Extraction, characterization, purification and catalytic upgrading of algae lipids to fuel-like hydrocarbons

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21 **Abstract**

22 The extraction, **characterization**, purification and upgrading of algal lipids was examined,
23 utilizing *Scenedesmus acutus* microalgae grown with flue gas from a coal-fired power plant.
24 Lipid extraction was achieved using a procedure based on the Bligh-Dyer method, modified so
25 as to utilize a significantly decreased solvent:biomass ratio than the original protocol. Both
26 activated carbon and K10 montmorillonite were found to function as efficient adsorbents for the
27 removal of chlorophyll, phospholipids and sterols from the crude algae oil. The yield of purified
28 lipids using this approach was similar to that obtained by in situ transesterification of the lipids in
29 *Scenedesmus acutus*, confirming that adsorption is an effective method for the removal of non-
30 esterifiable lipids. During the deoxygenation of the purified algae oil at 260 °C over a Ni-Al
31 layered double hydroxide catalyst, deactivation of the catalyst was observed, attributed to the
32 presence of highly unsaturated lipid chains which can act as poisons by adsorbing strongly to the
33 catalyst surface and/or acting as precursors to coke formation. However, upgrading at 300 °C
34 gave better results, the liquid product consisting of ~99 wt% hydrocarbons, diesel-like (C10-
35 C20) hydrocarbons constituting 76 wt% of the liquid after 4 h on stream.

36

37 **Keywords**

38 Algae; lipids; extraction; purification; **characterization**; upgrading

1. Introduction

Microalgae present a variety of advantages over terrestrial plants for the production of renewable fuels and chemicals. Chief among these is the fact that many species of algae exhibit high growth rates and can be grown on non-arable land using wastewater, thus avoiding undesirable competition with food crops for land and water resources [1]. Of late, the genetic manipulation of algae has also attracted interest as a means of making these organisms a better feedstock for the manufacture of a wide range of industrial products, including pharmaceuticals, aquaculture feed and biofuels [2].

Microalgae can be processed through a variety of methods to yield a diverse set of products depending on local demand and profitability. One approach involves the hydrothermal liquefaction (HTL) of whole algal biomass to produce bio-oil – a viscous, corrosive and unstable mixture of organic compounds that can be subsequently upgraded into liquid fuels. HTL has the advantage of utilizing wet algal biomass with little to no pre-processing [3]; however, if microalgae are to become a financially viable feedstock for the production of fuels, fractionation must be undertaken to extract high value components which are typically destroyed during HTL. These compounds include protein, pigments such as carotenoids, and nutraceuticals such as polyunsaturated fatty acids (PUFAs). Given the valuable nature of many of these compounds, any viable biofuel production process must be accompanied by the isolation and monetization of these commodities [1].

Another important step in the development of an economically sound algal fuel production scheme is the implementation of an efficient and cost-effective lipid extraction methodology.

Various techniques have been utilized to isolate lipids from algal biomass, including solvent extraction, supercritical fluid extraction, ultrasonication, and expelling/pressing [4]. Among the assortment of both emerging and mature extraction technologies, solvent-based extraction is still the most prevalently utilized technique for isolating lipids from oleaginous biomass. This type of process is considered attractive not only due to its simplicity and effectiveness, but also because the extraction solvent can typically be recycled. Remarkably, the extraction method first published by Bligh and Dyer in 1959 [5] which utilizes a chloroform-methanol-water solvent system, is still in widespread use today – along with its several variants [6, 7]. However, while the Bligh-Dyer method is considered a standard method for total lipid determination [8], it is not used at commercial scale due to the large amounts of solvent required and that fact that it is only effective for dry algal biomass.

Extracted algal lipids contain typically a number of compounds that are unsuitable for upgrading to fuels, as well as potentially valuable compounds which ideally should be isolated prior to catalytic upgrading. The most prevalent of these undesirable compounds in crude algae oil is chlorophyll, a magnesium-porphyrin complex. Similar metallic complexes have been demonstrated to poison the type of metal-based deoxygenation catalysts typically used to upgrade lipids to fuels through the adsorption of the metal center, which is followed by accelerated coke formation due to the highly aromatic nature of the chelating porphyrin ring [9]. As a result, the development of a lipid purification strategy is essential to render algal lipids a viable feedstock for deoxygenation processes.

