Report as of FY2006 for 2006AZ160B: "Advanced Biotechnology for Recycling Dairy Wastewater"

Publications

- Articles in Refereed Scientific Journals:
  - Zhang,C., K. Hanson, M. Sommerfeld, and Q. Hu, Dairy wastewater-fed algae feedstock production for biofuel. (in preparation)
- Dissertations:
  - Hanson, Karyn, 2006, Dairy Wastewater Nutrient Remediation Using Microalgae, MS Thesis, the Department of Applied Biological Sciences, College of Science and technology, Arizona State University at Polytechnic campus, Mesa, AZ 85212 (pp: 104)

Report Follows

A. Problem and Research Objectives

Concentrated dairy feeding operations, a billion-dollar industry in Arizona, not only provide a healthy and high-quality food supply, but also are essential to the state's economic stability and the viability of many rural communities. However, the growing scale and concentration of dairy feeding operations in the state has contributed to concerns about environmental and human health impacts. It is particularly relevant to the 12,250 square-mile Middle Gila Watershed surrounding the metro Phoenix area that has a fragile landscape and biodiversity. Over 100,000 dairy cows, or more than 70% of the total dairy cows in Arizona, are confined on this watershed. These dairy cows produce a total of more than 3 billion kilograms of manure waste per year, including some 12 million kilograms of nitrogen and 4 million kilograms of phosphorous (based on data from Van Horn et al. 1994). The vast amounts of waste nutrients produced by dairy cows overwhelm the ability of local soils and crops to absorb the nutrients, and result in degradation of water quality, threaten drinking water sources, and may influence air quality in the region. For instance, manure containing high concentrations of ammonia is highly toxic to fish and other aquatic organisms at low levels. On the other hand, increased amounts of nitrogen and phosphorus from dairy wastewater that could enter rivers and streams can cause algal blooms, and deplete the oxygen as the algae decompose, which in turn can devastate the aquatic food chain. Unpleasant algal blooms can also affect water recreational activities.

When nutrients from dairy wastewater seep into underground sources of drinking water, the amount of nitrate (converted from ammonia through nitrification) in the ground water supply can reach unhealthy levels. The Middle Gila River watershed with its highly permeable soil facilitates pollutant transport and increases the risk of ground water contamination. 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 ≥ 18 mg L^{-1} has also been reported to contribute to the risk of non-Hodgkin's lymphoma (Ward et al. 1996). In addition, dairy farms may also cause air quality problems due to gases emitted from the decomposition of animal wastes and by the dust generated by animal activity and farming practices. These air pollutants can cause respiratory illness, lung inflammation, and increase vulnerability to respiratory diseases, such as asthma (U.S. EPA Animal waste home, www.epa.gov).

Concentrated dairy feeding operations are under increasing pressure to develop best management practices (BMPs) that address water conservation along with water quality. Currently, facultative lagoons and land application are standard nutrient management methods used in the Gila River Watershed. However, a multi-year monitoring of 6 dairy feedlots revealed that significant contamination of nitrate, ammonia, chloride, and total dissolved solids occurred in groundwater due to leakage and seepage from lagoons (Arnold and Meister 1999). Some waste lagoons, even with clay liners, allow contaminants to leach into the groundwater below the lagoon. For instance, it was estimated that a 3-acre lagoon could legally leak more than a million gallon of wastewater a year at the maximum allowable rate (Weida 2000). Applying too much dairy wastewater to fields too rapidly or by inadequate methods can also cause the contaminants in dairy waste to pollute streams or ground water before they can be completely absorbed by the land and crops. Although aeration, anaerobic digestion, and constructed wetlands have been suggested to mitigate wastewater pollution, these techniques alone cannot remove sufficient nutrients to make treated wastewater reusable. Therefore, environmentally friendly and cost-affordable sustainable technologies for dairy wastewater remediation is needed.

The goal of this research project was to demonstrate the technical feasibility of removing waste nutrients (mainly ammonia) from dairy wastewater and recycling the treated water on the farm using an advanced microalgal biotechnology. To achieve this goal, two specific technical objectives were identified below:

1) Construct and evaluate a novel vertical column photobioreactor. The system was designed to reduce high concentrations of ammonia-N in dairy wastewater to <10 mg N L^{-1}, and return treated, reclaimed water back to the dairy operation.

2) Evaluate performance and nutrient uptake efficiency of isolated and native wastewater-born algal strains. The superior algal strain was used in the vertical column photobioreactor to demonstrate effectiveness and efficiency of the system for sustainable water conservation.

B. Methodology

Photobioreactor: A vertical photobioreactor was fabricated and operated for nutrient removal from dairy wastewater.

