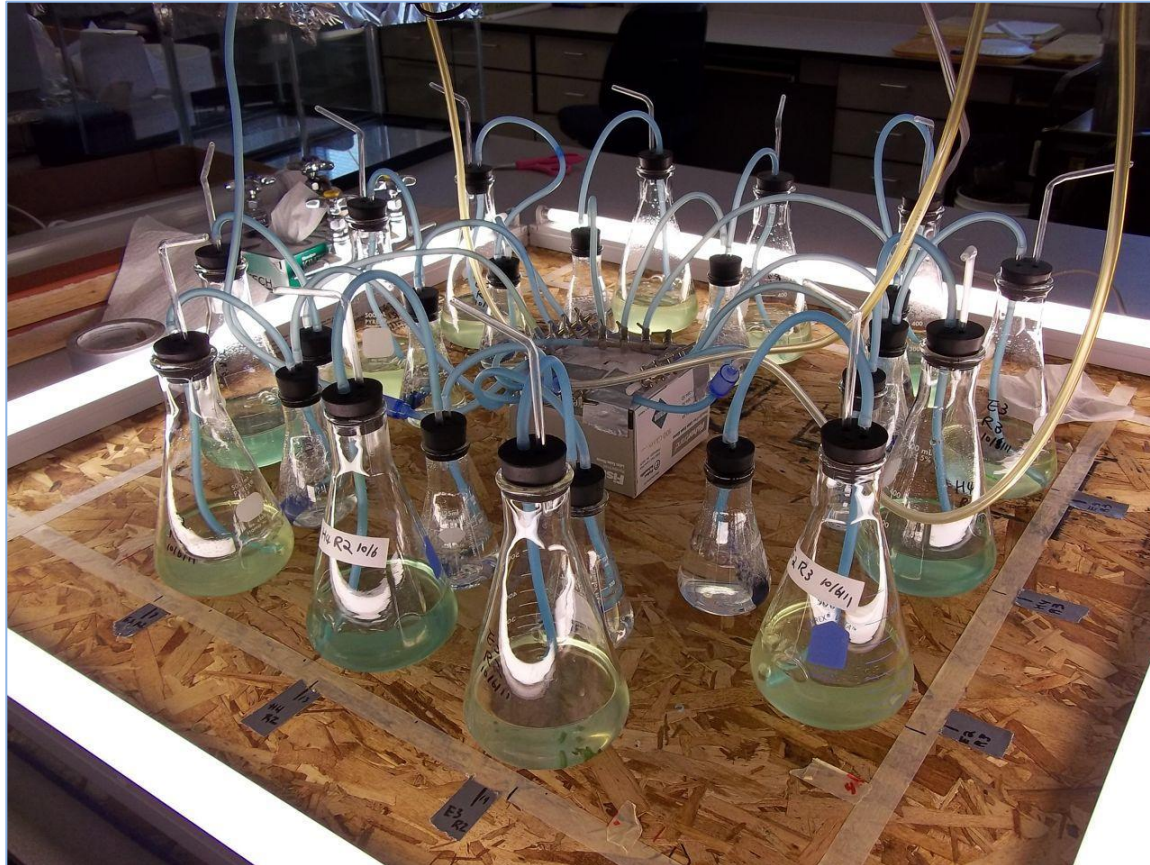


WSARE 2012 Final Report Figures and Tables

# **MATERIALS AND METHODS**



**Fig. 1:** Lightbox technique. Erlenmeyer flasks were filled with media and inoculated with culture. Flasks were bubbled constantly with filtered air and illuminated using florescent lights placed along each side of the box. Treatments were applied in a randomized complete block design. Experiments were typically conducted for 2 weeks.

**Table 1:** List of media treatments and possible organic substitutes for the prohibited chemicals in AA that could be used in an organic media formulation of AA (OAA<sub>1</sub>).

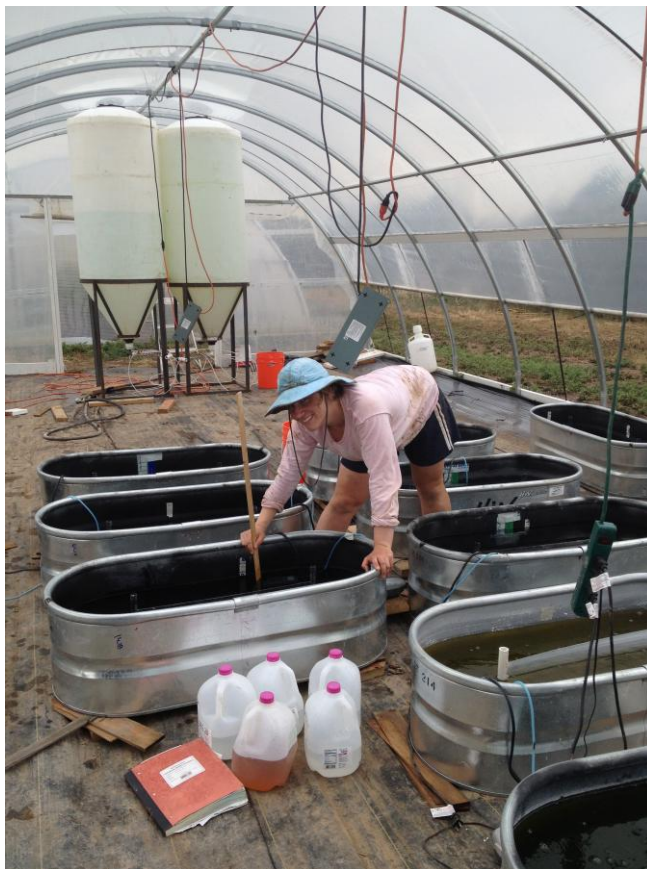
Media Treatments	Purpose
Allen and Arnon (AA)	Control
Organic AA (OAA <sub>1</sub> )	To replace prohibited chemicals of AA with allowed chemicals
AA w/o Vanadium (AA-V)	To determine whether differences from the control could be attributed to the substituted organic media components or the absence of vanadium

Prohibited AA Chemical	Supplied Nutrient	Organically Certified Substitutes*
K <sub>2</sub> HPO <sub>4</sub> anhydrous	P and K	<b>bone meal and KCl</b>
CaCl <sub>2</sub> •2H <sub>2</sub> O	Ca	<b>CaCl<sub>2</sub> Briners Choice Anhydrous</b>
MnCl <sub>2</sub> •4H <sub>2</sub> O	Mn	<b>MnSO<sub>4</sub>•H<sub>2</sub>O</b> or Mn <sub>2</sub> O <sub>3</sub>
CoCl <sub>2</sub> •6H <sub>2</sub> O	Co	<b>CoCO<sub>3</sub></b> or C <sub>4</sub> H <sub>6</sub> CoO <sub>4</sub> or CoO or CoO <sub>4</sub> S
FeNaEDTA	Fe	<b>FeSO<sub>4</sub>•7H<sub>2</sub>O</b> or C <sub>6</sub> H <sub>5</sub> FeO <sub>7</sub> or Fe <sub>2</sub> (C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> )

\*The chemicals in bold were selected as the organic substitutes for the indicated prohibited AA chemicals.



**Fig. 2:** In the field in 2011, we compared growth of four 100-gallon prototype ponds on a local organic farm. The cultures grown in the hoop house had superior growth to those grown outdoors under direct sunlight.



