



## Short communication

Decolorization improves the fuel properties of algal biodiesel from *Isochrysis* sp.Gregory W. O'Neil<sup>a,\*</sup>, Gerhard Knothe<sup>b</sup>, John R. Williams<sup>a</sup>, Noah P. Burlow<sup>a</sup>, Christopher M. Reddy<sup>c</sup><sup>a</sup> Department of Chemistry, Western Washington University, Bellingham, WA 98225, United States<sup>b</sup> National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, IL 61604, United States<sup>c</sup> Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, United States

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## ABSTRACT

Results from the comprehensive fuel testing according to American Society for Testing and Materials International (ASTM) standards of an alkenone-free and decolorized biodiesel produced from the industrially grown marine microalgae *Isochrysis* sp. are presented. Fatty acid methyl ester (FAME) profiles of the non-decolorized and subsequently decolorized biodiesel fuels were nearly identical, yet the fuel properties were remarkably different. Significant positive impacts on the cetane number, kinematic viscosity, and lubricity were observed, indicating a potential deleterious effect of pigments like chlorophylls and pheophytins on these fuel properties. The decolorization process using montmorillonite K10 gave on average 90% mass recovery, and allowed for an otherwise unobtainable cloud point determination. Oxidative stability of the decolorized *Isochrysis* biodiesel remained well below the minimum prescribed in biodiesel standards due to elevated content of highly polyunsaturated fatty acids, however other values were in the range of those prescribed in the ASTM standards. Overall, decolorization improved the fuel properties of biodiesel from *Isochrysis* and may provide a path toward improved biodiesel fuels from other algal species.

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## 1. Introduction

Following an approximately ten year hiatus after the United States Department of Energy's Aquatic Species Program (U.S DOE ASP) ended in 1996 [1], there has been a great resurgence of interest in algae as a potential source of biofuel in the last decade [2–4]. Some species of algae have reportedly very high oil contents (15–300 times more oil for biodiesel production than traditional crops on an area basis) [5–7], can be grown on brackish- or wastewater and otherwise non-cultivable land [8], and may achieve higher CO<sub>2</sub> sequestration capabilities than terrestrial plants [9–11]. Another suggested benefit described by both critics [12] and proponents of algal biofuel programs and within the United States Department of Energy's National Algal Biofuels Technology Roadmap [13], is the potential for other high-value co-products that may be used to offset production costs of the fuel.

However, the properties of the fuel produced must also be considered because it has been discussed that many algal biodiesel

fuels may have problematic properties [14]. In order to be commercialized, biodiesel must meet a variety of specifications described in the standards ASTM D6751 and EN 14214 in the U.S and Europe, respectively. Requirements include minimum values for oxidative stability and cetane number, allowable ranges for kinematic viscosity, maximum free and total glycerol and heteroatom (Na, K, S, P, Ca, Mg) contents, while cold-flow properties are addressed by a report of the cloud point (CP) in ASTM D6751 due to different requirements dictated by time of year and geographic location. Others have noted the challenges associated with examining the fuel properties of algal biodiesel due to the small experimental quantities that are generally produced compared to the larger amounts required for each individual test [15]. For this reason, many of the reports describing algal biodiesel fuel properties are incomplete with algal biodiesel blended with petrodiesel to complete the fuel testing [16] or have relied on predictions [17] and simulations such as using a mixture of biodiesel from vegetable oil and fish oil to model algal biodiesel fuel properties [18].

Our group has been investigating biodiesel and other co-products from the marine microalgae *Isochrysis* sp. [19–21]. *Isochrysis* is one of only a few species of algae currently grown industrially, harvested for purposes of mariculture, and available internationally on multi-kilogram scale from several suppliers

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[22]. Crude *Isochrysis* biodiesel, prepared from the total lipid extract of dried *Isochrysis* biomass, suffers from severe cold-flow issues (i.e. it is solid at room temperature) [19]. We showed that these cold-flow problems are likely associated with contamination of this material by a unique suite of lipids known as polyunsaturated long-chain alkenones biosynthesized by a few haptophyte algae including *Isochrysis* (Fig. 1) [23–25]. Owing to their long carbon-chain length (36–40 carbons), non-methylene interrupted *trans*-double bonds, and resulting high melting points, we demonstrated that even minor amounts of alkenones can have a deleterious impact on the CP of a B20 biodiesel blend [19].

