Report for 2005AZ90B: An Outdoor Multi-Stage, Continuous-Flow Photobioreactor for Bioremediation of Nitrate-Contaminated Groundwater

Publications

- Conference Proceedings:
  - Hanson, Karyn, Qiang Hu and Milton Sommerfeld (2006). Prospects for nutrient removal from dairy wastewater by microalgae. Oral presentation at the Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page11.
  - Case, Natalie, Milton Sommerfeld and Qiang Hu (2006). Utilizing microalgae to remove nitrate-contaminated groundwater. Poster presented at the Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page12.
  - Bellefeuille, Michael, Qiang Hu and Milton Sommerfeld (2006). Use of the green microalgae Scenedesmus for nitrate bioremediation, poster presented in Fourth Annual Graduate Student Research Conference (April 14, 2006), Arizona State University at the Polytechnic campus, Mesa, AZ 85212, Proceedings, Page18.
  - Bellefeuille, Michael, Qiang Hu and Milton Sommerfeld (2006). Biological nitrate removal from contaminated agriculture runoff water in an outdoor photobioreactor by the microalga Scenedesmus sp. Oral presentation at the Arizona-Nevada Academy of Science 50th Anniversary Meeting (April 7-8, 2006), University of Arizona, Tucson, Arizona, Proceedings, Page 11.
- Dissertations:
  - Bellefeuille, Mike (2005) “Use of Selected Microalgae for Environmental Bioremediation”, MS Thesis, School of Life Sciences, College of Liberal Arts and Sciences, Arizona State University, Tempe, AZ, pp. 74.
  - Case, Natalie (in progress) “Screening and characterization of high-performance microalgae for bioremediation of nitrate-contaminated waters,” Ph.D. Dissertation, Department of Applied Biological Sciences, Arizona State University, Mesa,

AZ.

- Hanson, Karyn (in progress): “Removal of Nutrients from CAFO Wastewater by Microalgae,” M.S. Thesis, School of Life Sciences, Arizona State University, Tempe, AZ.

Report Follows

A. Problem and Research Objectives

Groundwater contributes more than 40% of the Arizona's drinking water supply, making it a precious, yet vulnerable resource critical to Arizonans' health, and to the State's and region's economic prosperity. Nitrate contamination in groundwater has been identified by several federal agencies (e.g., USEPA, USDA) as one of the most widespread and severe environmental problems in the state of Arizona and many other parts of the country. In Arizona, over 10 percent of the groundwater wells tested (more than 1,000 wells) have been reported to exceed the maximum recommended concentration of 10 milligrams per liter (mg L^{-1}) of nitrate as nitrogen (NO^{-3}-N) in drinking water (Pontius 1993). This is equivalent to 45 mg L^{-1} of nitrate (NO^{-3}). The major groundwater pollutant sources include agricultural activities, industrial waste, leaking underground storage tanks, septic tanks, landfills, mining and wastewater treatment plants. Many of the groundwater quality problems are located in the Phoenix and Tucson metropolitan areas, although groundwater quality problems are found in all of Arizona's 10 watersheds. Particularly large portions of the aquifers within the Salt River Valley, including areas of Glendale, Mesa, Chandler and Phoenix, contain groundwater with nitrate concentrations high enough to render the water unfit for potable use. In addition, high nitrate levels occur in Marana, St. David, Quartzsite, Bullhead City, Lake Havasu City and other areas. Animal feeding operations and septic tank discharges are common nitrate sources in rural areas of Arizona and have contaminated many drinking water wells. Quartzsite, Bullhead City and Lake Havasu City are just a few locations with documented nitrate problems from septic tanks (*ADEQ's FY '02 Groundwater Assessment*, http://www.azdeq.gov).

High concentrations of nitrate in groundwater can pose a serious health risk to the State's residents, particularly in places where residents rely on groundwater supplies for drinking water. It has been shown that high levels of nitrate can be fatal to infants when nitrate is reduced to nitrite in the stomach, and the latter combines with hemoglobin in the blood to form methemoglobinanemia, leading to a condition known as "blue baby syndrome" (Gangolli et al. 1994). Reduction of nitrate to nitrite can also represent a risk to adults deficient in glucose-phosphate dehydrogenase (Pontius 1993). Moreover, nitrite can react with secondary amines or amides in water or food to form *N*-nitroso compounds that are potential animal carcinogens (Gangolli et al. 1994). Long-term consumption of drinking water containing nitrate concentrations of $\geq$ 18 mg L^{-1} has also been reported to contribute to the risk of non-Hodgkin's lymphoma (Ward et al. 1996).

