Algal Lipids and Omega-3 Production via Autotrophic and Heterotrophic Pathways at Cellana's Kona Demonstration Facility, Hawaii

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introduction

Cellana's Kona Demonstration Facility (KDF) is a 2.5 hectare facility, with 17,000 sq. ft. under roof and 1 hectare of cultivation systems. KDF is designed to execute and support all stages of the production process at pilot scale, from cultivation through extraction. Since Feb. 2009, KDF has been producing up to 0.7MT dry weight of algal biomass per month, while at the same time optimizing processes of cultivation, harvesting, dewatering and extraction. The cultivation system at KDF uses ALDUO™ technology, a hybrid system of photobioreactors (PBRs) and open ponds. All fluid transfers related to KDF cultivation and harvesting processes are operated and monitored by a remote Process-Control System. Fluid transfer data, together with biochemical data, enable the mass balance calculations necessary to measure productivity.

This poster summarizes methods to improve both biomass and lipids yield by 1) alleviating light limitation in open ponds, 2) de-oxygenation and 3) heterotrophic lipid production for post-harvesting cultures.

Photobioreactors (PBRs)

Open Ponds

methods

Top algal strains, selected by strict high throughput screening, were grown in PBRs before transferring to open ponds for biomass and lipid production.

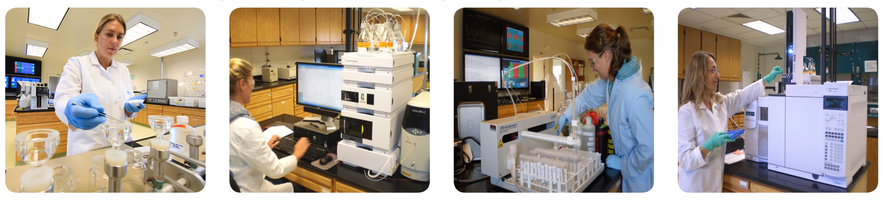
It has been found that autotrophic lipid production is often limited by light availability due to self-shading at the later stage of growth. A stepwise approach to investigate this production challenge was undertaken which allowed for biomass accumulation first and lipid accumulation later under increased light availability by splitting biomass into two ponds.

One of the top strains, C323, was tested for heterotrophic lipid production with multiple organic carbon sources. Glycerol was proved to be the most effective for lipid production in C323. Culture at post-harvesting concentration was tested under both light and dark conditions.

The mid-scale system at KDF, which was designed to simulate the large-scale ALDUO™ production system, is a powerful tool for algal research and development. It serves to screen algae stains and optimize production. Because it is located outdoors, light and temperature bias were greatly reduced compared to any indoor screening system. At 200L, its scale is large enough to yield highly robust and reproducible data, while being small enough to be efficient and cost-effective. The design of water circulation in the mid-scale systems enables it to predict behavior in the large ALDUO™ system accurately. Consequently, numerous rounds of controlled experiments were conducted using this system for strain screening and optimization.

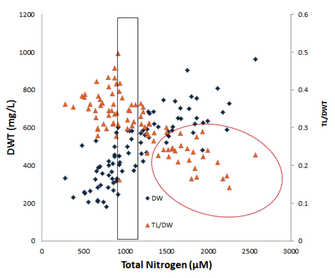
Multiple Omega-3-rich strains were scaled in triplicate and the strain with the best characteristics was selected. The growth conditions of this strain were then further optimized for Omega-3 production, including stock density indicated as total nitrogen loading, salinity, and physiological conditions indicated as C/N ratio.

High throughput screening of algal strain candidates

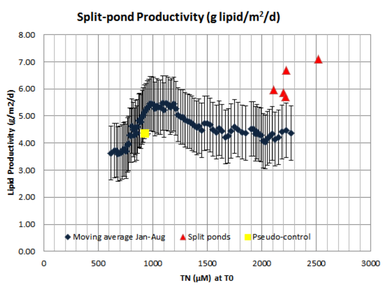


results and discussion

1. Ultimate limiting factor for autotrophic lipid production



Low lipid productivity in open ponds, caused by inverse relationship between biomass and lipid content due to self-shading. Split pond model allows for biomass accumulation on day 1-2, and lipid on day 3 under high light availability.