We therefore developed a protocol for preparing an “alkenone-free” *Isochrysis* biodiesel based on saponification/extraction techniques, and sufficient quantities were produced to allow for a range of fuel tests according to ASTM standards [20]. Unfortunately, however, we were unable to measure a CP due to the dark color of this material and thus confirm our original hypothesis connecting alkenones to the obvious poor cold-flow properties of our crude biodiesel. Herein we report the production and comprehensive fuel testing of a decolorized alkenone-free *Isochrysis* biodiesel, with pigments removed using montmorillonite K10 clay. In addition to allowing for CP analysis, a comparison of other fuel property values for our decolorized vs. non-decolorized biodiesel fuels revealed important insights about the potential impact of common pigments on fuel properties, along with remaining challenges for the production of a biodiesel from *Isochrysis* meeting ASTM standards as well as from presumably other algal feedstocks.

## 2. Materials and methods

### 2.1. Microalgae and sample preparation

The marine microalgae *Isochrysis* sp. “T-iso” used in the present study was obtained as strain CCMP1324 from Reed Mariculture (San Jose, CA) [27]. Approximately eight kilograms of wet biomass (20% biomass w/w) was freeze-dried in ~100 g batches, which resulted in an *Isochrysis* sp. as a greenish, dark-brown solid with an earthy seaweed-like smell. The resulting dry *Isochrysis* was then processed batchwise (100–200 g dry biomass) into biodiesel samples (e.g. D-Iso-1, D-Iso-2, and D-Iso-3) that were analyzed separately (*vide infra*).

### 2.2. Extraction and processing of lipids to produce an alkenone-free biodiesel

The dry *Isochrysis* sp. biomass was extracted and the obtained lipids processed into isolated alkenones and biodiesel as previously described [19–21]. Briefly, dry biomass is extracted with hexanes in a Soxhlet extraction apparatus. Hexanes were removed with a rotary evaporator giving a dark green near-black solid. The hexanes-extractable material (“hexane algal oil”) is then saponified (KOH, MeOH, CHCl<sub>3</sub>) allowing for separation of the resulting free fatty acids (FFAs) and alkenone-containing neutral lipids. Acid-catalyzed esterification (MeOH, cat. H<sub>2</sub>SO<sub>4</sub>) of the FFAs gives alkenone-free “non-decolorized” biodiesel (i.e. fatty acid methyl esters, FAMES).

### 2.3. Biodiesel decolorization [26]

To the dark green colored biodiesel obtained above (15 g) at 60 °C was added montmorillonite K 10 (MK10) powder (3.0 g, 20% w/w of the biodiesel) and the mixture was stirred for 1 h. The solution was then filtered through Celite with hexanes and the hexanes were removed on a rotary evaporator to produce an orange/red biodiesel (13 g, on average 90% w/w mass recovery). Samples were stored at 4 °C during which time some settling of insoluble material (<10% w/w) occurred. Decanting gave a clear homogeneous biodiesel that was analyzed separately.

### 2.4. Analysis by gas chromatography with flame ionization detection (GC-FID)

Fatty acid profiles were determined by gas chromatography utilizing a Perkin-Elmer Clarus 580 gas chromatograph equipped with a DB-88 [(88% cyanopropyl) methylarylpolysiloxane] column (30 m × 0.25 mm ID × 0.20 μm film thickness) and otherwise as described in the literature [27]. Common fatty acid methyl esters were verified by retention time comparison with authentic samples obtained from Nu-Chek Prep, Inc. (Elysian, MN). Additionally, gas chromatography-mass spectrometry (GC-MS; Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973 mass selective detector) was performed under identical conditions of temperature program and column to analyze components such as C18:4 for which no authentic standards are available with the results applied to GC quantitation.

