and Rittmann, 2003; Haugen et al., 2002), nitrite accumulation was shown to be a sensitive indicator of process stress due to high pH. This is contrary to what was seen in this study where nitrite accumulation was observed at both low and high pH. In the batch cultures, nitrite accumulation was observed at a pH of 9.5, therefore for further experiments a target pH of approximately 9.0 was determined. On day 80, 0.5 g/L of sodium bicarbonate was added to the influent feed water and periodic sparging of carbon dioxide to the HFMB was stopped to more consistently control pH in the system. After the addition of bicarbonate to the feed water, the effluent pH stabilized between 8.6 and 9.3 and the nitrite concentration decreased to 0.5 mg NO$_2^-$-N/L.

Figure 9: Influent and effluent nitrate-nitrogen concentrations in Configuration I HFMB. Changes in HRT for the system is shown at the top of the graph
One of the objectives of this research was to test the performance of the system when dissolved oxygen (DO) was present in the feed water. On day 107, sparging of the influent feed with nitrogen gas was discontinued. When DO was allowed to enter the reactor the effluent nitrate concentration initially increased to 12 to 14 mg/L NO₃⁻-N and then gradually decreased to below 10 mg/L NO₃⁻-N. The NO₂⁻-N concentration remained at approximately 1 mg/L. It is known that denitrification rates decrease with increases in dissolved oxygen concentrations and the process ceases with oxygen concentrations greater than or equal to 1.0 mg/L (Whitmyer et al., 1991, Metcalf and Eddy, 2004), however, in this study denitrification was observed in the presence of dissolved oxygen in the feed water. In the presence of oxygen, denitrifying bacteria prefer oxygen as an electron acceptor over nitrate (Madigan et al, 2003). Many hydrogenotrophic (hydrogen oxidizing bacteria) grow best microaerobically and are most successful in oxic/anoxic interfaces. Typically, oxygen levels of about 5-10% of saturation have been shown to support the best growth for hydrogen bacteria (Madigan et al, 2003). Table 4 summarizes the nitrate removal rates in other studies.

**Figure 10:** Effluent nitrite-nitrogen concentration in Configuration I HFMB
Table 4: Nitrate removal rates using various electron donors

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Denitrification rate per unit surface area (g NO₃⁻-N/m² d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.240</td>
<td>Liessens et al., 1992</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.0</td>
<td>Mansell and Schroeder, 2002</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>2.7-3.5</td>
<td>Mansell and Schroeder, 2002</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.312</td>
<td>Kurt et al., 1987</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.792</td>
<td>Islam et al., 1994</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>2.0</td>
<td>Gantzer, 1995</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>1.392</td>
<td>Benedict et al., 1998</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.01</td>
<td>Lee and Rittmann, 2003</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>2.2</td>
<td>Ergas and Reuss, 2001</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.4</td>
<td>Current study (between days 100-125)</td>
</tr>
</tbody>
</table>

The system was further evaluated using real wastewater. The flow rate for the HFMB was modified from the previous setting to mimic a typical onsite wastewater flow system (Table 5). Composite samples of non-chlorinated wastewater effluent were obtained from the Amherst, MA wastewater treatment plant. The facility uses an activated sludge system without denitrification to treat their wastewater. Since the nitrate concentrations in the treated effluent were less than 6 mg/L NO₃⁻-N, the wastewater was spiked with 50 mg/L NO₃⁻-N. Typical Chemical Oxygen Demand (COD) levels and pH of the wastewater were 35 mg/L and 6.25, respectively.

Table 5: Percentage flow distribution adopted from typical onsite systems for HFMB

<table>
<thead>
<tr>
<th>Clock time in hrs</th>
<th>Percentage of total influent flow to the HFMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 hrs to 11:00 hrs</td>
<td>40</td>
</tr>
<tr>
<td>11:00 hrs to 13:00 hrs</td>
<td>No flow</td>
</tr>
<tr>
<td>13:00 hrs to 16:00 hrs</td>
<td>20</td>
</tr>
<tr>
<td>16:00 hrs to 19:00 hrs</td>
<td>No flow</td>
</tr>
<tr>
<td>19:00 hrs to 22:00 hrs</td>
<td>40</td>
</tr>
<tr>
<td>22:00 hrs to 8:00 hrs</td>
<td>No flow</td>
</tr>
</tbody>
</table>

The effluent concentrations were monitored for pH, COD, NO₃⁻-N, NO₂⁻-N and turbidity over a period of 24 hours (Figure 11). Overall nitrate mass percent removal was between 74-82 %. Due to varying nitrate loadings over a period of 24 hours, the effluent nitrate concentrations were higher than 10 mg/L NO₃⁻-N during latter part of the day (16:00 hrs). This was attributed to high nitrate loading and less time for denitrification. The system
showed signs of stabilization when left with no flow overnight (22:00 hrs to 8:00 hrs), i.e. effluent NO$_3^-$-N concentration at 8:00 hrs was less than 10 mg/L NO$_3^-$-N. Increase in effluent COD, which was observed during certain times of the day, could be attributed to a release of soluble microbial by-products (SMP). In Figure 11 a higher effluent COD is observed at 1 pm at which time the flow was initiated. SMP might have accumulated over period of time which was then released. This phenomenon was also observed in a study by Mo et al., 2005, which studied hydrogenotrophic denitrification using a suspended growth membrane bioreactor. An average effluent COD of 14 mg/L was observed by the authors.

