Introducing Rationale

- The sufficient supply of water and nutrients have been identified as potentially limiting factors in the large scale production of algal biofuels.
- Nitrogen fertilizer production is energy intensive and may compromise the energy balance for biofuel production.
- The use of low quality or recycled water and nutrient sources for algae cultivation is of interest.
- The primary inorganic nitrogen forms of interest are nitrate, ammonium, and urea.
- Nitrate has received the most attention in algal biofuel research, but is likely the most cost prohibitive of the three.
- Ammonium and urea are also of interest as they are the dominant forms of inorganic nitrogen in many waste streams (municipal wastewater, piggy wastewater).

Objectives

- Assess the growth and lipid accumulation of Chlorella vulgaris UTEX 395 when cultured using different nitrogen sources (nitrate, urea, ammonium, and a combination of all three).
- Evaluate the effect of nitrogen stress and bicarbonate amendment at the time of nitrogen stress on lipid accumulation of C. vulgaris UTEX 395 under different nitrogen growth conditions.

Methods

- C. vulgaris UTEX 395 was cultured under eight conditions (Table 1) using Bold’s Basal Medium modified with three different nitrogen sources.
- The initial nitrogen concentration for all conditions was approximately 40mg-N/L.
- The cultures were grown in 1.25L tube reactors (Figure 1) with a 14:10 light/dark cycle.
- All reactor systems were aerated at 0.4LPM. During the light period, prior to nitrate depletion, the air being supplied to all conditions was supplemented continuously with 0% (v/v) CO₂.
- The ammonium control and bicarbonate conditions were pH controlled at pH 6.8 using KOH.

Results

- Figure 2. Cell concentrations for all conditions. Vertical dotted lines indicate the time of the bicarbonate amendment for each of the conditions. Blue=nitrate; orange=urea; purple-ammonium; green=mixed nitrogen (The purple and green dashed lines are overlapping). Error bars represent data range for each of the conditions.
- Figure 3. Nitrogen concentrations for each of the experimental conditions. The bicarbonate amended and control conditions for each of the nitrogen scenarios are plotted together. Error bars represent the range of experimental data for each of the nitrogen conditions. The inset graph presents a breakdown of nitrogen specification for the mixed nitrogen conditions.
- Table 1. Nitrogen concentrations for each of the experimental conditions as a function of time. The vertical dashed lines represent the time of the bicarbonate amendment for each of the bicarbonate amended conditions. Blue=nitrate; orange=urea; purple-ammonium; green=mixed nitrogen (The purple and green dashed lines are overlapping). Error bars represent data range for each of the conditions.
- Figure 4. Culture pH for each of the experimental conditions as a function of time. The vertical dashed lines represent the time of the bicarbonate amendment for each of the bicarbonate amended conditions. Blue=nitrate; orange=urea; purple-ammonium; green=mixed nitrogen (The purple and green dashed lines are overlapping). Error bars represent data range for each of the conditions.

Discussion

- Growth: The growth of C. vulgaris UTEX 395 was similar under all conditions (Figure 2). All of the conditions, with the exception of the ammonium control condition, reached stationary phase prior to or at nitrogen depletion. The ammonium control condition continued to grow exponentially for one day beyond nitrogen depletion.
- Nitrogen Uptake: Ammonium was the most readily utilized nitrogen species evaluated, followed by nitrate and then urea (Figure 3). This was observed in the mixed nitrogen conditions and when comparing the single nitrogen species conditions.
- Lipid Accumulation: The addition of bicarbonate just prior to nitrogen depletion resulted in an increase in lipid production for all of the bicarbonate amended cultures (Table 3).
- Experimental Issues/Concerns:
  - (1) A malfunction with the pH control system for one of the ammonium cultures resulted in the loss of an experimental replicate. Additional replicate data will be collected in a subsequent experiment.
  - (2) Lipid sample analysis is currently still in progress.

Conclusions

- C. vulgaris UTEX 395 was able to grow comparably under all of the experimental conditions.
- The combination of nitrogen stress and a sodium bicarbonate amendment resulted in an increase in lipid accumulation under all of the experimental conditions.

Future Work

The current study serves as a foundation for continuing to investigate the effects of using different nitrogen species for the cultivation of microalgae. Future research will:

- (1) Reevaluate the current experimental conditions in order to increase confidence in the current findings.
- (2) Evaluate the growth and lipid accumulation of C. vulgaris UTEX 395 using primary clarifier effluent collected from the Bozenman Wastewater Reclamation Facility.
- (3) Evaluate the effect of nitrogen stress and bicarbonate amendment at the time of nitrogen stress on lipid accumulation of C. vulgaris UTEX 395 using primary clarifier effluent as a growth medium.

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Literature Cited


