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

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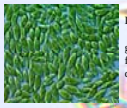
INTRODUCTION:

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 10 percent of the 8, 864 wells tested in Arizona exceed the maximum recommended concentration of 10 milligrams per liter (mg/l) of nitrate-nitrogen (NO_3-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 advanced biological system for bioabsorbtion of nitrogen and phosphorus using microalgal mass culture. 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 photobioractor demonstrate the ability of our remediation process.

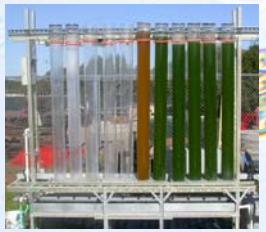
Materials and Methods:

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. Asexual reproduction is performed 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.



Photomicrograph of *Scenedesmus* sp. grown under favorable conditions.

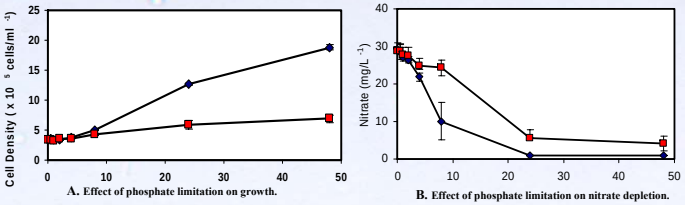
Methods: The cells were maintained in either Mesa canal or ground water spiked with nitrate and phosphate to an equivalence of 50 mg N/L or 30 mg N/L and 3.5 mg P/L. Cultures were illuminated with cool-white fluorescent lamps at 125 mmol m-2 s-1 in our indoor cultures. Outdoor cells were subject to natural conditions in our photobioreactor. Optical density at 730 nm (OD750nm) of the culture was used to determine algal growth. Nitrate and phosphate levels were determined using a Bran-Luebbe TrAAcs 800 auto analyzer.



Outdoor tubular photobioreactor taken during a recent January, 05 experiment. Here we are testing growth rates during the coldest month of Arizona's winter season.

Outdoor Photobioreactor:
The design of our research tubular photobioreactor allows us to conduct multiple experiments simultaneously in a natural outdoor environment. The clear tubes on the left are not in use. The green tubes are healthy *Scenedesmus* sp. cells. The orange tube on the left demonstrates how *Scenedesmus* sp., under nutrient starvation and stress accumulates carotenoids.

Addition of Phosphate on Growth and Nitrate Depletion

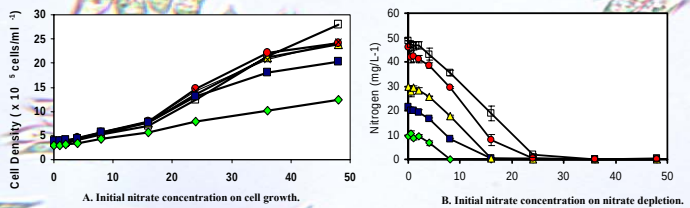


A. Effect of phosphate limitation on growth. B. Effect of phosphate limitation on nitrate depletion.

Legend: □=nitrogen plus phosphate ■=nitrogen minus phosphate

Cells were grown in media containing nitrate (30 mg/L) and phosphate (4 mg/L) and in media with nitrate (30 mg/L) And zero phosphate. Cells grown without the addition of phosphate in comparison to cells growth with additional phosphate exhibited slower growth and a reduced ability to deplete nitrate from the water.
Phosphate depleted cells after 48 hours had reduced nitrate levels to 5 mg/L whereas phosphate enriched cells reduced nitrate levels to approximately zero after 24 hours. Previous experiments have shown that phosphate levels of 4 mg/L are rapidly reduced to approximately zero with the first two hours of growth in healthy cultures.

Effect of Initial Nitrate Concentration.