![Figure 11: Influent and effluent COD and NO$_3^-$-N concentrations for Configuration I HFMB using real wastewater spiked to 50 mg/L NO$_3^-$-N](image)

There is a need for documentation on onsite nitrogen removal system performances (Whitmyer et al., 1991). Addition of real wastewater to a bench scale system using hydrogen has not been reported in literature. Also, changes in nitrate loading rates at shorter intervals, typical of that of an onsite system, has also not been reported. Table 6 gives a summary of onsite systems for nitrogen removal with their mass removal efficiency rates (Whitmyer et al., 1991).
Table 6: Typical nitrogen removal performance at onsite systems

<table>
<thead>
<tr>
<th>System</th>
<th>Mass removal efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended Aeration Package Plant</td>
<td>25-35</td>
</tr>
<tr>
<td>Aerobic/Anaerobic Trickling filter plant</td>
<td>45-50</td>
</tr>
<tr>
<td>Peat Filter</td>
<td>50-60</td>
</tr>
<tr>
<td>RUCK</td>
<td>40-60</td>
</tr>
<tr>
<td>Recirculating sand filter</td>
<td>40-70</td>
</tr>
<tr>
<td>HFMB (Current study-bench scale with onsite system protocol)</td>
<td>78-80</td>
</tr>
</tbody>
</table>

Biofilm thickness

Biofilm growth was observed along the complete length of the membrane module. The thickness was uniform because the system was equipped with a flow through membrane. Non uniformity of biofilm thickness has been reported in various studies (Lee and Rittmann, 2000, Ergas and Reuss, 2001). The membranes used in both these studies were dead end membranes.

After the system was shutdown, the membranes were removed from the system and the threads were cut and observed under the microscope for biomass thickness. Figure 12 shows the biomass accumulation on the hydrophobic membrane. The biomass developed was greater than 1mm each side of the membrane. However, confocal laser scanning microscopy (CSLM) was conducted to increase the understanding of the biofilm structure on the membrane surface (Figures 13 and 14).

![Figure 12: Biomass accumulation on the membrane under microscopy.](image)

Biofilms constitute extracellular polymeric substances (EPS), multivalent cations, biogenic and inorganic particles as well as colloidal and dissolved compounds (Lawrence et al, 2003). EPS are mainly responsible for the structural and functional integrity of biofilms and are considered as the key components for the physicochemical and
biological properties of biofilms (Madigan et al, 2003). Confocal laser scanning microscopy (CSLM) in conjunction with fluorescent chemical probes enabled examination of the three-dimensional structure of fully hydrated and intact bacterial biofilms (Wingender et al, 1999).

In this study, biofilm attached on the hollow fiber membrane surface were observed using the CSLM. The biofilm community is seen to be homogenous in nature (Figure 13) not attached to the membranes because the membranes are hydrophobic in nature. Studies have shown the biofilm attached to hydrophlic membranes (Min et al, 2005) are more dense which is not observed in the above pictures. The lectin stain reveals glycoconjugate, sugar and carbohydrates and the SyproOrgane stain reveals the proteins present in the biofilm (Figure 14).

Figure 13: Confocal Laser Scanning Microscopy photographs of hollow fiber membrane stained with Lecti-FITC, Concanavalin
Field-Scale Studies with Sulfur Oxidizing Denitrification

Composite samples of influent to the bioreactors and the effluent from each bioreactor were collected twice every week and analyzed for pH, Total Alkalinity, NO$_3^-$ - N, NO$_2^-$ - N, SO$_4^{2-}$, COD, BOD$_5$, and TKN. Figures 15 – 22 provide the profile of each of the above-listed parameters. Although clogging was not a problem in the field-scale bioreactors, a water-based backwash system was successfully tested. Three times the total volume of the reactors was passed through the reactors in a reverse direction to dislodge accumulated biomass. Results of gravimetric analysis of the backwash effluent from each field-scale bioreactor are presented in Table 7.
Figure 15: Profile of pH of Composite Sample from Both Field-Scale Bioreactors

Figure 16: Profile of Total Alkalinity of Composite Sample from Both Field-Scale Bioreactors
Figure 17: Profile of Nitrate - Nitrogen of Composite Sample from Both Field-Scale Bioreactors

Figure 18: Profile of Sulfate of Composite Sample from Both Field-Scale Bioreactors
**Figure 19:** Profile of Nitrite - Nitrogen of Composite Sample from Both Field-Scale Bioreactors