Shortage of surface water supplies, especially due to the continuing drought across the State in the recent years, coupled with rapid increase in population, has already placed heavy pressure on Arizona's cities and water supply utilities to treat available groundwater. To convert nitrate-contaminated groundwater into acceptable human drinking water resources, a number of treatment options have been proposed or tested. These options include microbial-based nitrification and denitrification, and chemically and physically-based technologies, such as ion exchange, reverse osmosis, electrodialysis and catalytic denitrification (Kapoor and Viraraghavan 1997). However, these treatment processes are often difficult to accomplish on a large scale and very expensive. They require not only a large capital investment, but also have high operation and maintenance

costs. Additionally, input of chemical additives (e.g., organic carbon sources, salts, acids, or base solutions) generates concentrated waste-streams that then must be treated and properly disposed. High costs have prevented many cities, especially those small communities in remote areas from adopting these treatment strategies to treat their groundwater. Therefore, development of innovative, environmentally-friendly, and cost-effective sustainable technologies for treating nitrate-contaminated groundwater is becoming increasingly critical.

A novel photobioreactor-based algal biotechnology has been proposed by the ASU investigators for high efficient, sustainable removal of nitrate and possibly other contaminants from groundwater, while concomitantly producing renewable biomass. Two critical challenges to this concept were identified: 1) finding high performing algal species that can thrive in groundwater and take up nitrate at rates substantially higher than those previously reported; and 2) developing a large-scale photobioreactor to accelerate the biological process in a sustainable manner. In a previous Water Resources Research Center research grant (Grant No. 01-HO-GR-0113), the first critical challenge has been successfully met. As a result, four high-performance algal species have been isolated and evaluated in terms of nitrate removal rate. It was demonstrated that one of the species, a *Scenedesmus* strain, can remove 50 mg L^{-1} nitrate as nitrogen from groundwater within 24 hours, a record high rate set for algae-based nitrate removal potential. In this grant research (Grant Number: 01-HQ-GR-0113), we have focused on the second challenge, i.e., development of a highly efficient, cost-effective photobioreactor. A Multiple-stage, Continuous-Flow Photobioreactor (MCP) has been designed, fabricated, and operated under outdoor conditions. The high-performance *Scenedesmus* strain was used as a model organism to evaluate the performance of microalgae in the MCP. Future R&D issues about the system scale-up, automation, and optimization are also discussed.

B. Methodology

Photobioreactor: A prototype MCP system consisted of 6 flat-plate bioreactor units arranged in a linear fashion and located at a series of heights with one end bioreactor unit being at the highest position, whereas the other end bioreactor unit being at the lowest position. Individual bioreactor units were made of glass measuring ca. 210 cm long, 50 cm height, and 15 cm depth, and having a volume capacity of about 150 liters. The total culture volume capacity of the MCP prototype was slightly over 900 liters. Culture mixing was provided by a compressed air stream containing 0.5-1.0% CO_2 through tubing submerged at the bottom of the tank. All 6 bioreactor units were connected by piping, through which the culture suspension can overflow or cascade down from one reactor unit to another by gravity. An evaporative cooling system was installed in the MCP and cooling water was collected and reused.

Organism: The high-performance *Scenedesmus* strain was used to evaluate the performance of the prototype MCP. *Scenedesmus* cells were grown in the lab-scale bioreactor to generate sufficient inoculum. Then, the *Scenedesmus* cells were transferred

into the outdoor MCP system to pre-culture for up to 2 to 3 days to allow adaptation of the algal cells to the natural conditions before starting trials.

Growth measurement: Algal growth was measured using optical density. Optical density of the culture was measured with a UV-Vis spectrophotometer at a wavelength of 750 nm.

Nutrient analysis: NO_3^- measurement wwas performed on a Bran-Luebbe TrAAcs 800 Autoanalyzer, a continuous flow wet chemistry autoanalyzer using the cadmium reduction method (APHA, #4-89). The instrument was operated according to the standard operating procedure provided by the manufacturer. The standards and reagents were prepared fresh the day of analysis. The standards were made from a 100 ppm concentration of sodium nitrate ranging from 0.01, 0.02, 0.05, 0.2, 0.8, 2.0, and 5.0 ppm. After every six samples, the blank and drift were measured.