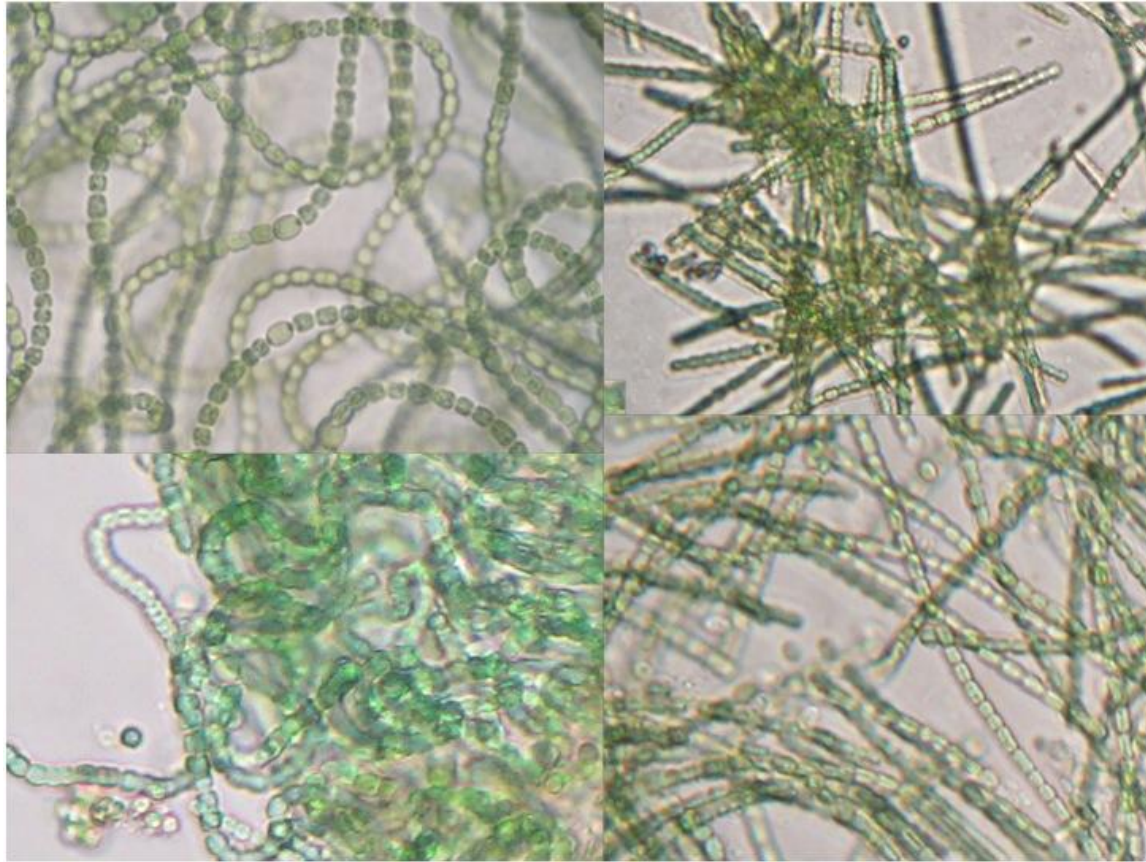
**Fig. 3:** Graduate student setting up an experiment with the pilot-scale raceways inside the hoop house on CSU's Horticultural Research Farm.



**Fig. 4:** 625-gallon raceway constructed with wood, lined with plastic, powered by a 6-blade paddle wheel.

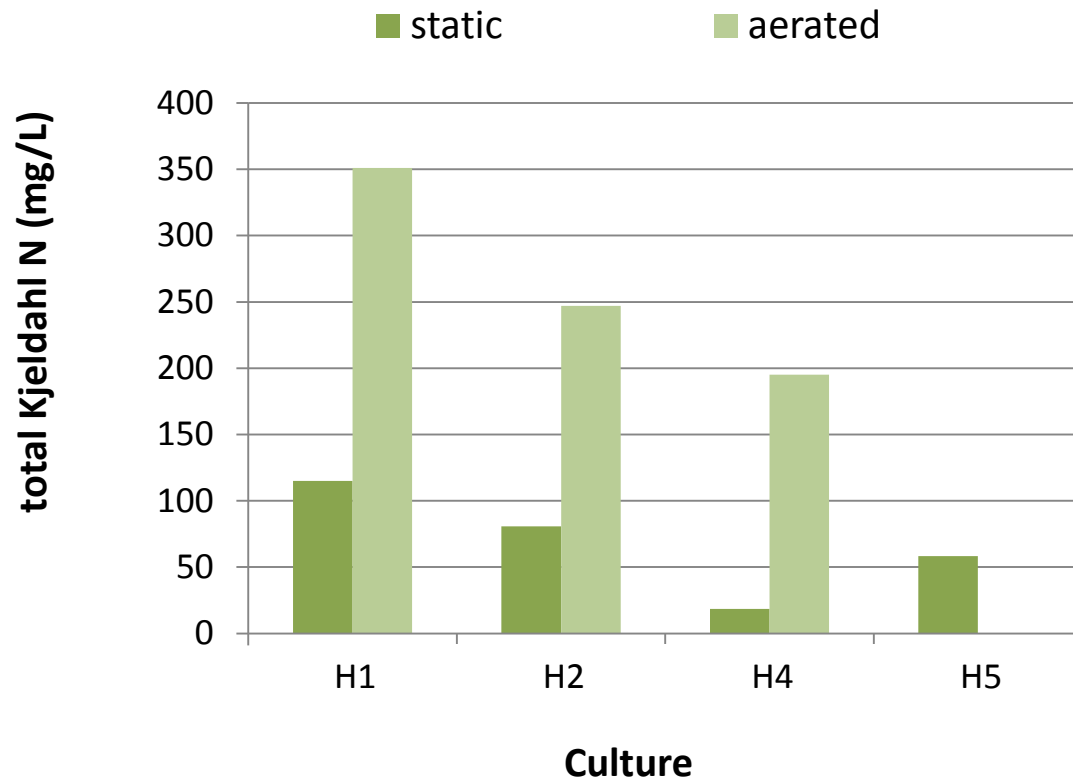
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# **RESULTS AND DISCUSSION**