**Figure 20:** Profile of Chemical Oxygen Demand of Composite Sample from Both Field-Scale Bioreactors
**Figure 21:** Profile of Biochemical Oxygen Demand of Composite Sample from Both Field-Scale Bioreactors

**Figure 22:** Profile of Total Kjeldahl Nitrogen of Composite Sample from Both Field Scale Bioreactors
Table 7. Characteristics of Backwash Effluent from Each Field-Scale Bioreactor

<table>
<thead>
<tr>
<th>Water Quality Parameter</th>
<th>Bioreactor # 1</th>
<th>Bioreactor # 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.05</td>
<td>5.85</td>
</tr>
<tr>
<td>Total Alkalinity (mg/L as CaCO₃)</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Total Solids (mg/L)</td>
<td>414.0</td>
<td>498.0</td>
</tr>
<tr>
<td>Fixed Solids (mg/L)</td>
<td>260.0</td>
<td>318.0</td>
</tr>
<tr>
<td>Volatile Solids (mg/L)</td>
<td>154.0</td>
<td>180.0</td>
</tr>
<tr>
<td>Total Suspended Solids (mg/L)</td>
<td>103.52</td>
<td>176.38</td>
</tr>
<tr>
<td>Fixed Suspended Solids (mg/L)</td>
<td>14.12</td>
<td>54.16</td>
</tr>
<tr>
<td>Volatile Suspended Solids (mg/L)</td>
<td>89.40</td>
<td>122.12</td>
</tr>
</tbody>
</table>

Discussion

Solid-Phase Buffer

From Figure 5 it is clear that crushed oyster shell is a more suitable solid-phase buffering agent than marble chips. Additional investigations were performed to understand this phenomenon. Figures 23 & 24 provide results of Energy Dispersive X-Ray Analysis (EDX) for Marble chips and Figures 25 & 26 provide the same for crushed oyster shells.

Figure 23: EDX Analysis of Marble Chip
Figure 24:  Elemental Weight % of Constituents in Marble Chip

Figure 25:  EDX Analysis of Crushed Oyster Shell
From Figures 23-26 it is clear that marble chips constitute a high percentage of Mg(OH)$_2$ and CaCO$_3$ whereas crushed oyster shell is overwhelmingly just CaCO$_3$. At the near-neutral pH prevalent in the bioreactor (Figure 15), CaCO$_3$ (or more precisely, the HCO$_3^-$ that results from Equation 4) is a much better buffering agent than OH$^-$. 

\[
\text{CaCO}_3(s) \rightarrow \text{Ca}^{2+} + \text{CO}_3^{2-}
\]
\[
\text{CO}_3^{2-} + \text{H}^+ \rightarrow \text{HCO}_3^-
\]  

Moreover, a Scanning Electron Microscope (SEM) analysis of the two buffering materials indicated that the surface of the crushed oyster shells (Figure 27) have a much higher percentage of nanoflakes than the surface of marble chips (Figure 28). These two reasons combined result in faster and longer-lasting buffering action of crushed oyster shells compared to marble chips.
Figure 27: SEM Microphotograph (1000X) of Crushed Oyster Shell

Figure 28: SEM Microphotograph (1000X) of Marble Chip
Technology Transfer and Management Application

The following presentations were based on the results of this project:

1. 36th Mid-Atlantic Industrial & Hazardous Waste Conference at the University of Connecticut, Storrs, on October 10, 2004. This conference had about thirty participants.

2. Water Resources Research Center Conference at the University of Massachusetts, Amherst, on October 22, 2004.

3. Workshop on “Denitrification Systems: New Technologies and Alternatives” at the Waquoit Bay NERR on March 15, 2005 which was attended by approximately 50 persons.


5. Two presentations at the WEFTEC 2005 Annual Conference in Washington, DC, October 29-November 2, 2005. At the time of each presentation, the room had more than 50 attendees.


Technology Commercialization

A provisional patent application has been filed to protect intellectual property relating to this process: Sengupta, S. and Ergas, S.J. (2005) “Process for Autotrophic Denitrification Using Elemental Sulfur,” US Provisional Patent Application No. 60/753,992, filing date 12/23/05. Also, negotiations are continuing between University of Massachusetts Commercial Ventures and Intellectual Property Office and Wastewater Alternatives of New England to license this technology for commercial Title 5 systems in the New England states.

Achievement and Dissemination

Journal Publication:
Two papers have been published in the *Proceedings of the 78th Annual Meeting of the Water Environment Federation (WEFTEC 05)*, Oct. 29-Nov. 2, Washington, DC.

One paper has been published in the *Proceedings of the North American Membrane Society Annual Meeting*, Providence RI, June 11-15, 2005.

One graduate student, Ashish K. Sahu worked on this project full-time. His dissertation title is: Perchlorate and Nitrate Treatment Technologies Using Autotrophic Bacteria. Mr. Sahu expects to complete his dissertation in May, 2007.

Two graduate students, Erika Lopez-Luna and Kumaravel Palaniswamy worked on this project part-time.

One undergraduate student, Jacob Wood, worked on this project part time.
Literature Cited