### 2.5. Fuel properties

Cetane numbers were determined as derived cetane number (DCN) using an Ignition Quality Tester™ (IQI™) as described in ASTM D6890 at Southwest Research Institute, San Antonio, TX [28]. CP was determined with a Phase Technology (Richmond, BC, Canada) cloud, pour and freeze point analyzer. Kinematic viscosity was determined according to the procedure described in the standard ASTM D445, oxidative stability according to EN 14112 using a so-called Rancimat instrument, and lubricity with a high-frequency reciprocating rig (HFRR) lubricity tester according to ASTM D6079. Density was measured with an Anton Paar (Anton Paar USA, Richmond, VA) DMA 4500 density meter. Free and total glycerol as well as monoglyceride content were determined according to the gas chromatographic procedure described in the standard ASTM D6079

## 3. Results and discussion

Table 1 contains information on the fatty acid methyl ester composition of the decolorized biodiesel. Table 2 lists the fuel properties of this material.

### 3.1. Production of a purified non-decolorized *Isochrysis* biodiesel

Results for the extraction and subsequent separation of FFAs and neutral lipids from dry *Isochrysis* biomass, followed by esterification of the FFAs to biodiesel were consistent with those previously reported [19–21]. The hexane algal oil obtained was a glossy,

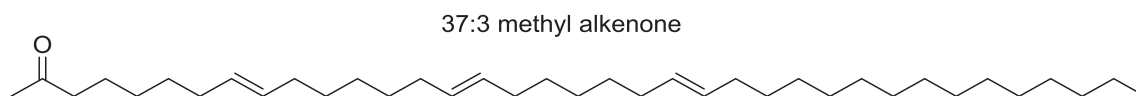


Fig. 1. Structure of a common alkenone produced by *Isochrysis* sp. Nomenclature is # of carbons: # of double bonds, where methyl refers to a methyl ketone.

**Table 1**FAME composition of non-decolorized (Non-D),<sup>23</sup> and decolorized *Isochrysis* biodiesel (D-Iso) samples.

FAMES	Non-D <sup>a</sup>	D-Iso-1	D-Iso-2 <sup>d</sup>	D-Iso-3
14:0	13.9	14.3	17.1	16.4
15:0	0.2	ND	0.4	Trace
16:0	11.0	10.1	12.4	10.1
16:1 $\Delta^9$	5.9	7.0	6.6	7.6
16:2	0.6	ND <sup>f</sup>	Trace	ND
16:3	0.7	ND	ND	ND
18:0	Trace	ND	1.1	Trace
18:1	11.4 <sup>b</sup>	10.8 <sup>b</sup>	11.0 <sup>b</sup>	12.1
18:2	7.4	9.9	6.5	8.1
18:3	6.8 <sup>c</sup>	10.3 <sup>c</sup>	6.9 <sup>c</sup>	8.5
18:4	20.7	21.0	18.3	19.8
18:5	1.6	ND	ND	ND
20:5	1.6	ND	ND	ND
22:5	1.1	ND	ND	ND
22:6	10.6	8.4	8.2	6.9
$\Sigma$	94.9	91.8 <sup>e</sup>	88.5 <sup>e</sup>	89.8 <sup>e</sup>

<sup>a</sup> Average values from three separate samples.<sup>b</sup> Combined 18:1  $\Delta^9$  + 18:1  $\Delta^{11}$ .<sup>c</sup> Combined  $\Delta^6$ , 9, 12 and  $\Delta^9$ , 12, 15 isomers.<sup>d</sup> Prepared from an older hexane algal oil that was stored at  $\sim 20^\circ\text{C}$  for two years before being used for this study.<sup>e</sup> The remaining material is roughly 50:50 other FAMES and non-FAME components (Total  $\sim 95\%$  FAME).<sup>f</sup> ND = not detected.

dark green/near-black grease-like material (typically 20% w/w of the dry biomass). Saponification of hexane algal oil with KOH in methanol then allowed for separation of the saponified FFAs from alkenones and other non-polar compounds with yields consistently quantitative (60% FFAs +40% neutral lipids). Acid-catalyzed esterification of the FFAs produced a dark green colored biodiesel (“non-decolorized biodiesel”) that has previously undergone extensive analysis (see “Non-D”, Table 2) [20].