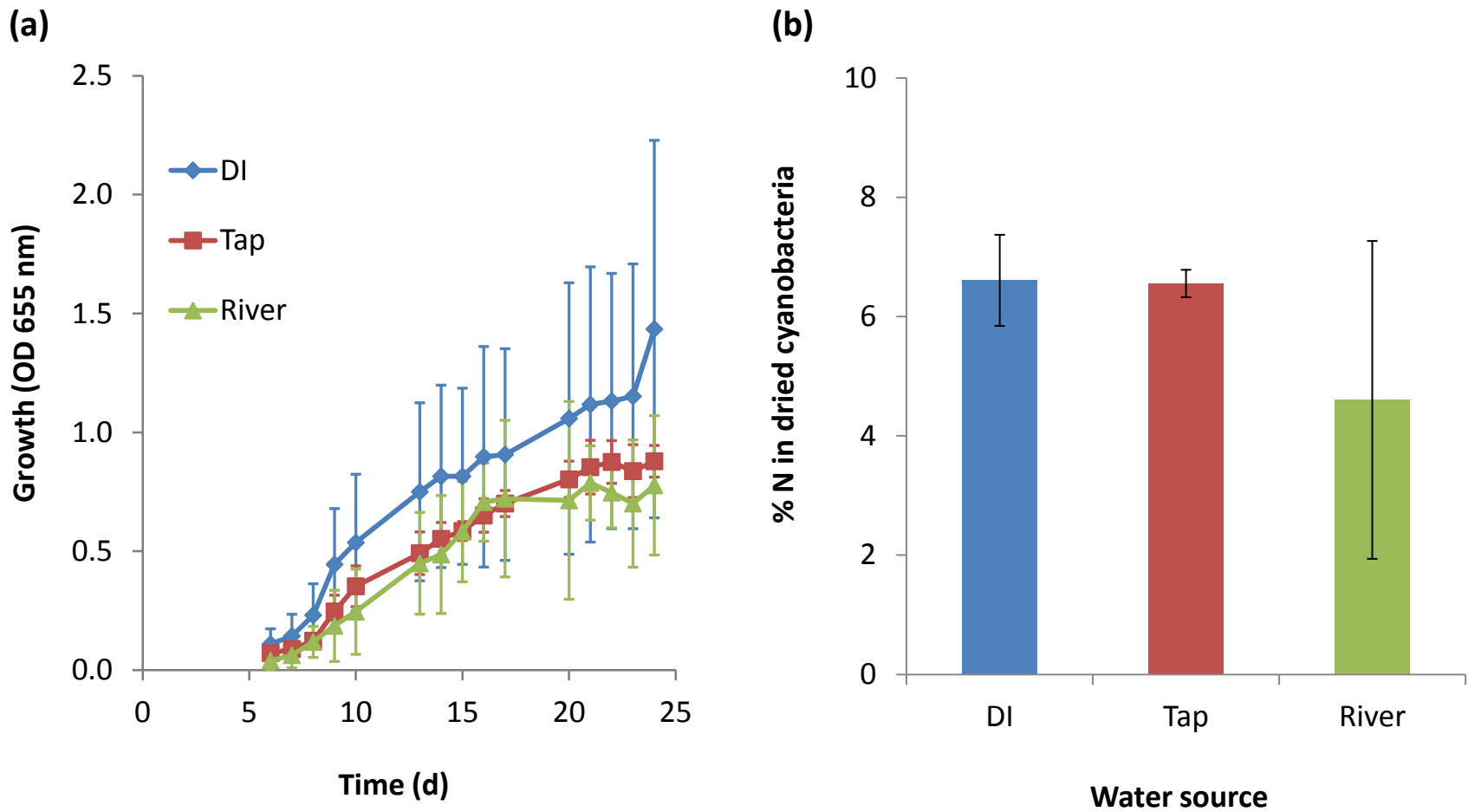


**Fig. 5:** Various morphologies of N-fixing cyanobacteria represented in our N-fixing culture collection.

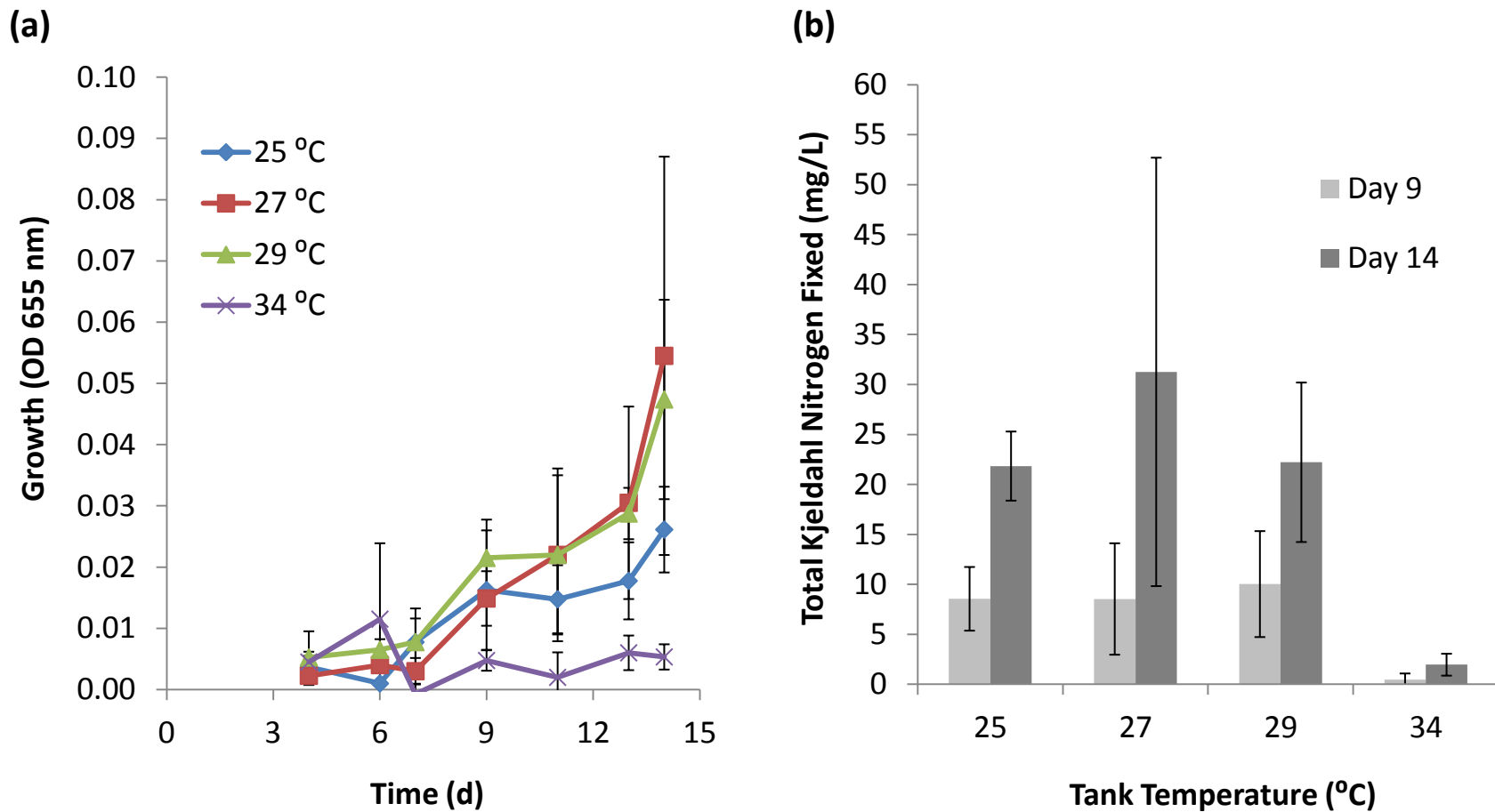




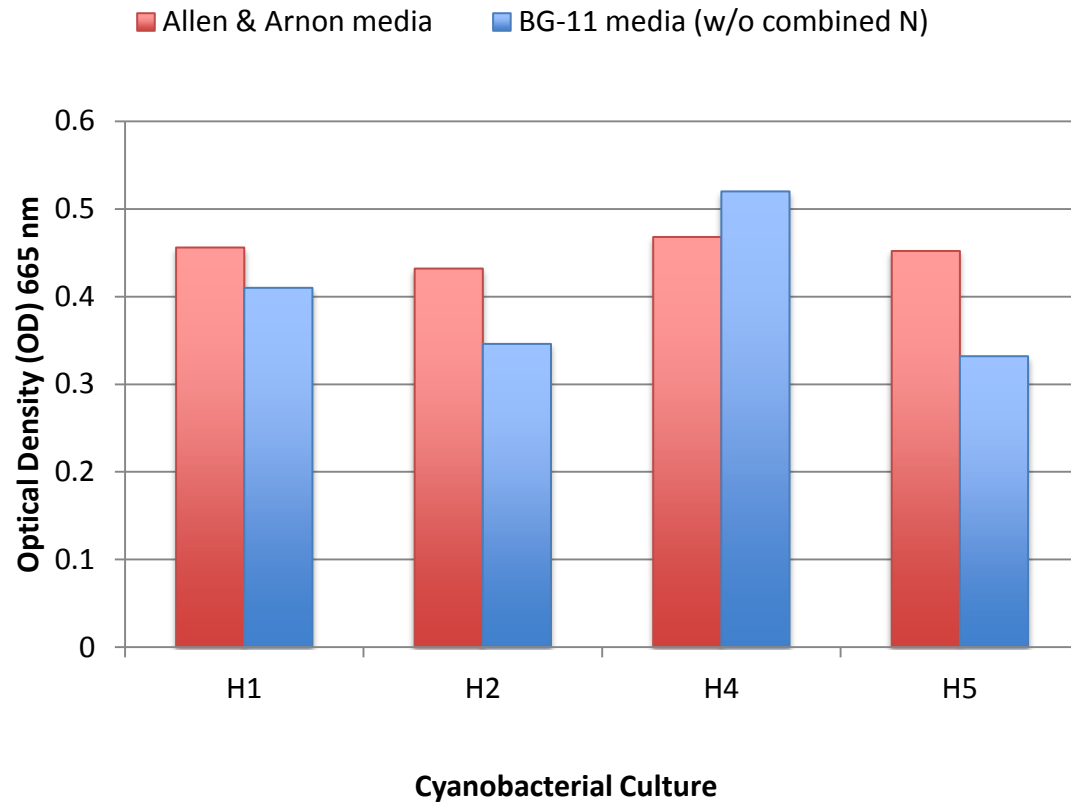
**Fig. 6:** Comparing the N-fixation of cultures when grown either statically or with aeration.