Organisms: *Scenedesmus* sp. and *Chlorella* sp. are two green algal strains used in this study. The *Scenedesmus* strain was isolated from a lake near Phoenix, while the Chlorella strain was obtained from native dairy wastewater. Our previous USGS/WRRC project on screening of high-performance microalgae for remediation of nitrate-contaminated groundwater already identified the *Scenedesmus* sp. to be a suitable candidate for rapid growth and nitrate uptake potential. The newly isolated *Chlorella* strain from a dairy waste lagoon also exhibited growth in full strength dairy wastewater, although its nutrient uptake rate has yet to be determined.

Indoor algal culture: Laboratory study of algal nutrient uptake rate was conducted in mini-glass column bioreactors each containing 300 ml of culture volume. Algal cultures that contained either full-strength or diluted (by 25 and 50%) dairy wastewater were maintained at 25 ^{0}C and with a light intensity of ~ 200 mol m^{-2} s^{-1}. Growth rate and concentrations of ammonia, nitrate and phosphate were measured in each column daily.

Outdoor algal culture: Algal performance in the outdoor column photobioreactor was assessed in a batch culture mode. The column photobioreactor was filled with diluted dairy wastewater (e.g., 5 to 15% wastewater) and inoculated with pre-cultured algal cells. When ammonia concentrations in the column are reduced to below 10 mg L^{-1} N, the whole culture was removed from the column and subjected to downstream processing to remove algal cells from treated water. The growth rate, as indicated by changes in biomass (e.g., cell dry weight), was monitored using a UV-VIS spectrophotometer. Aliquots of culture were taken from individual columns at certain time intervals for nutrient analysis, using an automated nutrient analyzer (Lachat QuickChem 8000 Flow Injection Nutrient Analyzer).

C. Principal Findings and Significance

Fabrication of the vertical column photobioreactor:

The vertical column photobioreactor consisted of 32 clear acrylic columns arranged in four rows, each consisting of eight columns. All columns were 6-inch in diameter and 6-foot in height, and each with a capacity of 32 liters. The total culture volume of the system is over 1,000 liters. Eight columns in a row were interconnected through the base caps, which were designed such that wastewater and algal suspension in individual columns can be filled or drained simultaneously or separately. All rows of columns were also interconnected using manifold connectors (Fig. 1).

Fig. 1. Overview of a vertical column photobioreactor for dairy wastewater bioremediation. The bioreactor design student team members: from left to right: Chad Jackson, Brian Blum, Shaun Whitney, Andrew Miles, and Andres Dale.

Aeration mixing system:

Algal culture mixing was was provided by a compressed air stream containing 0.5-1.0% CO_2 through tubing inserted into the base cap of the column.

Temperature control system:

An evaporative cooling system was installed on the columns and cooling water was collected and reused.

Algae harvesting and wastewater refilling system:

Dairy wastewater was supplied to individual columns using a water pump. The same system was also used to harvest algal culture suspension and refill the columns with growth medium or wastewater.

Growth kinetics of *Scenedesmus* sp. and *Chlorella* sp. in dairy wastewater

The green algae, *Scenedesmus* sp. and *Chlorella* sp., were able to grow in the dairy wastewater with or without dilution with tap water. However, the concentration of dairy wastewater did affect growth. The higher the dairy wastewater, the slower the growth and longer the leg phase before growth resumed to grow. Compared with *Chlorella* sp., *Scenedesmus* cells exhibited a faster growth rate and a higher final cell density at every dilution rate tested (**Fig. 5**). Based upon these findings, the *Scenedesmus* strain was selected for further investigation in the vertical column photobioreactor outdoors.