Nitrate uptake rate: Cellular nitrate uptake rate of individual algal species was calculated using the following equation:

Nitrate uptake rate (mg N L^{-1} h^{-1}) = $(LnN_2 – LnN_1)/(t_2 – t_1)$
Where t_1 and t_2 represent different time points, and N_1 and N_2 represent nitrate concentration in the growth medium at time t_1 and time t_2, respectively.

C. Principal Findings and Significance

Fabrication and Installation of the Prototype Photobioreactor Module

***Overview*:** The prototype MCP module is shown in **Figure 1**. The MCP module consisted of 6 separate, identical culture tanks. The volume capacity of the MCP module was 900 liters. The module is supported on a welded steel frame, which rests on 9 small concrete pads. Six glass tanks were set on the frame in stair-step fashion to allow full solar illumination. The tanks were each aerated continuously and CO_2-rich air was fed into the aeration system from a CO_2 cylinder during daylight hours. Evaporative cooling was provided by spraying water on the front surface of the tanks. Culture flowed from the top tank through intermediate tanks to the lowest tank, where it was harvested. Essentially nitrate-free water from the last reactor unit will be subjected to a separation process where algal biomass will be removed from the water by means of centrifugation, sand/membrane filtration or dissolved air flotation. The purified groundwater can then be discharged into rivers, canals or delivered into the conventional water treatment plant for regular water treatment. While a small portion of harvested algal cells can be used as inoculum for the individual reactor units, bulk quantities of algal biomass generated from the process will be subjected to downstream processing to obtain wet/dry algal biomass for various potential applications.

Figure 1. an outdoor multi-stage, continuous-flow photobioreactor (MCP) for groundwater nitrate removal.

Design and construction details are presented below:

***Support Frame*:** The module was supported on a welded steel frame made of 1" square tubing, primed and painted to retard corrosion. Tube ends are sealed shut to exclude moisture (**Figure 2**). The frame is supported on 9 small concrete pads to prevent movement with wet soil or erosion (**Figure 3**). Six reactor units were set on the frame in stair-step fashion to allow full solar illumination. Reactor units had a narrow base, and each one was attached to an upright post on the frame, to prevent tipping.

Figure 2. A welded steel frame made of 1" square tubing for bioreactor support.

Figure 3. Nine small concrete pads to support the steel frame.

***Reactor tank units*:** Each tank is measuring ca. 210 cm long, 50 cm height, and 15 cm depth. Standard window glass is used, with a thickness of ¼ inch (**Figure 4**). All sides were glass, but the top was open. Glass panes were cemented together with silicone adhesive, and the glass portion was self-supporting as many aquariums are. A hole was provided in the bottom pane for plumbing. Tanks had a protective trim around the top and bottom edges, and flexible pads isolated them from the steel frame, to accommodate thermal stresses and unevenness in the frame. Clear plastic top covers were provided to reduce airborne contamination and water evaporation, while allowing additional solar radiation to enter algal culture to enhance photosynthesis (**Figure 5**). A PVC piping

system allowed water to flow from higher to lower tanks as groundwater was added, and the piping system had provision for draining the tanks when needed.

Figure 4. A glass tank.

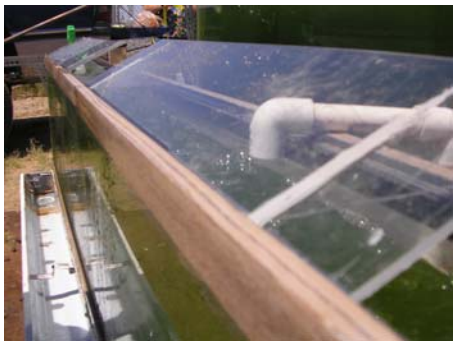

Figure 5. Clear polycarbonate sheet cover on tope of the glass tank.

***Aeration*:** Aeration was necessary to maintain algal cells in suspension for effective photosynthesis and also to facilitate cellular nutrient uptake and enhance gas mass transfer for O_2 and CO_2. Aeration was provided by a Sweetwater regenerative blower at 70 cfm. The blower was bolted to a concrete pad behind the reactor (**Figure 6**). The blower was shaded to limit overheating. The potential for overheating of the blower on hot summer days needs to be evaluated.