**Fig 7:** Growth (a) and N-fixation (b) of H4 cultures of cyanobacteria grown in different water sources: ultrapure deionized water (DI), tap water, and Poudre River water (River).



**Fig 8:** Growth over time (a) and TKN (b) on day 9 (midpoint) and day 14 (end) of the study when H4 culture of cyanobacteria was grown over a range of temperatures: 25°, 27°, 29°, 34°C.

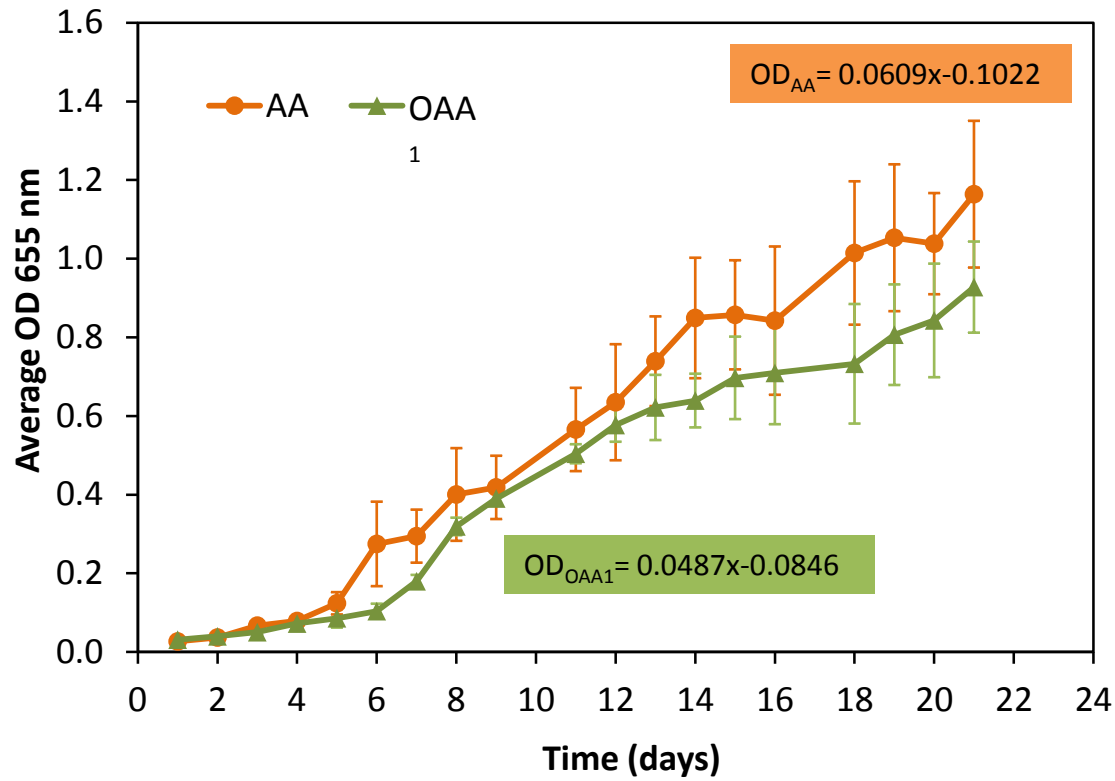


**Fig. 9:** Comparison of final optical density (OD) of 4 cultures grown for 14 days in two types of media: Allen and Arnon and BG-11 (without combined N).

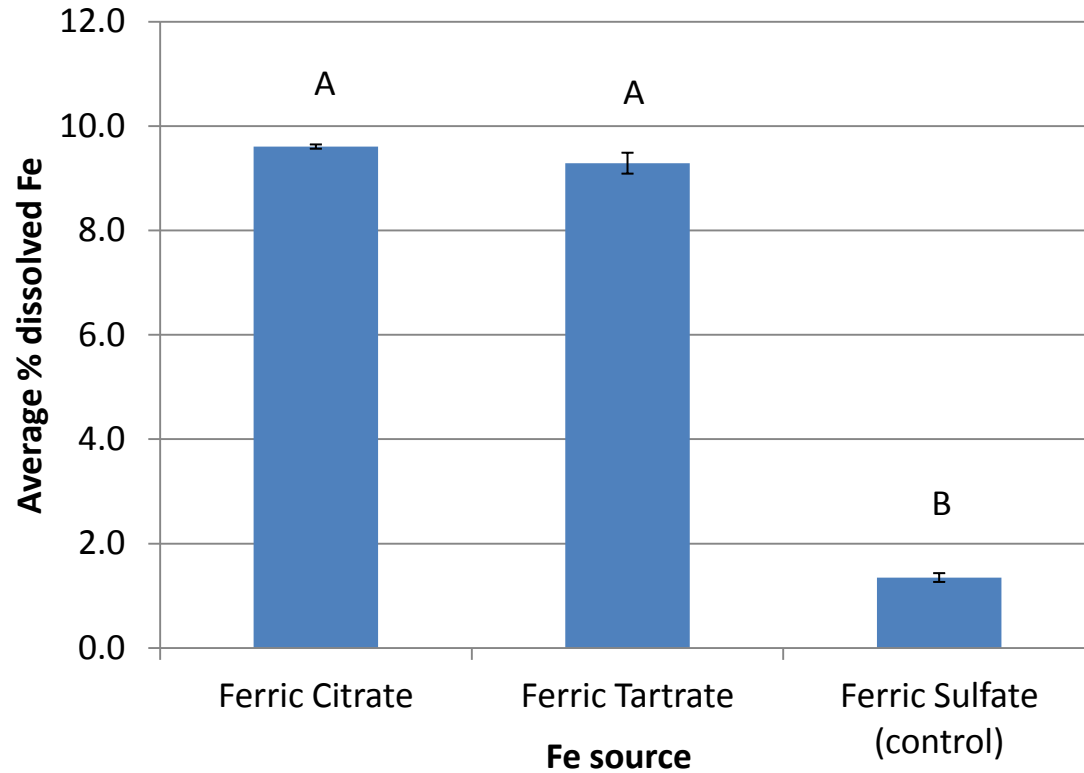
**Table 2:** Theoretical and Measured Concentrations (mM) of key nutrients in the media treatments Allen and Arnon (AA), AA without Vanadium (AA-V), and Organic AA (OAA<sub>1</sub>).