### 3.2. Decolorization of the biodiesel using montmorillonite K 10

Based on the work of Dalai [26], stirring our green biodiesel over 20% (w/w) MK10 for 1 h at  $60^\circ\text{C}$  proved highly effective in removing pigments, visualized by the color change of the biodiesel and the MK10 itself going from a white powder to black (Fig. 2). After decolorization, a small amount ( $<10\%$  w/w) of “grainy material” previously described became evident [21]. Upon storage these compounds settled to the bottom of the vessel and could be easily removed by decanting or, if necessary, centrifugation. Mass

**Fig. 2.** Comparison of non-decolorized (left) and decolorized (right) *Isochrysis* biodiesel fuels.

recoveries of particulate-free biodiesel after decolorization were generally 85–95% (w/w).

### 3.3. FAME analysis of decolorized *Isochrysis* biodiesel

The FAME profiles of the non-decolorized biodiesel and subsequently decolorized biodiesel were nearly identical as an indication of a successful decolorization process (Table 1) [20]. For each, the major fatty acid was 18:4, at approximately 21 mg/g of crude FAME. Polyunsaturated fatty acid (PuFAMES, more than two double bonds) content was highest for the non-decolorized biodiesel (Non-D, 42.4%) but in the same range for all samples (e.g.  $39.7\% = 10.3$  (18:3) +  $21.0$  (18:4) +  $8.4$  (22:6) for D-Iso-1). Docosahexaenoic acid (DHA; 22:6), was also present to a large extent (6.9–8.4 mg/g of decolorized FAME). Other individual FAMES for the different batches are listed in Table 1. The sample produced from a hexane algal oil that had been isolated and stored at  $20^\circ\text{C}$  for approximately two years (D-Iso-2) had a slightly higher proportion of saturated FAMES (30.6% vs. 26.5% for D-Iso-3) and lower unsaturated FAME content, consistent with stability trends for these compounds. For all of the samples, at least 95% of the material could be identified as a FAME. It may be noted that the present *Isochrysis*-derived biodiesel would not meet the European biodiesel standard EN 14214 concerning its specification  $<1\%$  fatty acids with more than three double bonds but the American standard ASTM D6751 does not have this restriction, so that the

**Table 2**Fuel Properties of Non-decolorized (Non-D)<sup>23</sup> and Decolorized *Isochrysis* (D-Iso) biodiesel fuels.

Property <sup>b</sup>	Non-D	D-Iso-1	D-Iso-2 <sup>d</sup>	ASTM D6751	EN 14214
Cetane number	36.53	42.3	48.4	47 min	51 min
Kinematic viscosity ( $40^\circ\text{C}$ , $\text{mm}^2/\text{s}$ ) <sup>a</sup>	2.46	3.38	3.76	1.9–6.0	3.5–5.0
Oxidative stability ( $110^\circ\text{C}$ ; h)	0.06	0.35	0.05	3 min	6 min
Cloud point ( $^\circ\text{C}$ )	ND <sup>e</sup>	−6.0, −6.0 <sup>c</sup>	−5.8, −5.6 <sup>c</sup>	Report	
Pour point ( $^\circ\text{C}$ )	ND	−8.6, −8.4 <sup>c</sup>	−6.0, −6.0 <sup>c</sup>	Report	
Density ( $15^\circ\text{C}$ , $\text{kg}/\text{m}^3$ )	934.92	895.52	898.54	–	860–900
Lubricity ( $\mu\text{m}$ ; $60^\circ\text{C}$ ; HFRR)	260	131, 125 <sup>c</sup>	136, 133 <sup>c</sup>	520 max (ASTM D975)	460 max (EN 590)
Free glycerol (mass%)	0	0.004	0.0045	0.020 max	0.02 max
Total glycerol (mass%)	0.029	0.025	0.033	0.240 max	0.25 max
Monoglycerides (mass%)	0.034	0.029	0.020	0.40 max	0.70 max
Acid Value ( $\text{mg KOH g}^{-1}$ )	ND	3.029	5.139	0.50 max	0.50 max
Moisture	ND	345 ppm	ND	0.05% max (v/v)	500 max mg/kg