Figure 6. A Sweetwater regenerative blower for culture aeration.

Figure 7. A PVC manifold system conveys compressed air into individual glass tanks for aeration.

Compressed air from the blower was delivered to individual tanks through a PVC manifold system at a pressure of 1 psi. The amount of air entering the tank was controlled by a valve installed right above the tank (**Figure 7**). A Fiskar 5/8" garden soaker hose was placed at the bottom of the tank to deliver air bubbles in individual tanks. Fiskar soaker hose is used for pond aeration applications and produces medium-size bubbles. The hose weighted with steel rod encased in PVC tubing, and it was removable from the top of the tank for service. Carbon dioxide from a gas cylinder was added into the inlet of the air manifold system connected to the blower. Injection of CO_2 into the air stream at a final concentration of ca. 0.1 liter of CO_2 per liter of air per minute (vvm) took place during the daylight hours. A timer shut off the CO_2 supply at night (**Figure 8**).

Figure 8. CO_2 supply system.

***Cooling System*:** A cooling system is an important design component of an outdoor photobioreactor to prevent potential overheating of algal culture by solar radiation and to a lesser extent, heat from aerated air contributes to water heating. A low-cost, simple evaporative cooling system was used to maintain the culture temperature below 36^{o}C. Misting heads spray water on the front faces of the glass tanks, cooling the tanks as it evaporates. Water was sprayed from a distance of 25 cm in front (south) of each tank, using six standard misting heads per tank (**Figure 9**). The misting heads were mounted on ½" copper tube. Excess water running down the glass flowed into a standard rain gutter and was recycled through a 7.5 cm drain line that flowed into a 170-liter underground reservoir (**Figure10**). A sump pump in the reservoir pressurized the water for delivery to the copper tube. Periodically, water was added to the reservoir to maintain the constant water level by an automatic water leveling device. A thermostat in one tank actuated the cooling pump to feed cooling water when the temperature rises above 35^{o}C. It appears that the evaporative cooling system installed in this bioreactor was sufficient to maintain the culture temperature at an optimal temperature range of 25 to 35^{o}C during daylight hours.

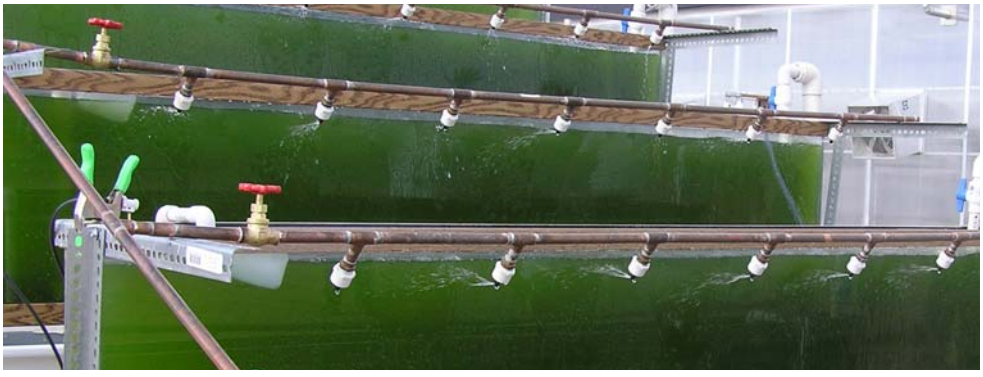

Figure 9. A evaporative cooling system for culture temperature control.

Figure 10. A underground cooling water reservoir receives the water from and delivers to glass tanks using a sump pump.

***Power*:** Electricity was required for the aeration pump, cooling pump, and control boxes. Power was provided through a standard 15-amp 1-phase 120V line.