Nutrient	Theoretical Concentration	Measured Concentration		
	AA	AA	AA-V	OAA <sub>1</sub>
P	0.76	0.88	0.84	0.69
K	1.5	1.6	1.5	1.5
Ca	0.13	0.04	0.04	0.42
Fe	0.018	0.016	0.014	0.0029
Mn	0.0023	0.0025	0.0024	0.0018
V	0.000050	0.00020	0.00020	0.00039
Co	0.000042	<0.00017	<0.00017	<0.00017

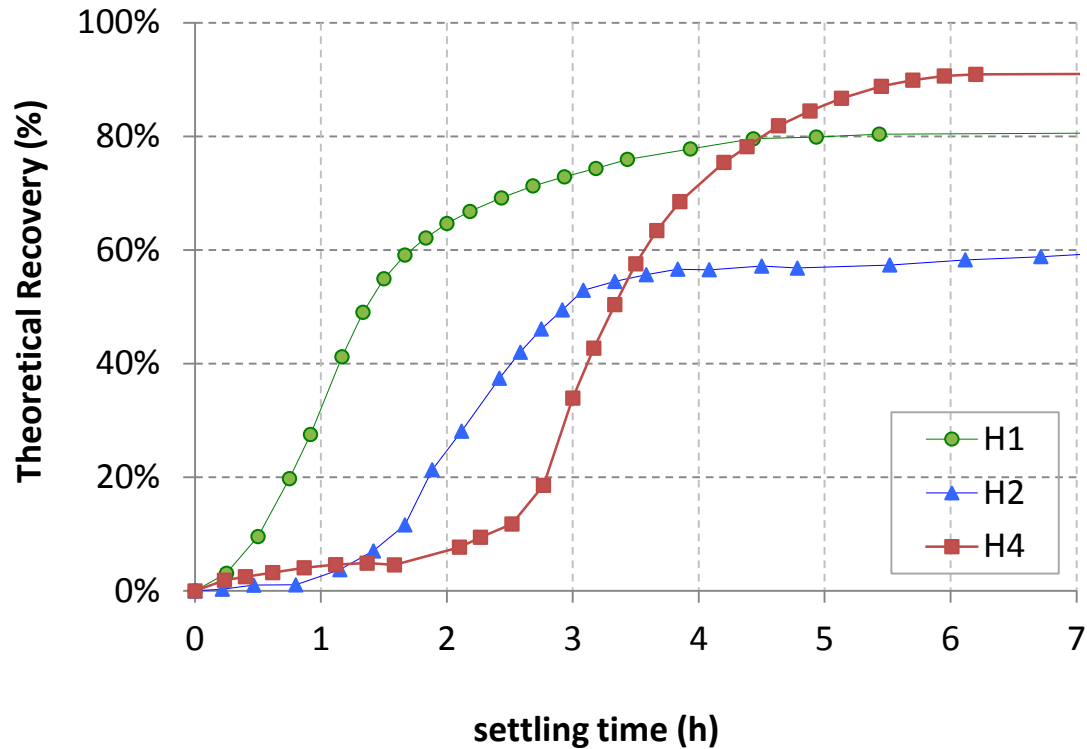
\*Vanadium (V) concentrations of AA-V were not different from AA, therefore AA-V results are not further discussed.



**Fig. 10:** The growth rate of AA (slope= 0.0609) was significantly higher than OAA<sub>1</sub> growth rate (slope=0.0487) ( $p < 0.0001$ ).

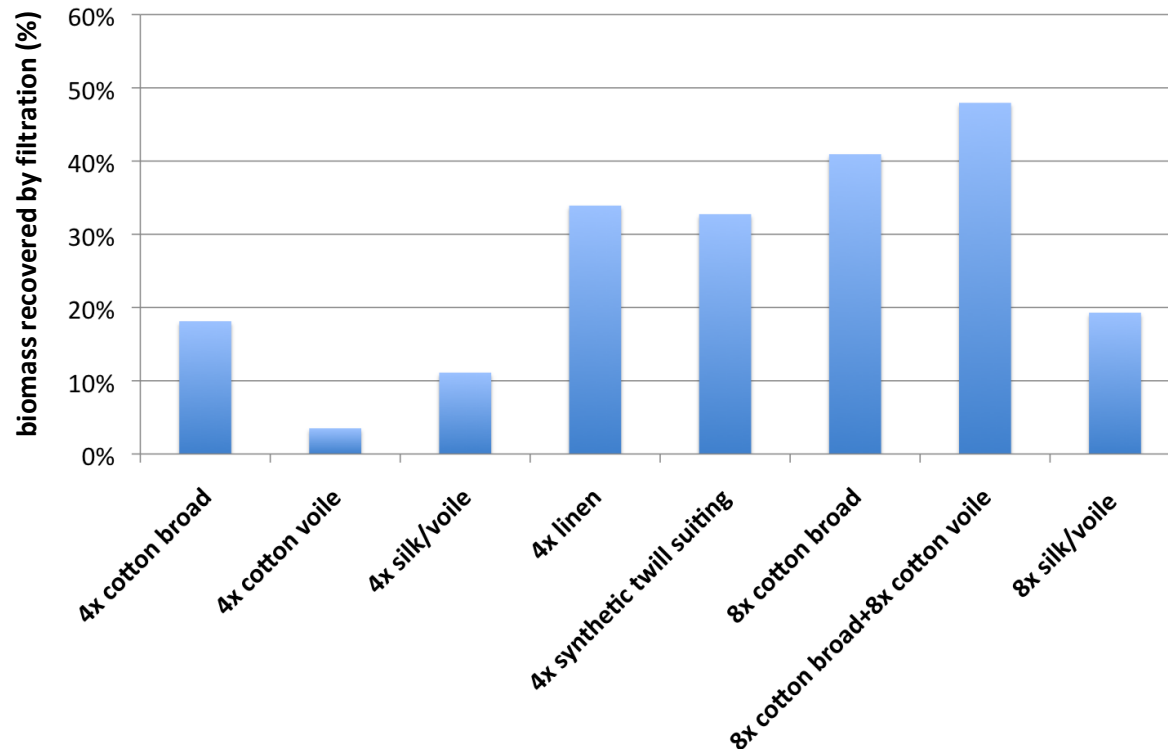


**Fig. 11:** Ferric citrate and ferric tartrate yield greater % dissolved iron (Fe) compared to the control ferric sulfate, the Fe source used in OAA<sub>1</sub>. Fe sources with a common letter were not significantly different from each other ( $p < 0.05$ ) according to Tukey's test for mean separation.

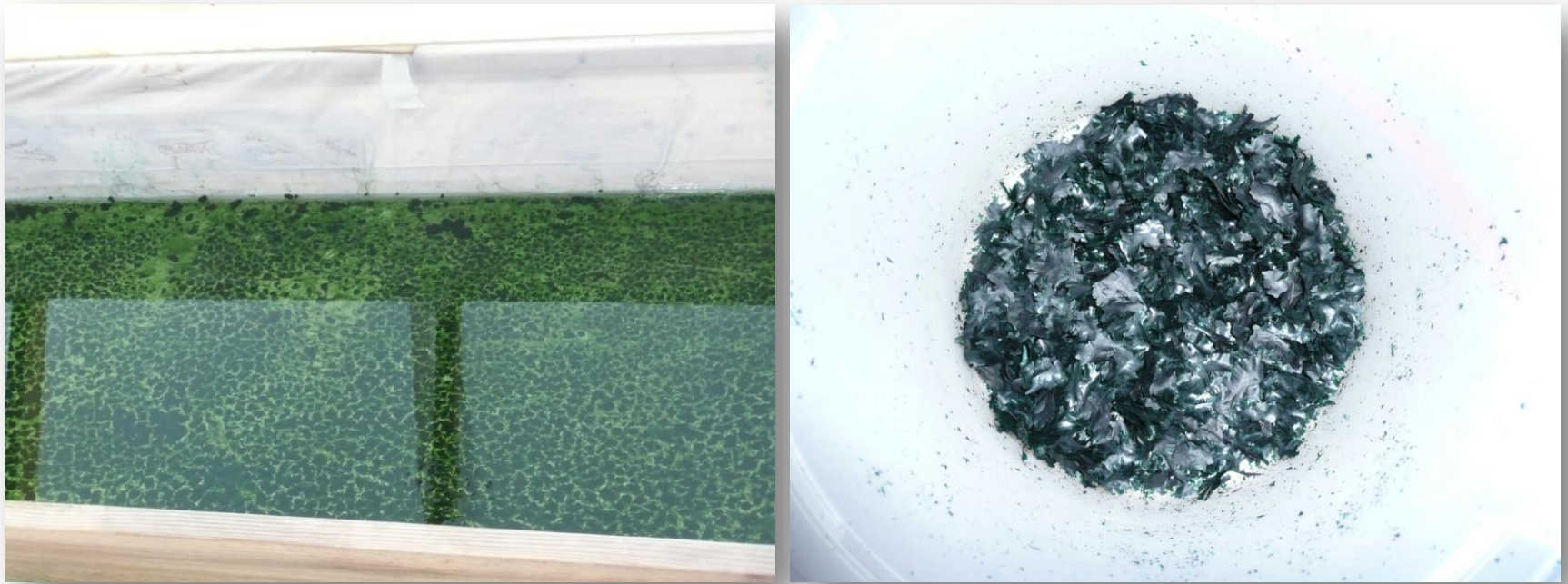


**Fig. 12:** Theoretical recoveries (calculated as the percentage of the original culture that settled) of three N-fixing cyanobacterial cultures allowed to naturally settle over 7 hrs.

