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Satellite culture methods crop protection research

Satellite culture methods are essential to pond monitoring practices and especially useful for crop protection research since they allow the study of pest infection without losing large cultures. Thus they are powerful tools for developing pest treatment and tracking options, and understanding of pest life cycles. In order to do this the culture systems must produce algal growth or death from pests that mimics that observed in the pond environment. For this reason, whenever new strains, media conditions, or pests are used/discovered, all satellite culture methods must be tested for suitability to research such things.

Developing satellite culture methods for *Nannochloropsis*

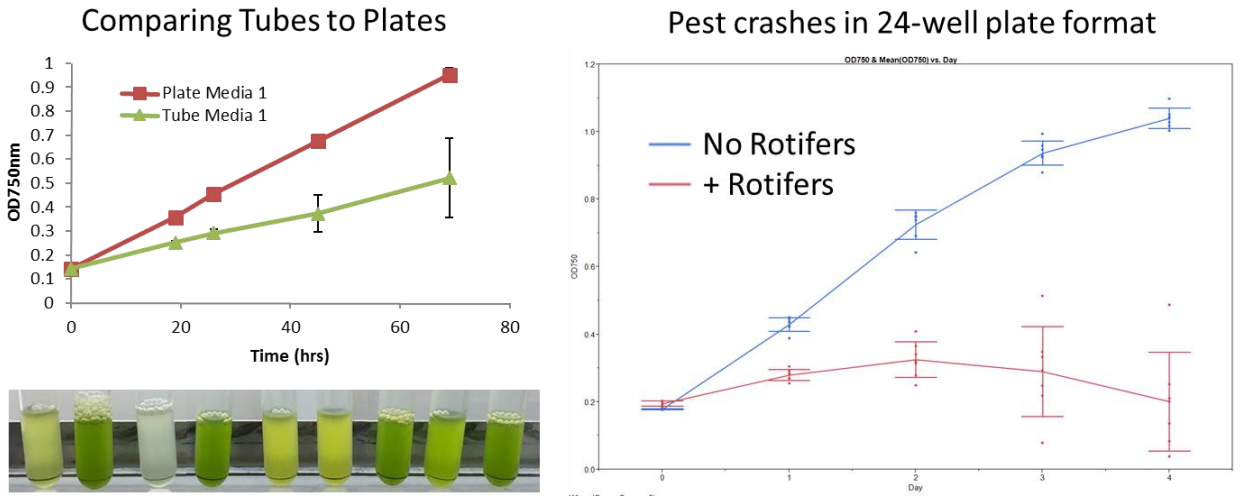

Figure 1. Comparing old and new methods for laboratory satellite culture. Left graph shows OD 750nm over time for identical culture grown in plastic test tubes (Tube) vs 24-well plates (Plate). Decreased and inconsistent growth was observed with tube cultures (note error bars in graph). Picture shows tubes cultures taken on the same day. Right graph shows pest induced culture crash in 24-well plates. Culture was grown with or without rotifers as shown.

Historically, culture in test tubes has been used to monitor growth of pond samples in the laboratory and preview potential treatment options. For this type of satellite culture, the goal is to speed up what is happening outside rather than mimic it precisely. For this reason, the cultures are maintained at constant temperature and light. Depending on the pest and time of year this can be predictive of a pest crash, up to 7 days in advance of it happening in the field, allowing proactive treatment. When *Nannochloropsis*

was cultured under standard conditions in tubes, the consistency of growth was poor (Fig. 1). It is unclear what the reason for this inconsistency is but it represented an opportunity to improve lab processes.

Alternative methods for cultivating satellite cultures in the laboratory were developed. The result of this was the use of 24-well deep well plates that contain 5ml of culture. This system consistently performed well in terms of algal growth and a number of pest models were shown to be pathogenic in this growth system. The advantages of using these plates is that it allows use of a multichannel pipette for many of the sample handling steps, increasing ease and throughput for many uses. The decision was made to use this method for all satellite culture experiments in the laboratory. The concerns with this system are evaporation, which could be solvable with investment in low evaporation lids. Currently the solution to this is to add water at routine intervals (e.g., after sample collection). The other concern is contamination and cross contamination since all the cultures are in close proximity and there is only one lid for all 24 samples. With careful sample handling and use of a flow hood it is possible to mitigate contamination.

Satellite methods used in 2015

Preview tubes

Laboratory satellite cultures were routinely used throughout the year and an example is shown in Figure 2. Potential crop protection treatments (including pesticides and chemicals identified in screening) were tested in this system and data was directly used to determine field actions on a weekly basis throughout the summer. The 24-well plate method performed well throughout the year for these and other experiments.