<sup>a</sup> Determined on an individual batch ( $\sim 10$  g) prior to blending.<sup>b</sup> Limits listed as given in the standards.<sup>c</sup> Duplicate measurements.<sup>d</sup> Prepared from an older algal oil, stored at  $\sim 20^\circ\text{C}$  for two years (ref. Table 1).<sup>e</sup> ND = not determined.

addition of antioxidants may overcome this issue for the North American market.

### 3.4. Pigment removal and cloud point analysis of an *Isochrysis* biodiesel

Cloud point has been shown to be the most stringent specification regarding cold flow properties but can be correlated with other tests such as the cold filter plugging point [29]. Experimental CP data for a biodiesel from an algal feedstock are quite scarce with exceptions being [30,31]. For instance, a CP value is absent from otherwise fairly extensive testing of biodiesel from the microalga *Schizochytrium limacinum* [32]. While these authors do not state the reason for this omission, the overall unavailability of this data may be due to the same difficulties we encountered with our initial *Isochrysis*-derived product being too dark in color to measure a CP.

Like other land and aquatic photosynthetic organisms, *Isochrysis* contains chlorophylls and pheophytins that can degrade into compounds like pheophorbides [33]. It was reported that chlorophyll and its derivatives have a negative effect on the stability of vegetable oils [34]. Various solid materials such as clays and activated carbon can be used to selectively remove chlorophylls and pheophytins from these mixtures [35]. For instance, Issariyakul and Dalai demonstrated the effectiveness of montmorillonite K 10 (MK10) clay for decolorizing greenseed canola oil in connection with biodiesel production [26]. Applied to our green non-decolorized *Isochrysis* biodiesel, we found that stirring over 20% (w/w) MK10 at 60 °C for 1 h resulted in a dramatic reduction in pigment content by visual inspection (ref. Fig. 2). Moreover, absorbance peaks corresponding to chlorophylls and pheophytins were no longer observed in the now orange/red biodiesel [See Supplementary Material]. Presumably it is the Lewis basic porphyrin structures within these compounds interacting strongly with the acidic MK10 clay that accounts for the selectivity of this process [36], giving on average 90% mass recoveries for now decolorized biodiesel.

The measured CP values for our decolorized biodiesel samples were as expected low, however the values obtained (−6.0 and −6.0, duplicate analysis for D-Iso-1; −5.8 and −5.6 °C duplicate analysis for D-Iso-2) were lower than what would be predicted based on the FAME profile [37]. The pour points (PP) were also exceptionally low (−8.6 and −8.4, duplicate analysis for D-Iso-1; −6.0 and −6.0 °C, duplicate analysis for D-Iso-2) given the fairly large amounts of saturated FAMES (e.g. ~15% C16:0) in the material. For comparison, soybean biodiesel (SME) contains roughly 10% methyl palmitate (C16:0) and has CP and PP values of 1 °C and 0 °C respectively [38]. SME, however, also contains approximately 5% of even higher melting methyl stearate (C18:0) of which there are only traces in *Isochrysis*. CP is sensitive to minor amounts of higher melting components [19,39] so that the different methyl stearate contents may at least partially explain the large disparity in CP between SME and our decolorized *Isochrysis* biodiesel. Efforts are ongoing to better understand the cold-flow properties of our *Isochrysis* biodiesel toward further refined predictive CP models.