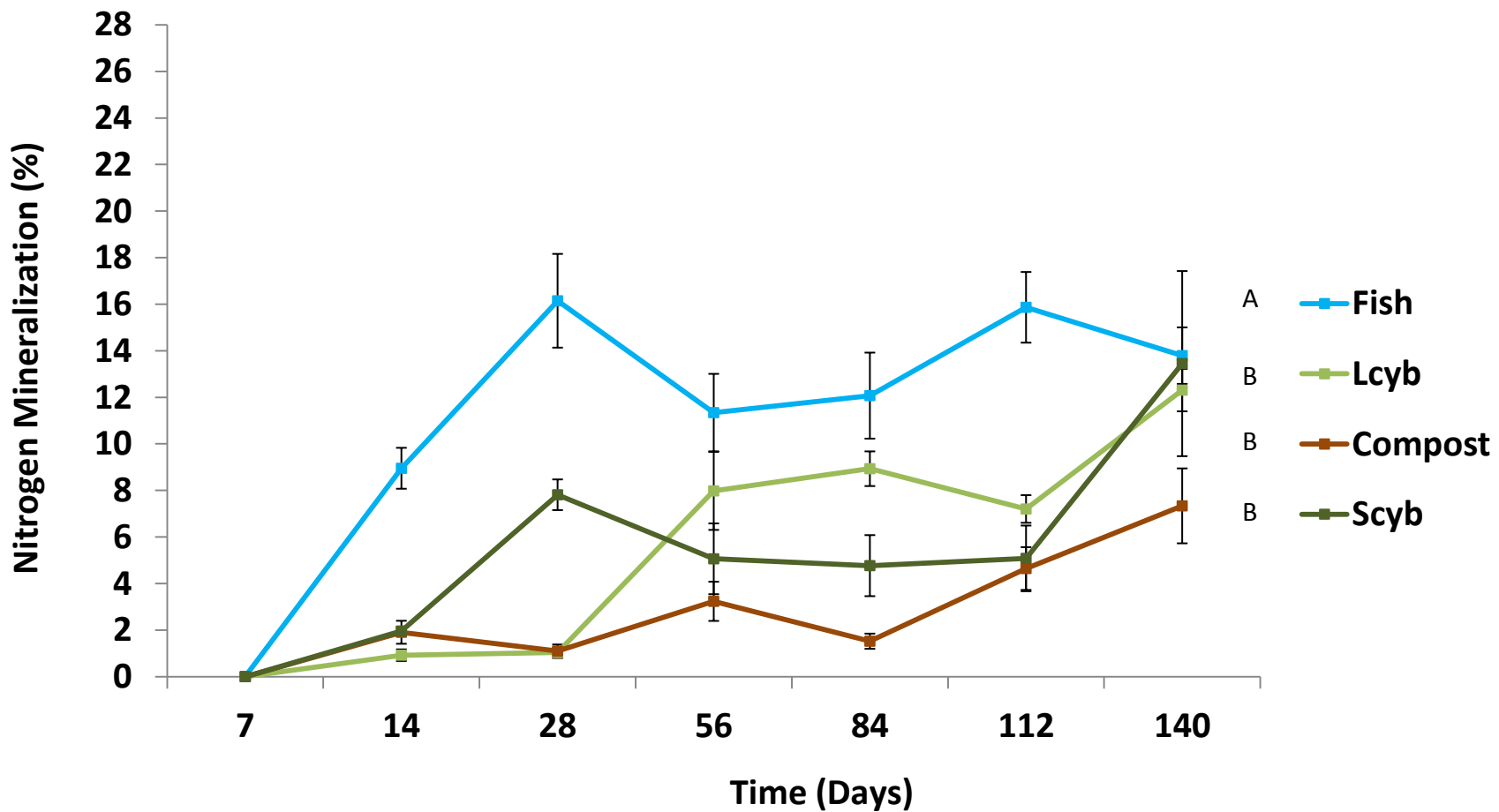




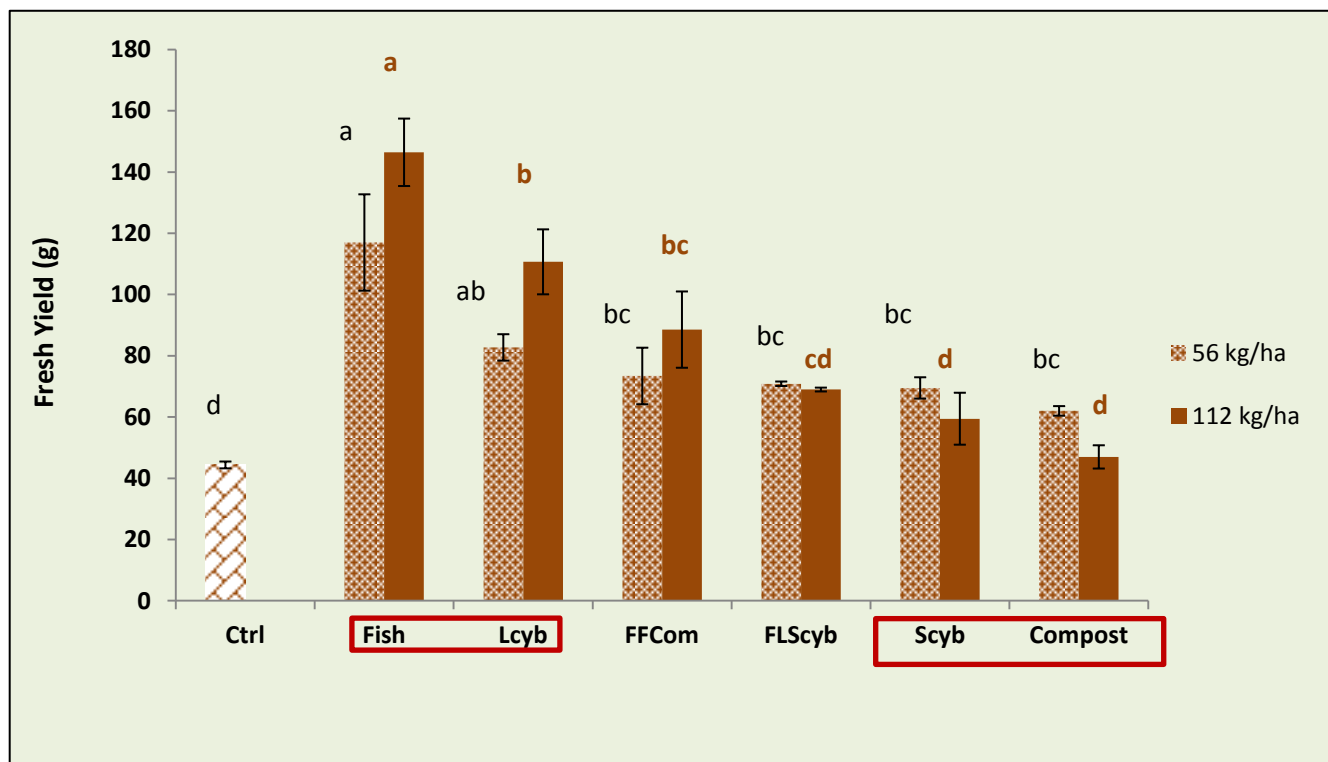
**Fig. 13:** Results from fabric filtration study.



