

3115 Merryfield Row San Diego, California 92121 www.sapphireenergy.com

| File Name: |                | Version: |                 |            |  |
|------------|----------------|----------|-----------------|------------|--|
| Title:     | Recycled Media |          |                 |            |  |
| Status:    | Draft          |          | Effective date: | 10-24-2011 |  |

## **SIGNATURES**

Signed by Date Justification

Douglass Fenton 10-24-2011

## I. EXECTUTIVE SUMMARY

This report analyzes the best way to condition our media, after we perform a harvest using polymer, before it is returned to the pond. We found that not conditioning the media was the best for the pond health and production.

# II. PURPOSE

The purpose of this report is to validate the way media is conditioned at the Las Cruces Test Site (L.C.T.S.). After a "dissolved air flotation" (D.A.F.) harvest has been performed our media is conditioned, before returning it to the pond, to see if the process affects the health or growth of the algae.

# III. BACKGROUND

Currently, the L.C.T.S. media is conditioned by adding bleach at 125ppm for an hour to neutralize the polymer, and then Sodium Thiosulfate is added at 90ppm for an hour to neutralize the bleach, before being returned to the pond after a D.A.F. harvest. Polymer is a chemical flocculent, which causes algae to stick and clump together and float, making it easier to harvest through the D.A.F. system on site. The polymer used in this experiment to polymerize SE107 is "Ashland 859BS". This process seems to be effective, but sometimes ponds will become sick and/or die after the conditioned media is returned, and research is needed to locate what step(s) in our process could be causing these problems.

## IV. HYPOTHESIS

Not conditioning the media before it is returned to the pond will give an increased productivity/growth rate and biomass accumulation when compared to the conditioned returned media.

#### V. EXPERIMENTAL DESIGN AND RESULTS

For this experiment 15 flasks are inoculated with SE107 into 250ml of 6AC3-SC2 at an O.D. of ~0.2. When the flasks reach an O.D. of 0.5 or above 100ml (2/5 of culture) are harvested out of each flask, conditioned, and returned back to the flask. The O.D. of each flask is recorded before and after each harvest to keep track of growth rates and total biomass production. In addition, the flasks are supplemented with a stock solution containing: nitric acid, phosphoric acid, and trace metals. The supplements are added when the majority of the flasks drop below 150ppm of nitric acid, just as they would supplement a pond at the L.C.T.S. Throughout the experiment biomass and media samples are given to analytical, typically when a set of flasks or a couple sets of flasks appear to be sick or dying, to run various tests to see what could be causing this anomaly. When media sample are submitted new media would be added back into the flask.

For this experiment five different processes were used to condition media, before returning it to the pond, testing to see the media's effect on the health and productivity of the culture.

| Flocc-Bleach            | Polymer is added to harvest the algae on the D.A.F., then bleach is added to neutralize polymer for one hour at 125ppm, then Sodium Thiosulfate is added to neutralize the bleach for one hour at 90ppm, and then returned to the pond.            |
|-------------------------|--|
| Flocc-No Treatment      | Polymer added for harvest and then immediately returned to the pond.   |
| Centrifuge-Bleach       | A centrifuge is used to harvest the algae (5 min, 3000rpm), then bleach is added to neutralize the polymer for one hour at 125ppm, then Sodium Thiosulfate is added to neutralize the bleach for one hour at 90ppm, and then returned to the pond. |
| Centrifuge-No Treatment | A centrifuge is used to harvest the algae (5 min, 3000rpm), then immediately returned to the pond.   |
| Centrifuge-Polymer      | A centrifuge is used to harvest the algae (5 min, 3000rpm) then immediately returned to the pond, with polymer added.  |

Table 1: Five different media conditioning process were tested during this experiment

Unfortunately, we do not have a D.A.F. at the San Diego test site. Therefore, to mimic the D.A.F there, polymer would be added to the harvested media and allowed to mix for 10 min, and then settle for 10min, before centrifuging out the biomass. This allows the polymer to mix in with the media and cells as it would in the D.A.F. at the LCTS. In addition, we hope that centrifuging does not pack down the polymer with the biomass and stays suspended in the media while being centrifuged.

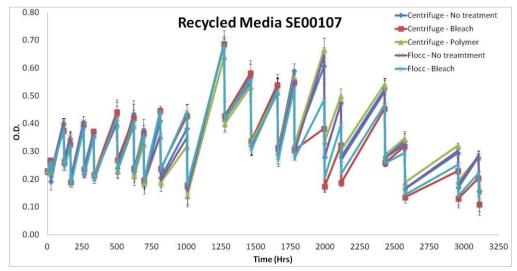


Figure 1: Growth curve of the culture during the duration of the experiment.

All the cultures survived the length of the experiment without crashing. However, as the experiment progresses the conditions using bleach tended to have a lower O.D.s than the other conditions

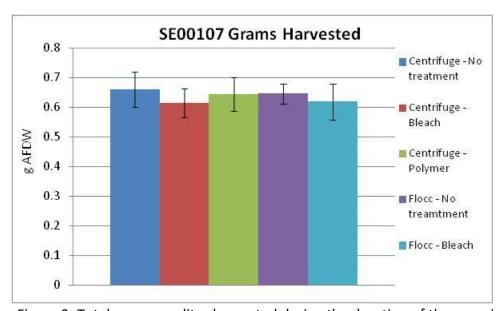


Figure 2: Total grams per liter harvested during the duration of the experiment.

This figure shows that during the experiment the conditions that involved bleaching the media produced the same or less than the three other conditions where media was not conditioned and just added back into the flask.

# VI. CONCLUSION

Both the similarity in the growth curve and the total grams per liter harvested shows that the conditioning of the recycled media after a harvest has little to no observable effect on the productivity or harvest-ability of a culture of SE00107.