### 3.5. Cetane number (CN)

The ASTM D6751 and EN 14214 standards prescribe minimum CN values of 47 and 51 respectively for commercial biodiesel. For our non-decolorized biodiesel, we had previously measured a CN of 36.5 (Table 2) [20]. After decolorization, both samples exhibited a higher CN and with one meeting the ASTM standard (42.3 and 48.4). Care must be taken, however, when calculating and comparing CN values as these numbers are not absolute and the results often variable [40]. In a recent comprehensive evaluation of this parameter for biodiesel, the CN of methyl oleate was at best

estimated to be in the range of 56–58 using data from 23 different measurements [40]. Nonetheless, pigment removal improved the CN. While too much emphasis cannot be placed on the actual value for reasons stated above, the CN of our decolorized *Isochrysis* biodiesel is likely close to the ASTM standard (avg. for samples = 45).

### 3.6. Kinematic viscosity

Algal biodiesel fuels with high PUFA content would be expected to have lower kinematic viscosity than vegetable oil-derived biodiesel, although some higher viscosities have also been reported for simulated algal FAME mixtures [41]. Previously we reported a kinematic viscosity of 2.46 mm<sup>2</sup>/s at 40 °C for our non-decolorized biodiesel [20], which was lower than the 3.2 mm<sup>2</sup>/s that would be estimated based on the FAME profile [20]. Attempts to check the accuracy of this result failed, however, as the samples consistently plugged viscometer tubes due to the presence of some insoluble material. Our final decolorized biodiesel product was free of these particulates and the kinematic viscosity measured (3.38 and 3.76 mm<sup>2</sup>/s) was similar to the predicted value (3.2 mm<sup>2</sup>/s). The measured kinematic viscosities are on the low end of the range prescribed ASTM D6751 standards and close to the minimum specified in EN 14214. However the technical justification for the higher minimum viscosity specification of 3.5 mm<sup>2</sup>/s in EN 14214 is not clear as conventional petrodiesel fuels generally exhibit viscosity values below 3.5 mm<sup>2</sup>/s.

### 3.7. Oxidative stability

All non-decolorized and decolorized *Isochrysis* biodiesel fuels exhibited poor oxidative stability, well below the ASTM minimum specification. This is due to the high PUFA contents of these fuels (approximately 40%, ref. Table 1) as increased unsaturation within a fatty acid carbon-chain results in decreasing oxidative stability [42]. The highest oxidative stability among the samples tested might therefore be expected for D-Iso-2 with a higher proportion of saturated FAMES and lack of pigments that have been reported to adversely affect biodiesel oxidative stability [26]. The oxidative stability of D-Iso-2 was in fact lower than for the decolorized D-Iso-1 (0.05 h vs. 0.35 h) and essentially the same as the non-decolorized biodiesel (0.06 h). However, the oxidative stability of all samples is very low so that the differences would likely not have any major effect under practical aspects. Furthermore, the percentage of identified FAMES was lowest for the decolorized D-Iso-2 (88.5%). Minor components of undetermined nature within this sample may affect the oxidative stability more than their minor amounts would indicate.

### 3.8. Lubricity and density

Lubricity for diesel fuels is becoming an increasingly recognized property with more stringent limitations on sulfur content for environmental reasons. The HFRR wear scars of the decolorized *Isochrysis* biodiesel samples were well below the maximum wear scars of 460 μm and 520 μm prescribed in the standards EN 590 and ASTM D975, respectively. These values (131 and 125 μm, duplicate analysis for D-Iso-1; 136 and 133 μm, duplicate analysis for D-Iso-2) represent an improvement from the non-decolorized biodiesel (260 μm), and are now more in line with measured lubricities for biodiesel from other feedstocks (e.g. soy-derived biodiesel around 130 μm) [43].

Fuel density relates to fuel performance (e.g. within the injection system). Differences in density between biodiesel and petrodiesel creates some concern about potential mismatching of engine parameters when using this type of fuel in engines optimized for petrodiesel [44]. For this reason, the European standard EN-590

establishes a density range for diesel fuels of 820–845 kg/m<sup>3</sup> at 15 °C. Biodiesel tends to have a higher density than petrodiesel [45], and accurate density data are needed to calculate appropriate blend ratios that will meet this specification.