**Fig. 14:** Harvesting solid cyanobacterial bio-fertilizer through settling, pumping supernatant off, and air-drying. The settled cyanobacteria can be seen on the left following settling and removal of supernatant. The air-dried bio-fertilizer is seen on the right.



**Fig. 15:** Time course for soil N mineralization percentage in clayey soil during a 140-day incubation experiment. Bars represent standard errors of the means. Fish=fish emulsion. Lcyb=Liquid cyanobacteria. Compost=composted manure. Scyb=solid cyanobacteria.



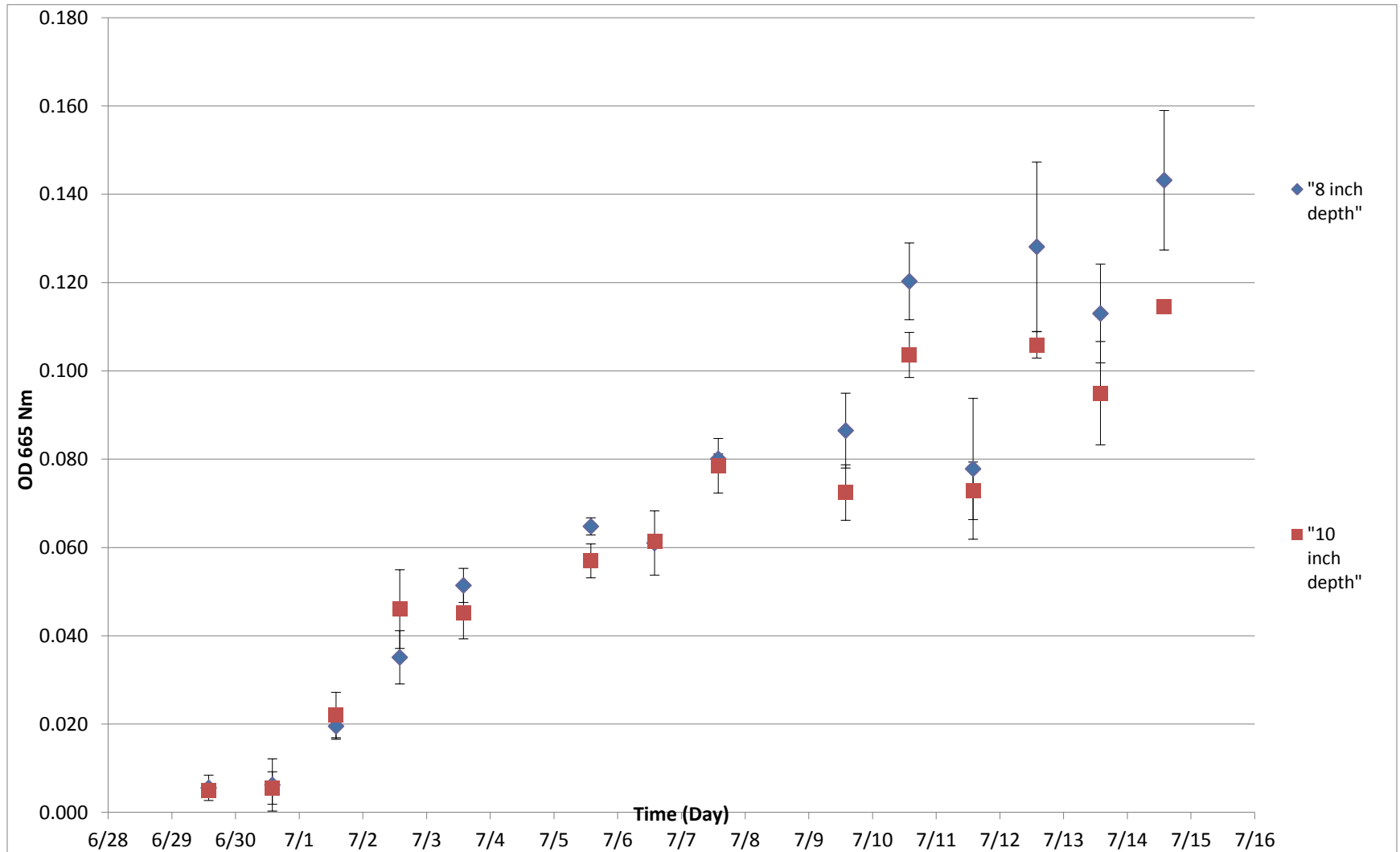
**Legend:**

- Ctrl= Control
- Fish= Fish emulsion
- Lcyb= Liquid cyanobacteria
- FFCOM= Fertilizer Combination (Fish emulsion + Compost)
- FLScyb= Fertilizer Combination (Liquid cyanobacteria + Solid cyanobacteria)
- Scyb= Solid cyanobacteria
- Compost = Composted manure

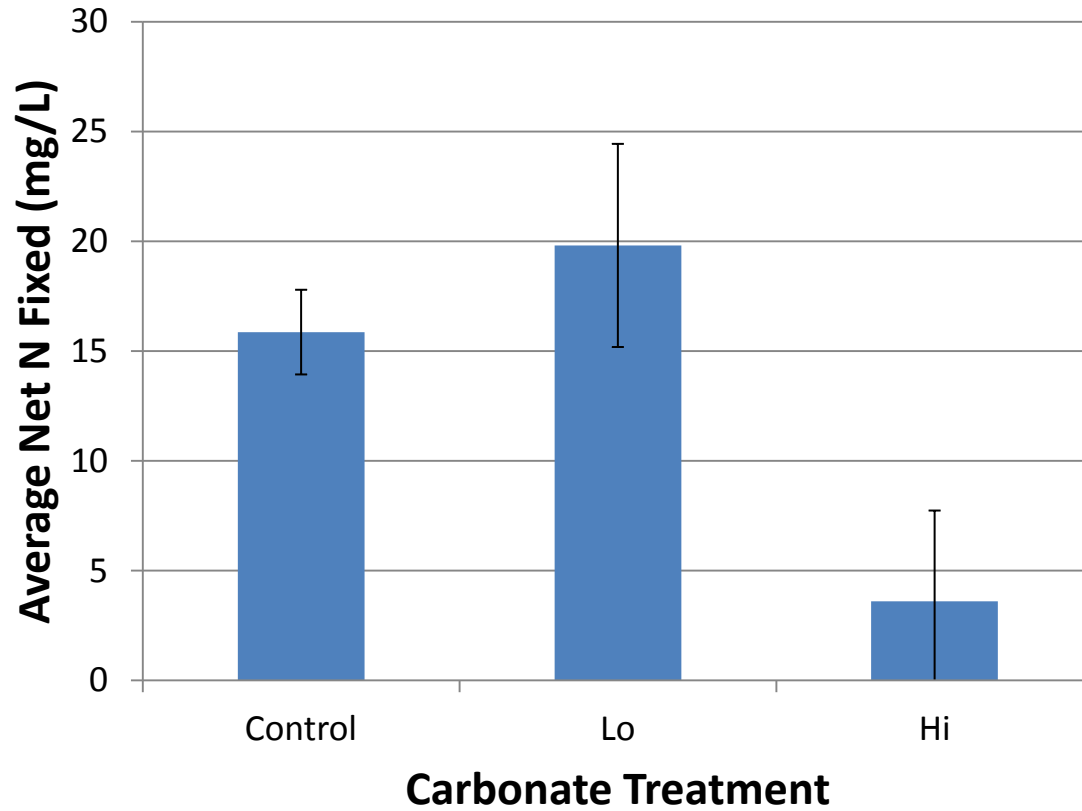
**Fig. 16:** Mean of fresh yield from soil and foliar applied organic fertilizer treatments on clayey soil at 56 and 112 kg N ha<sup>-1</sup> (e). Treatments with a common letter were not significantly different from each other (p<0.05) according to Tukey's test for mean separation.



