Use of the green microalga *Scenedesmus obliquus* for bioremediation of nitrate-contaminated agricultural runoff

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INTRODUCTION:
Monitoring of groundwater quality by several government agencies has found increasing problems with high nitrate and phosphate levels in Arizona. A large portion of this contamination is a result of surface water run-off percolating into aquifers from agricultural facilities. Over 80 percent of the 8,864 wells tested in Arizona exceed the maximum recommended concentration of 10 milligrams per liter (mg/L) of nitrate-nitrogen (NO₃-N) in drinking water set by the EPA. High levels of nitrate contamination in surface and ground drinking waters can pose health and environmental risks. Removing nitrates and phosphates from run-off water before they reach the watershed will reduce contamination of water resources.

We introduce an overall biological system for bioabsorption of nitrogen and phosphorus using microalgal mass cultures. The basis for this technology is that some microalgae exhibit nutrient uptake potential 10 to 20 times higher than other plants due to efficient light and nutrient utilization. In this study we investigated a green alga, *Scenedesmus obliquus*, that exhibits an extraordinarily high nitrate uptake rate and biomass yield. Experiments in our outdoor tubular photobioreactor demonstrate the ability of our remediation process.

Materials and Methods:

**Organism**: *Scenedesmus obliquus* consists of 2 or 4 linearly or distinctly alternating arranged cells. The cells are broadly spindle-shaped, tapering to slightly extended apices. The chloroplast is parietal in the cell, with a single pyrenoid. Axial reproduction is partitioned by autospores and released by fracture of lateral cell wall. Under laboratory culture conditions, the organism often becomes unicellular with the cells gradually becoming coccoidal with thick cell walls.

**Methods**: The cells were maintained in other Moss canal or ground water spiked with nitrate and phosphate to an approximate 10 mg N/L or 30 mg N/L and 3.5 mg P/L. Cultures were maintained with cool-white fluorescent lamps at 125 millecandles (mc) at 30°C in our indoor cultures. Outdoor cultures were subject to natural conditions in our photobioreactor. Optical density at 750 nm (OD750nm) of the culture was used to determine algal growth. Nitrate and phosphate levels were determined using a Bran-Luebbe TrAcx 800 auto analyzer.

**Effect of Initial Nitrate Concentration.**
For the first 24 hours, no difference in terms of growth rate was observed among most treatments. Rapid nitrogen uptake was observed in all cultures at a slower than the semi-saturation constant of ca. 2.2 mg N/L. In parallel to initial specific kinetic algal populations underwent a lag phase of about 4 hours. Differences observed in the 10 mg N/L culture and rates for other cultures after 24 hours is due to deprivation of the nitrate source for algal growth.

**Effect of Initial Cell Density.**
Cells were grown in canal water spiked with 50 mg N/L and 3.5 mg P/L at differing initial cell densities. Growth rates were similar for all cell concentrations. The effect of cell density in nutrient depletion was the most apparent. Cell cultures with lower initial cell densities required considerably more time to deplete nitrate to the desired level (50 mg N/L-1).

**Effect of Light Intensity.**
Cells were grown under conditions of differing light intensity. Growth rates indicated a direct relation between light intensity and cell density with the lowest light level expressing the least growth and the highest light level reaching the highest growth rate. Nitrate depletion occurred the fastest in cells grown under the highest light intensity and the slowest in the least intense light level. Direct sunlight can reach intensity levels as high as 2000 pound of photons m-2 h-1.

**Conclusions:**
Microalgae mass culture is a promising technology for bioabsorption of nitrate and phosphate contamination in surface water. Physiological characterization of *Scenedesmus obliquus* has determined that this species is a perfect candidate for mass culture and removal of nitrate and phosphate contamination from surface water run-off. To enhance nitrate removal it will be necessary to introduce a phosphate source into the culture system. Continued research on *Scenedesmus* using our photobioreactor remediation technology to optimize growth potential and nitrate reduction and to test the viability of our system through the extremes of Arizona’s weather conditions is underway. In the near future we plan to construct a multiple-continuous flow photobioreactor to further test the abilities of our water remediation technology.

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