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From: Gregory O'Neil

To: Nam Nguyen, Science Editor, JOVE

Date: January 21, 2016

Editorial and reviewer comments are appreciated and have been addressed in the revised manuscript as outlined below:

Editorial:

- 1. The document has been thoroughly proofread
- 2. All abbreviations have been defined at their first use within each section (e.g. FFAs in the Long Abstract, Protocol (Step 3.10), and Results sections)
- 2.1. Soxhlet specifications in Protocol Step 2.1 have been removed as requested.
- 2.2. The description of fatty acid quantitation (Protocol Step 4.6) is a note in the references section as requested (reference #29).
- 3. Protocol Steps 3.5-3.8 and 4.5 are highlighted as suggested.
- 4.1. Additional detail has been added to Protocol Steps 4.1 and 5.5 to make clear how the solvents are introduced and their purpose.
- 4.2. A reference to Protocol Step 3.8 has been added to Step 6.1 to remind the reader of when the neutral lipids were obtained.
- 5. The names Reed and Necton now only appear as a note in the Protocol section.
- 6. Possibilites for future work using the protocol described are given in the first paragraph of the Discussion (Lines 325 334). These include analyzing yields from various Isochrysis strains and commercial products and other extraction protocols.

Reviewer #1:

- 1. **Comment**: "It will also be interesting to see a small paragraph to explain what has been done and what was the need for this protocol." **Response**: A few sentences have been added (Lines 102-106) to explain the purpose for separating alkenones and fatty acids with reference to our previous work.
- 2. **Comment**: Request to "prove the consistency of the results from this protocol." **Response**: Wording has been added with reference to our three previous articles to demonstrate the consistency of yields from Reed *Isochrysis*. Table 1 shows duplicate results obtained from Necton *Isochrysis* (*Iso*-powder-1 and *Iso*-powder-2) and references our prior work as proof of consistency.
- 3. **Comment**: "Mention the moisture content after 24 h." **Response**: The moisture content after 24 h. varies depending on the actual "room temperature". A reference has been added (#40) to clarify.

We hope that you will find this revised manuscript worthy of publication in your prestigious journal.

Sincerely,

Dr. Gregory W. O'Neil

Associate Professor of Chemistry

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