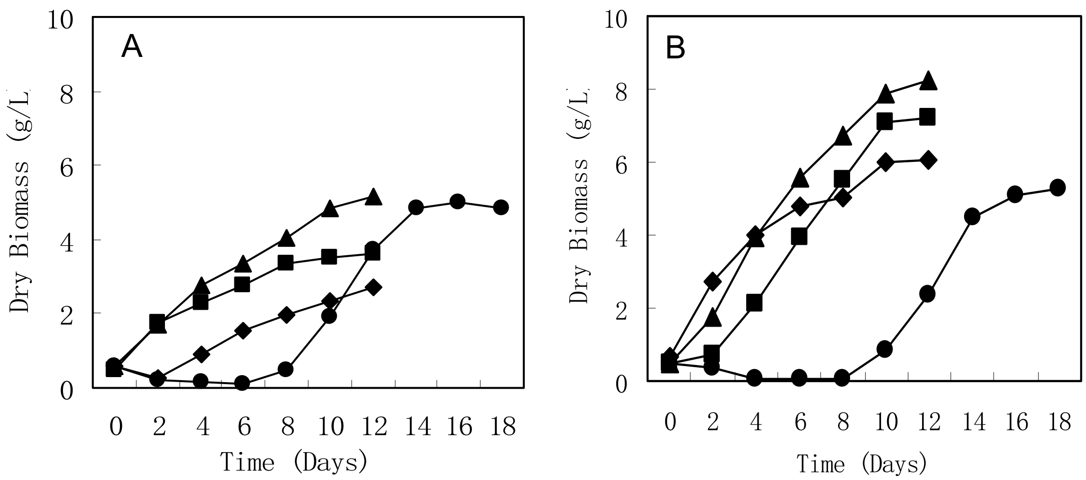

Fig. 5. Growth of *Chlorella* sp. (A) and *Scenedesmus* sp. (B) maintained in 25% (▲), 50% (■), 75% (◆), and 100% (●) of dairy wastewater.
Each data point represents an average of three independent cultures. Culture temperature: 25 ^{o}C, light intensity: 170 μmol m^{-2} s^{-1}, and CO_2 concentration: 1%

Nutrient removal from dairy wastewater by *Scenedesmus* sp.

The ammonia concentration was measured in *Scenedesmus* cultures maintained in dairy wastewater with different initial dilution rates. Rapid uptake of ammonia by *Scenedesmus* cells occurred in all the treatment cultures, yet the higher the initial wastewater concentration the longer it took for algal cells to remove ammonia from the culture medium (**Fig. 6**). On average, the uptake rate of ammonia by *Scenedesmus* cells was ca. 3.8 mg L^{-1} h^{-1}.

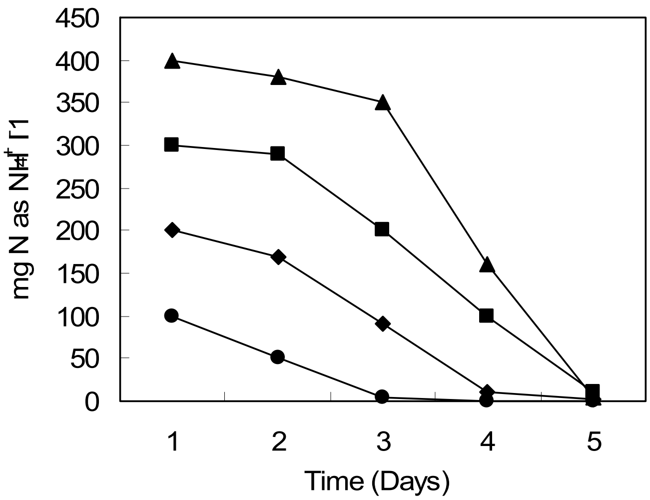

Fig. 6. Reduction in ammonium concentration in wastewater as a function of wastewater dilution rate. *Scenedesmus* cells grew in 25% (●), 50% (■), 75% (◆), 100% of wastewater (▲). Error bars represent confidence intervals α=0.05 (n=3).

Fig. 7 demonstrates the technical feasibility of ammonia removal from dairy wastewater using the vertical column photobioreactor outdoors. Quantitative assessment of the vertical column reactor for dairy wastewater bioremediation is underway.

Fig. 7. Sustainable dairy wastewater treatment by a vertical column photobioreactor.

Significance of the project:

The major accomplishments of the research project were the identification and evaluation of isolated algal strains that can thrive in dairy wastewater and rapidly remove ammonia, and the development and exhibition of an innovative column photobioreactor for cost-effective recycling dairy wastewater. Based upon the results obtained from this study, a commercial full-scale photobioreactor system should be able to treat and recycle 50,000 to 100,000 gallons/day of wastewater to meet the U.S. EPA standard, a capacity that can satisfy the needs of dairy operations of various sizes. While treating large quantities of wastewater, the innovative photobioreactor system would only need a small portion of the land (about 10%) otherwise required by dairy operators for land application.

Project beneficiaries included dairy producers throughout the Gila River Watershed and across the southwest and western regions of the US, regional water districts, local and state water quality regulators, and all stakeholders in regional water conservation efforts. If the ultimate goal of the project could be achieved through our continuous R&D efforts along this line, it is believed that this technology will not only benefit dairy producers by assisting them in meeting National Pollutant Discharge Elimination System (NPDES) requirements and exposing producers to a new innovative method for conserving water, but also enable dairy producers to incorporate the developed technology in their CNMPs to: a) reduce amounts of water, b) reduce amounts of nitrogen and phosphorous being discharged (corresponds with reduction in acres needed to land apply wastewater), and c) develop an improved odor control BMP for the dairy operation.

The technology developed through this project can benefit agricultural water districts and local water quality regulators by demonstrating a workable method for efficiently controlling waste nutrients from other agricultural and industrial sources.