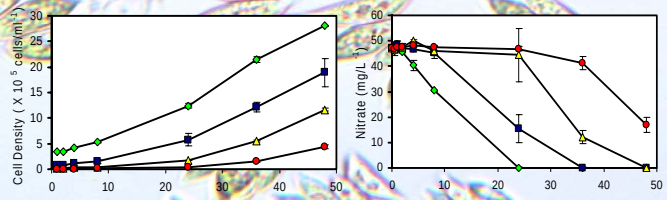


A. Initial nitrate concentration on cell growth. B. Initial nitrate concentration on nitrate depletion.

Legend: mg N/L^{-1} □=10 ■=20 ▲=30 ●=40 ☐=50

For the first 24 hours, no difference in terms of growth rate was observed among most treatments. Rapid nitrate removal occurred in all cultures at a more or less the same removal rate of ca. 2.2 mg N/L h^{-1} In parallel to nitrate uptake kinetics, algal populations underwent a lag phase of about 4 hours. Differences observed in the 10 mg N/L^{-1} 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

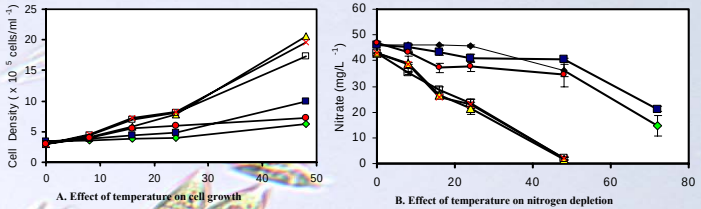


A. Effect of initial cell density on growth B. Effect of cell density on nitrate depletion

Legend: Cells/ml^{-1} (x 10^5) □= 3.33 ■=0.67 ▲=0.13 ●= 0.027

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 on nitrogen depletion was the most apparent. Cell cultures with lower initial cell densities required considerably more time to deplete nitrate to the desired level (10 mg N/L^{-1} <).

Effect of Temperature on Growth and Nitrate Depletion

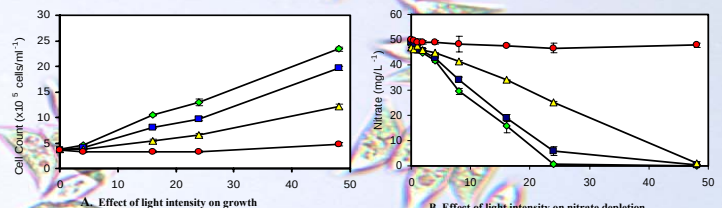


A. Effect of temperature on cell growth B. Effect of temperature on nitrogen depletion

Legend: □ = 15°C, ■ = 20°C, ▲ = 25°C, x = 30° ☐ = 35°C, ● = 40°C

In order to predict how cells might react to changing seasonal temperature conditions in our outdoor photobioreactor we conducted experiments at various temperatures. Temperatures for each set remained constant throughout the experiment. Cells grown at 25 and 30□ grew the fastest with the 35 □ culture following close behind. The colder 20 □ culture grew considerably slower followed by the highest temperature of 40 □ and the lowest temperature of 15 □.

Effect of Light Intensity on Growth and Nitrate Depletion

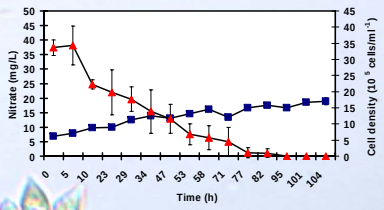


A. Effect of light intensity on growth B. Effect of light intensity on nitrate depletion

Legend: µmol of photons s^{-2} h^{-1} □=350 ■=200 ▲=75 ●=15

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 fasted 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 µmol of photons s^{-2} h^{-1}.

Growth in outdoor photobioreactor



Direct comparison of growth to nitrate removal.

Legend: ■= Cell growth ▲=Nitrate concentration

Cells were grown during the month of September in runoff water spiked with 35 mg N/L^{-1} from the 14th to the 18th. Outdoor temperature ranged from a 20 □ low and a 40 □ high. culture temperature did not go above 35 □ as our system is designed with cooling capabilities. Growth rate and nitrate removal was slower in the reactor compared to indoor growth primarily because growth did not occur at night.

Conclusions:

Microalgal mass culture is a promising technology for bioabsorbtion 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* sp. 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 Multi-stage Continuous-flow Photobioreactor to further test the abilities of our water remediation technology.

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