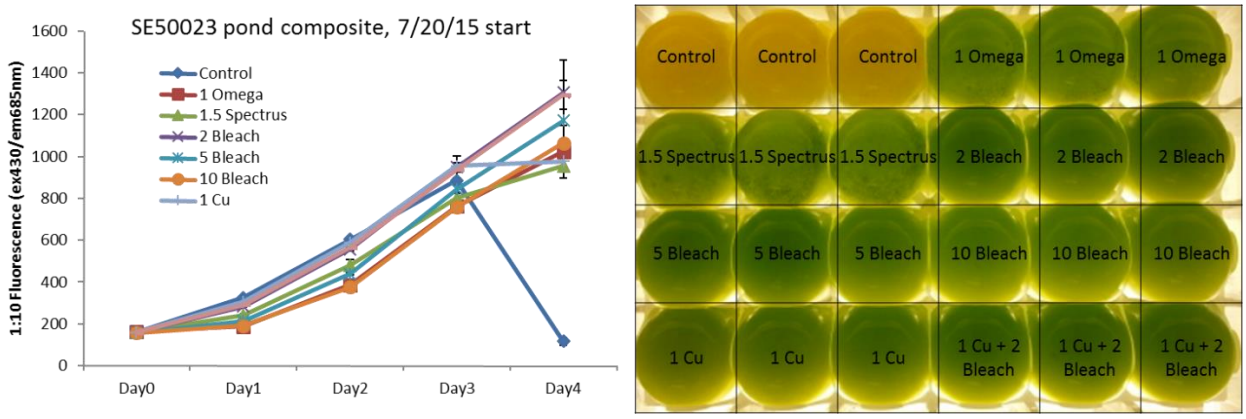

Figure 2. Example of "preview tube" satellite culture data used to determine crop protection action in *Nannochloropsis* ponds. Graph shows Fluorescence over time for culture from a composite sample of three raceway ponds sampled on 7/20/15 and cultured in 24-well plate with constant shaking, light, and temperature. Picture on right shows culture appearance after 4 days. The lack of fluorescence and brown color of the control on day 4 indicates a culture crash caused by pests. Multiple treatments delayed or prevented the crash. Chemical name and concentration (ppm) of active ingredient are shown.

Miniponds

Miniponds are a very valuable satellite culture method. Data from culture grown in miniponds can be directly compared to data from raceway cultures since this method represents the closest environment to the raceway culture (same environmental conditions and similar control mechanisms). A number of experiments this year showed that the data from miniponds and raceways with the same culture aligned very closely.

Reactors

Low volume reactors that mimic the pond environment offer similar advantages to miniponds, except with easier sample handling and more options for control of environmental conditions. In multiple experiments the reactors have shown close replication of field data (e.g., Fig. 4). This highlights their utility, particularly for research in winter season where "summer" experiments can continue to be used at small scale to prepare techniques for the following year.