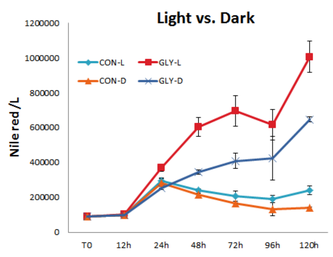


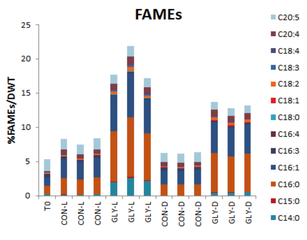
- 37% higher lipid areal productivity
- 78% higher in biomass production
- 66% higher in lipid production

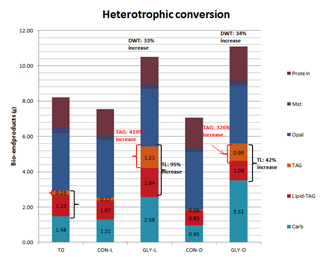
results and discussion continued

2: Mixotrophic and heterotrophic glycerol uptake

Post-harvested C323 biomass responded to glycerol addition under both light and dark conditions indicated by the significant enhancement of neutral lipids measured by Nile Red Fluorescence. Mass balance exhibited significant increase in both biomass and bio-products, including both lipids (mainly TAG) and carbohydrate. More carbon flux into lipids under light and more into carbohydrate under dark were observed. Total FAME increased 3.7 and 2.4 times under light and dark conditions, respectively. PUFA content decreased under glycerol uptake, but total PUFA remained the same due to the increase of biomass.







Increase under light:

- 33% in DWT
- 419% in TAG
- 95% in lipids
- 74% in carbohydrate

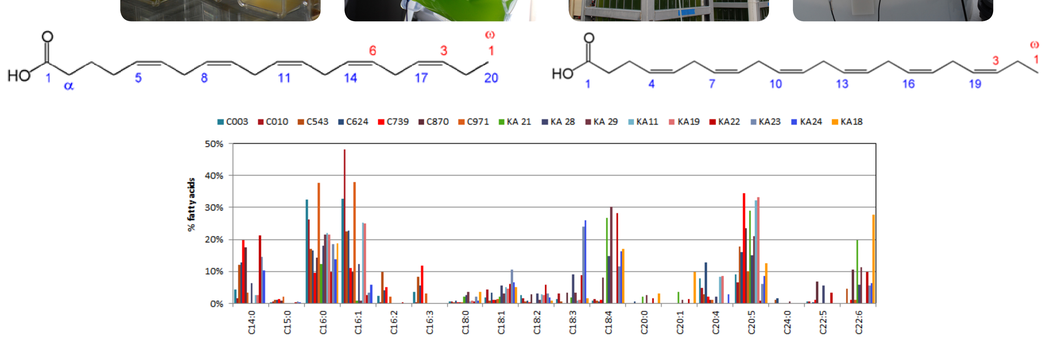
Increase under dark:

- -34% in DWT
- -326% in TAG
- -42% in lipids
- -138% in carbohydrate

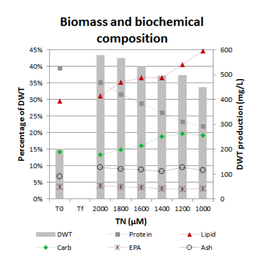
3. Omega-3 screening

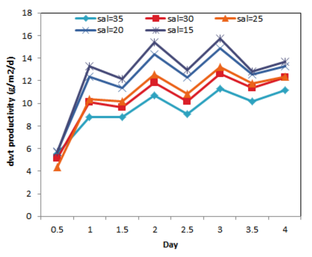
KDF has more than 500 strains, with numerous strains rich in EPA and/or DHA based on high-throughput screening data. Multiple algal strains were screened at mid-scale for Omega-3 production. One strain was fully optimized and scaled up for large strain production. So far, more than 1.5 MT biomass has been produced.

Scaling/testing of selected algae strains



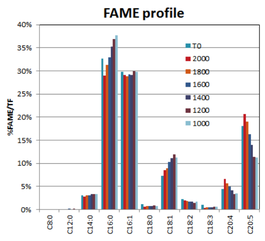
4.Omega-3 optimization

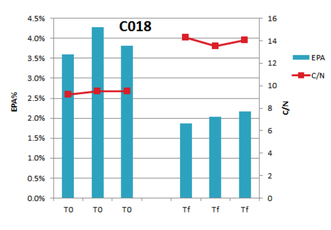




Omega-3 content was determined daily during a pond cycle, under different total nitrogen loading and physiological status.

- For both C018 and C970, the content of Omega-3 decreased over time.
- Both biomass yield and EPA content increased with increasing nitrogen loading.
- Similarly, EPA content was higher at lower C/N ratio.





conclusion

- Light availability is one of the major limiting factors for autotrophic lipid production. Split pond model optimizes both biomass and lipid production in open ponds.
- A diatom produced in open ponds is capable of heterotrophic organic carbon uptake. Glycerol, a biodiesel waste product, was proven to be highly favorable for lipid/TAGs/FAMEs production.
- Numerous strains at KDF are rich in EPA and/or DHA.
- One EPA-strain produced more than 1.5 MT at large scale.
- Another strain rich in both EPA and DHA is being prepared for large scale production at KDF in Sept. 2012.

acknowledgements

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