The availability of experimental density data for algal biodiesel fuels is limited [16,46,47], but can be predicted from FAME profiles using linear mixing rules and the known densities of neat FAMES [17]. The measured density for our non-decolorized *Isochrysis* biodiesel at 15 °C was 934.92 kg/m<sup>3</sup>, higher than the maximum (900 kg/m<sup>3</sup>) prescribed in EN 14214 [24]. Post-decolorization, the densities measured were 895.5 and 898.5 kg/m<sup>3</sup> for D-Iso-1 and D-Iso-2 respectively, which now just fall within the EN 14214 range (max. = 900 kg/m<sup>3</sup>). Because the FAME profiles and contents of the non-decolorized and decolorized biodiesel fuels were very similar (ref. Table 1), the data suggests that the presence of pigments results in a higher density. However, the extent of their impact has yet to be rigorously investigated and it remains to be determined if such low levels of these compounds (max. = 5% w/w) could account for the observed differences of our decolorized and non-decolorized biodiesel fuels.

### 3.9. Glycerol and FFA and moisture content

Our processing consistently produces a biodiesel that meets the free and total glycerol amounts as well as moisture content (345 ppm for D-Iso-1) according to ASTM D6751 and EN-14214. The acid values, however, exceeded the limitations according to these specifications. Problems associated with acidity for diesel fuels center on the possibility of corrosion and potential formation of engine deposits. For biodiesel, the acid value indicates FFA content [48]. Because our processing involves first converting all acylglycerols to FFAs for the purpose of separating alkenones and other neutral lipids, the acid value essentially represents the percent yield for our esterification step, or 98.6% (Acid Value = 3.029 = 1.383% FFAs) and 97.7% (Acid Value = 5.139 = 2.347% FFAs) for D-Iso-1 and D-Iso-2, respectively. The ASTM D6751 and EN-14214 acid value limit of 0.50 corresponds to an exceedingly low FA content of approximately 0.25%, meaning we would need to achieve an esterification yield of 99.75% or reduce the acid value of the final product by other means [49]. Future work will therefore include an optimization of this parameter toward the production of a commercially viable *Isochrysis* biodiesel.

## 4. Conclusion

Pigments such as chlorophylls and other derivatives can be efficiently removed from *Isochrysis* biodiesel using montmorillonite K10 clay. The process was performed on sufficient scale to allow for comprehensive fuel testing of the resulting decolorized biodiesel. This was made possible in part due to the commercial availability of *Isochrysis* in multi-kilogram quantities from several suppliers. Results from the fuel tests provided important experimental data that can be used to validate and refine often used predictive models for algal biodiesel fuel properties. For instance, oxidative stability remains an issue for our *Isochrysis* (and presumably other algal) biodiesel, and appears highly sensitive to even minor amounts of PUFAMES. Through the decolorization process we were now able to obtain a CP, with the measured CP for our decolorized biodiesel lower than what would be predicted based on the FAME profile. A comparison of the fuel testing results for our decolorized sample to that previously obtained for a non-decolorized *Isochrysis* biodiesel also revealed certain impacts of pigments on fuel properties. Specifically, pigment removal resulted in a 24% increase in CN (from 36.5 to 45.4 (avg.)), 40% increase in kinematic viscosity (from 2.5 to

3.5 mm<sup>2</sup>/s), a 50% decrease in lubricity (from 260 to 131 μm), and 4% decrease in density (from 935 to 897 kg/m<sup>3</sup>). There remains, however, approximately 5% unaccounted for material in the samples tested making any absolute claims about the role of pigments on fuel properties difficult. Work is therefore ongoing to fully characterize these mixtures, along with continued studies toward the production of an ASTM-certified *Isochrysis* biodiesel.

## Disclaimer

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fuel.2016.03.061>.

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