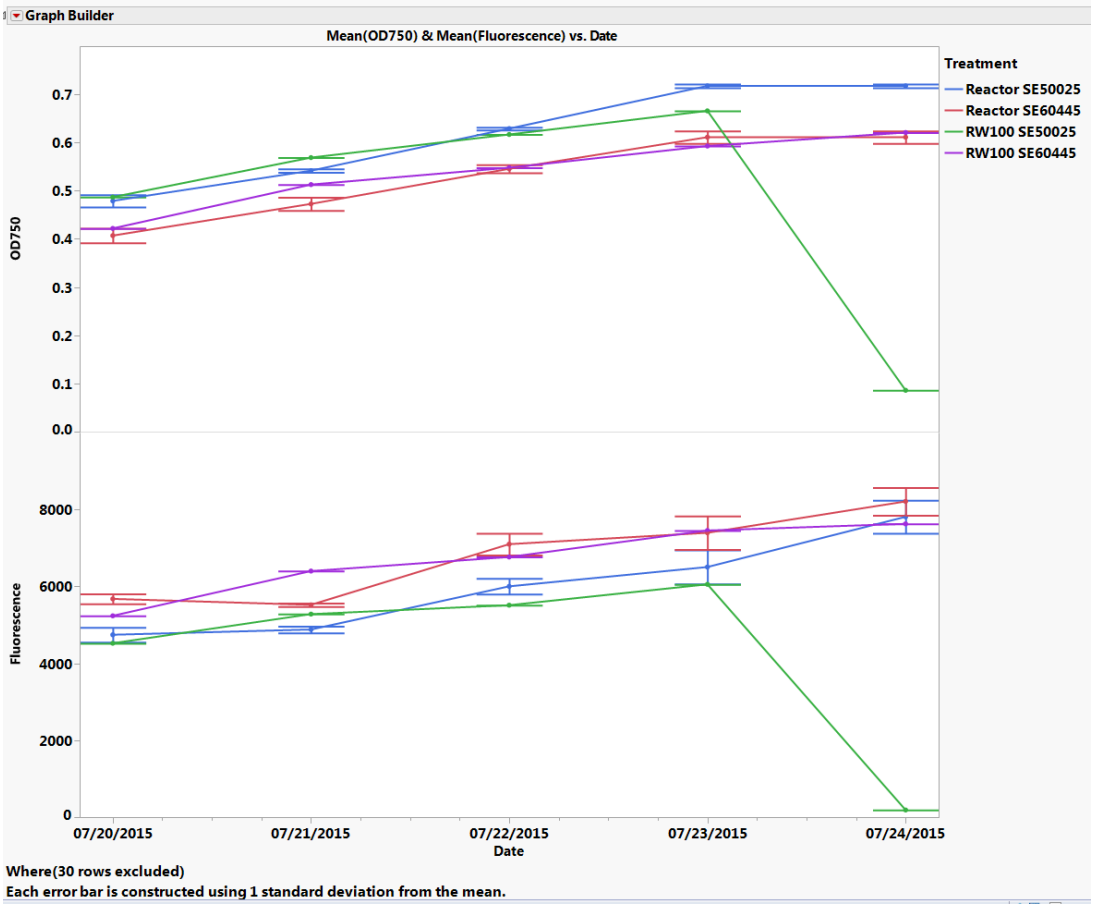

Figure 4. Comparing culture growth in raceways vs. reactors. Culture from two different raceways (RW100) was cultivated in reactors (6 reactors per pond sample is shown) at conditions similar to field conditions at the time. Graphs show OD 750nm (upper) and Fluorescence (lower) over time. On 7/23 RW100 SE50025 was harvested resulting in the drop in OD and fluorescence observed on 7/24.

One of the biggest advantages of reactors over other laboratory growth vessels is the ability to control pH directly in the culture. An observation was made during raceway experiments that suggested culture growth and/or health was increased at lower pH (Fig. 5. Left). Later, reactors were used to successfully test this hypothesis. Other experiments with clean culture (not shown) did not show an effect of pH. This and other observations led to the conclusion that pH was more likely affecting pests and/or crop protection.

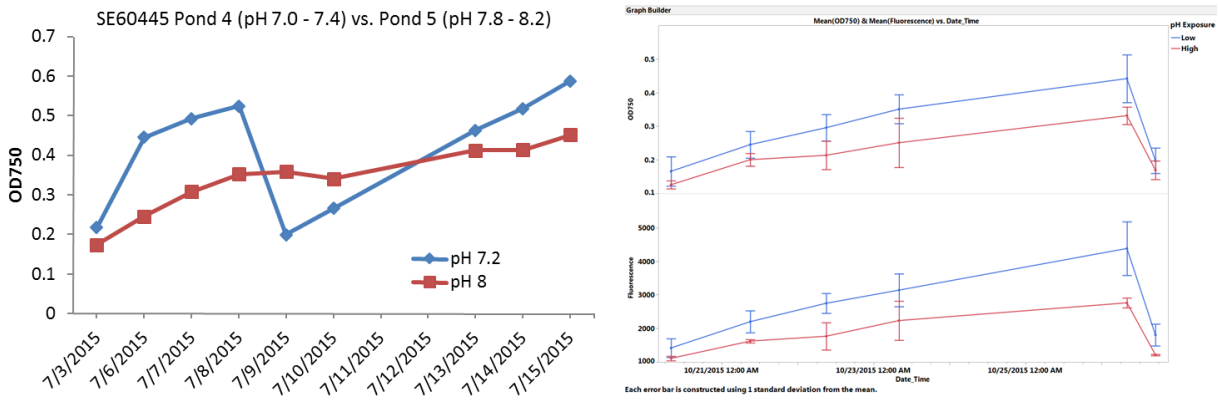

Figure 5. Effect of pH on culture in the presence of pests. Raceways containing pest FD111 were cultivated at two different pH setpoints. Left panel shows OD750nm over time for these cultures at pH 7.2 (blue) and 8.0 (red). Later, similar parameters were used in reactors mimicking conditions using culture from outdoors that also contained pest FD111. Right panel shows OD750nm (upper) and fluorescence (lower) over time for reactors (how many?).