Performance of Algal Culture in the Prototype MCP Module

Scenedesmus cells were first grown in a column photobioreactor outdoors for four days and then inoculated into the MCP module in a 1 to 10 ratio (one part seed culture added into 10 parts of groundwater). **Figure 11** shows a *Scenedesmus* culture maintained in the prototype MCP module two days after inoculum. Our preliminary results indicate that algal cells grew more rapidly in the MCP module than the column photobioreactor which was also developed by our research team (data no shown). **Figure 12** shows growth and the nitrate removal of *Scenedesmus* cells grown in the column photobioreactor in March 2006. The cell concentration in the culture, as indicated by optical density measured at a wavelength of 730 nm, increased gradually over a six-day period (**Figure 12A**). Note that the increase in cell concentration occurred during the daylight only, indicative of obligate photoautotrophic nature of the algal strain. As alga proliferated, nitrate levels in the groundwater decreased from 54 mg NO_3^--N L^{-1} to below 10 NO_3^--N L^{-1} within four days, representing a daily nitrate removal rate of ca. 10 NO_3^--N $L^{-1}d^{-1}$ (**Figure 12B**). Little nitrate removal occurred during the night, confirming that the nitrate assimilation is photosynthesis/growth-dependent. The nitrogen removal rate of 10 NO_3^--N $L^{-1}d^{-1}$ is at least 300% more efficient than other algal culture devices have reported previously (Blier et al. 1996). The higher the photosynthetic activity and growth, the higher the nitrate uptake rate. It is anticipated that the MCP module will facilitate superior algal performance and thus higher nitrate removal potential than other reactors. Quantitative evaluation of growth and nitrate uptake rate of *Scenedesmus* cells maintained in the prototype MCP module is continuing.

Figure 11. Operation of the multi-stage, continuous-flow photobioreactor for groundwater nitrate removal by *Scenedesmus* cells.

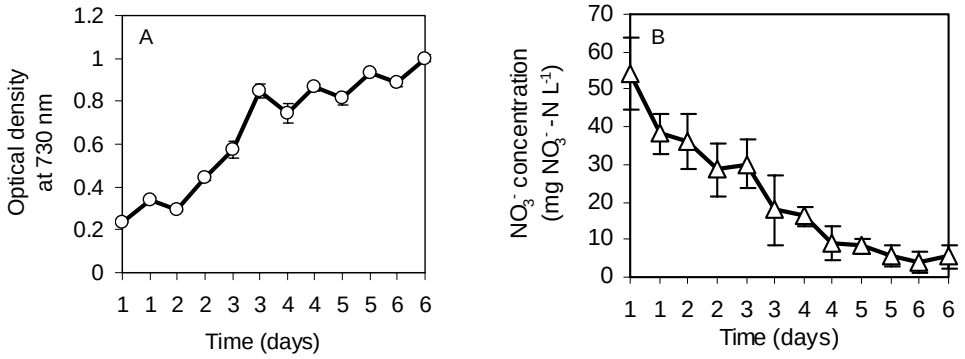

Figure 12. Growth (A) and nitrate removal (B) of *Scenedesmus* sp. grown in an outdoor column photobioreactor fed with groundwater.

Significance of the Prototype MCP Module Development

The major objective of the research project has been successfully fulfilled, and the work represents a major milestone in the effort to design, construct, and operate a commercial large-scale MCP modular photobioreactor. Continuation of this research using the prototype MCP module developed in this project and future designed large-scale MCP modular system is necessary to demonstrate that photobioreactor-based algal biotechnology has potential as an advanced engineered biological system for large-scale sustainable nitrate bioremediation.

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References

Blier et al. (1996) Production of the cyanobacterium *Phormidium bohneri* in parallel with epuration of a dairy anaerobic effluent. *Process Biochemistry*, 31: 587-596.

Gangolli, S.D., van den Brandt, P.A., Feron, V.J., Janzowsky, C., Koeman, J.H., Speijers, G.J.A., Spiegelhalder, B., Walker, R. and Wishnok, J.S. (1994) Nitrate, nitrite, and N-nitroso compounds. *Eur. J. Pharmacol. – Environ. Tox. And Pharm. Section,* 292:1-38.

Herrero, A. and Flores, E. (1997) Nitrate metabolism, p. 1-33. *In* A.K. Rai (ed.), *Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology*. Narosa Publishing House, New Delhi.

Kapoor, A. and Viraraghavan, T. 1997. Nitrate removal from drinking water - review. *J. Env. Eng.* 123:371-380.

Pontius, F.W. (1993) Nitrate and cancer: is there a link. *J. AWWA*, 85:12-14.

Ward M.H. et al. (1996) Drinking water nitrate and the risk of non-Hodgkin's Lymphoma. *Epidemiol*. 7:465.