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We would like to submit the following manuscript as an article to JOVE.

Experimental Protocol for Biodiesel Production with Isolation of Alkenones as Coproducts from Commercial *Isochrysis* Algal Biomass

Recently our group has been investigating biofuel production from a marine microalgae *Isochrysis*. This work has focused on a unique class of algal lipids known as long-chain alkenones that have been largely unexplored as a potential fuel source (O'Neil et al., *Energy Fuels* **2012**, 2434; *Energy Fuels* **2014**, 2677; *Energy Fuels* **2015**, 922). Alkenones are biosynthesized by a select number of microalgae that includes *Isochrysis* sp. *Isochrysis* is also one of only a few algae currently grown industrially, harvested for purposes of mariculture.

For all of our previous work we have used *Isochrysis* purchased from Reed Mariculture (San Jose, CA), who supplies a wet (80% water) paste that is dried prior to processing. In this manuscript we compare processing and results from Reed to another commercial source of *Isochrysis*, Necton S.A. (Olhão, Portugal). Multi-kilogram quantities of *Isochrysis* can also be purchased from Necton, but in this case as a dry (5% water) fine milled powder. The color of the algae from Necton is also strikingly different compared to Reed (yellow-brown vs. dark-green near black from Reed) yet all final products are similar in appearance. In addition to yields, we also compare fatty acid profiles which is of importance to the fuel properties of a corresponding biodiesel.

The availability of the algae utilized and standard methods employed, make the protocol presented widely accessible to other groups. We hope that you will find this manuscript worthy of publication and look forward to the opportunity to create a video reference that will encourage further investigations into important lines of research that emerged from the results we describe.

Sincerely,



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