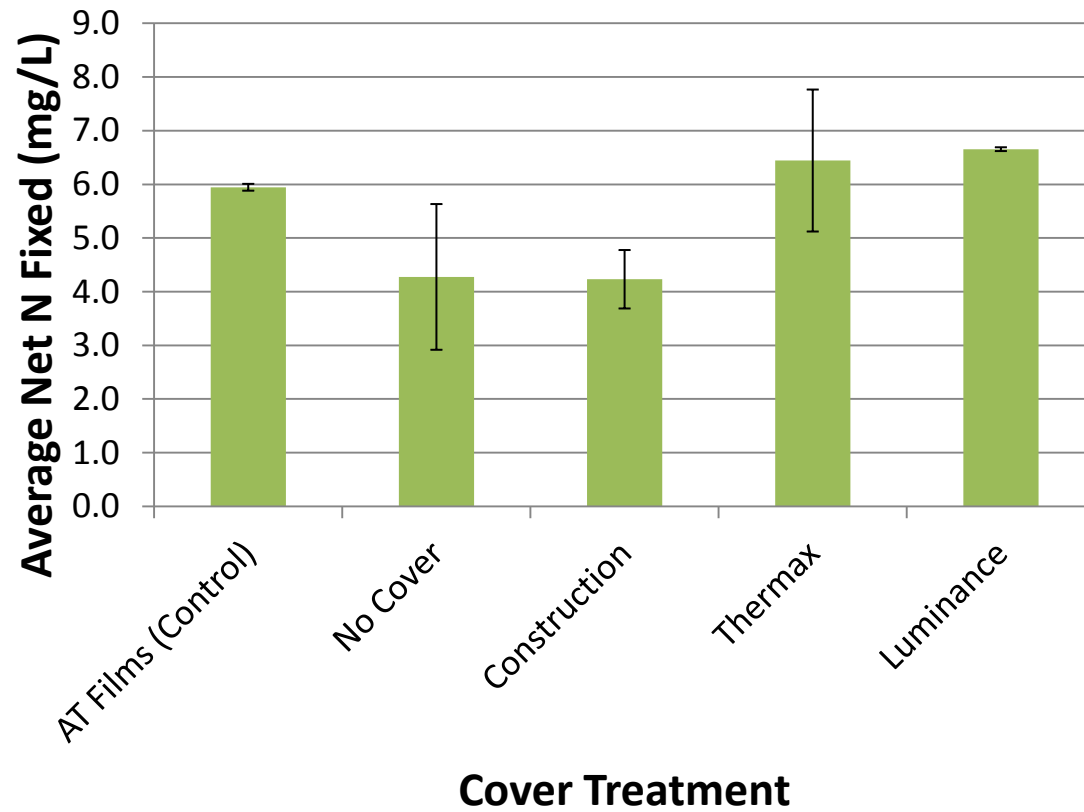
**Fig.17:** Lettuce grown with 100 lb N/acre application of a) composted manure, b) solid cyanobacteria, and c) liquid cyanobacteria.



**Fig. 18:** Optical density as a function of time and pond depth. The optical densities for the two pond depths were nearly identical until the last three days of the experiment, when the cyanobacteria growing in 8 inches of AA media seemed to surpass those growing in 10 inches of AA media.

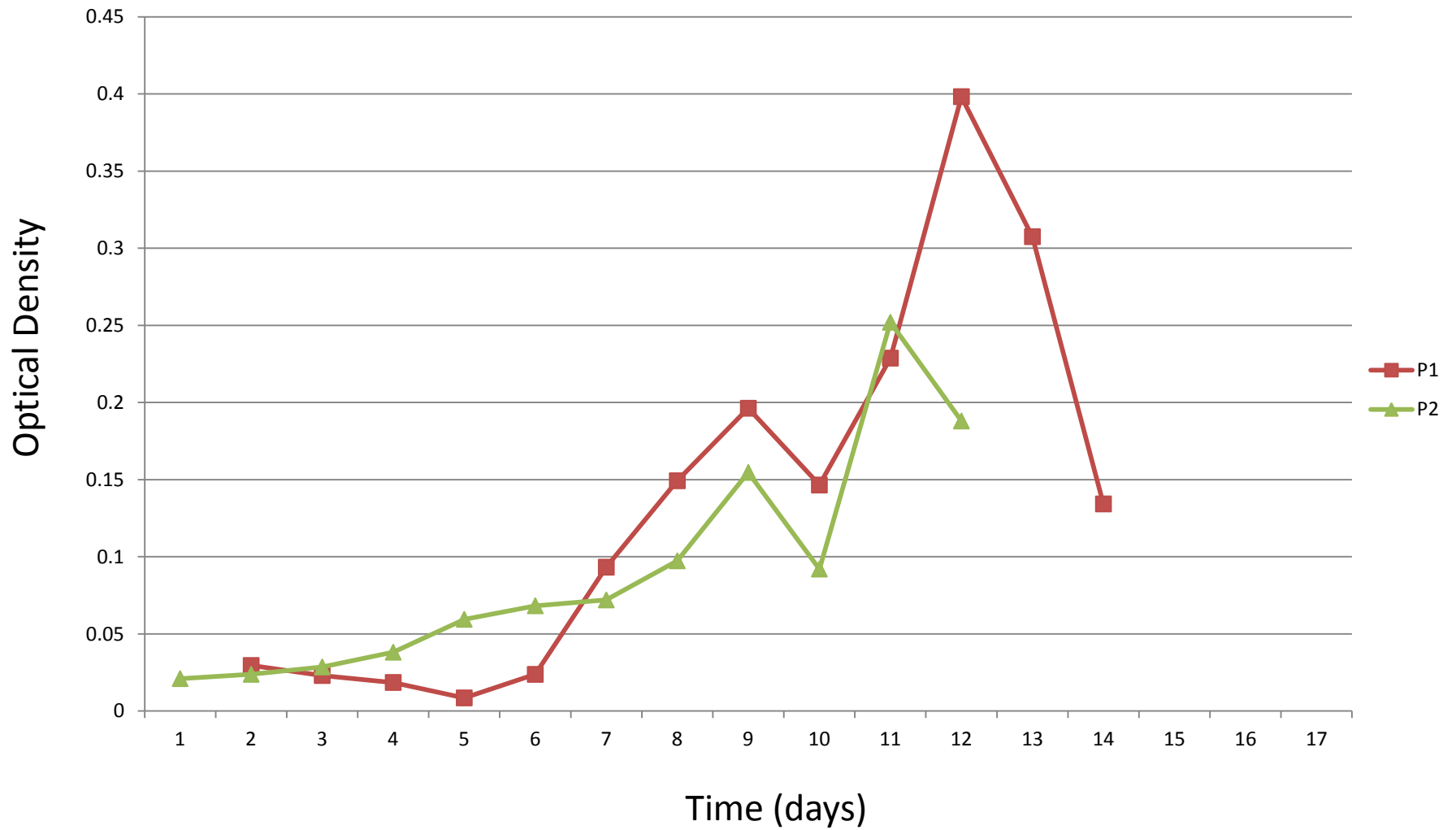


**Fig. 19:** Effect of carbonate addition on the Average Net N Fixed in the 50-gal pilot-scale raceways.



**Fig. 20:** Effect of hoop house film cover on average net N fixed.





**Fig. 21:** Optical density of cyanobacterial culture H4 grown in 625-gal prototype ponds. P1 is Prototype 1, and P2 is Prototype 2.

**Table 3:** Nitrogen fixation as a function of water use (lb N fixed/100,000 gal of water) for a variety of cover crops and cyanobacteria.

	lb N fixed/ 100,000 gal water
Forage soybeans	5.9
Red clover	9.2
Sweet clover	14.2
Hairy vetch	30.9
<b>Solid cyanobacteria</b>	<b>37.6</b>
Winter peas	51.0