While liquid transportation fuels can be obtained from lipid-based feeds by converting the latter to the Fatty Acid Methyl Esters (FAMES) that constitute biodiesel, the high oxygen content of FAMES results in a number of undesirable properties that make biodiesel a less than ideal biofuel. Consequently, attention has shifted to deoxygenation processes capable of converting lipids to drop-in hydrocarbon fuels [1, 10]. Hydrotreating – a process mostly based on the hydrodeoxygenation (HDO) reaction – represents the most commonly used approach to achieve this transformation. However, this approach has a number of disadvantages, as it requires high pressures of hydrogen and employs problematic sulfided catalysts that risk contaminating the products with sulfur and which deactivate in the presence of water, the latter being a product of the HDO reaction. Interestingly, it has recently been reported that supported iron nanoparticles constitute an efficient and sulfur-free catalyst for converting algae oil to green diesel [11]. However, the reaction proceeds mostly via HDO, meaning that this approach still has the drawback of high hydrogen consumption. Deoxygenation via decarboxylation/decarbonylation (deCO_x) represents a promising alternative to hydrotreating, since this reaction can proceed at lower temperatures over simple (non-sulfided) metal catalysts and under lower pressures of hydrogen [1, 10], thereby minimizing the process costs for fuel production from algae [12]. It is also worth noting that both the energy balance and the techno-economic analysis of deCO_x -based processes to convert lipids to fuel-like hydrocarbons have been performed, these processes being found to be 89.6% energy efficient, more economically competitive than hydroprocessing-based approaches and potentially competitive with petroleum depending on the relative prices of the lipid and petroleum feedstocks [13]. Admittedly, most work to date on the deCO_x of lipids to fuel-like hydrocarbons has focused on catalysts based on precious metals such as Pd and Pt, the cost of which may limit their industrial application [1]. However, recent work has shown that

comparable yields of fuel-like hydrocarbons can be obtained over inexpensive and recyclable Ni-based catalysts [14-17], the transformation of triglycerides and related compounds to green diesel over these formulations being the subject of a recent critical review by Kordulis et al. [18]. In addition, these catalysts have shown promise in the upgrading of algal lipids in both batch [19-21] and continuous mode [21-24].

While recent contributions from our group focused on the cultivation of microalgae using flue gas from a coal-fired power plant [22] and on the study of Ni-based catalysts capable of upgrading model and algal lipids to fuel-like hydrocarbons via deCO_x [23, 24], details of the extraction, purification and characterization of the algae oil fell outside of the scope of these articles. In this contribution, the extraction, characterization, purification and upgrading of algal lipids is presented, utilizing *Scenedesmus acutus* grown with flue gas as the CO₂ source. Lipid extraction was conveniently accomplished using the Bligh-Dyer method, albeit this cannot be considered an industrially viable method for the reasons indicated above. Rather, emphasis is placed on demonstrating the technical feasibility of using low-cost adsorbents to afford purified algal lipids amenable to catalytic upgrading. Finally, we report the continuous catalytic upgrading of the purified algae lipids to fuel-like hydrocarbons via deCO_x over a Ni-Al layered double hydroxide (LDH) catalyst.

2. Materials and methods

2.1 Reagents

The Ni-Al LDH catalyst employed was prepared using a previously described method [25] and was determined to be [Ni_{0.67}Al_{0.33}(OH)₂][CO₃]_{0.17}·mH₂O by elemental analysis. Reagents

purchased from Fisher Scientific included chloroform (HPLC grade), acetone (histological grade) and diethyl ether (laboratory grade). Dodecane (>99%) and cyclohexanone (>99%) were obtained from Alfa Aesar. Silica gel (SiliaFlash® P60) was purchased from SiliCycle. The Supelco® 37 Component FAME Mix, activated charcoal (Darco ® KB-G), K10 montomorillonite, toluene (anhydrous, 99.8%), methanol (anhydrous, 99.8%), glyceryl heptadecanoate (≥99%), methyl nonadecanoate (analytical standard), potassium methoxide (95%) and chloroform-d were procured from Sigma Aldrich. Phytol (distilled) was obtained from MP Biomedicals, LLC. D₂O was purchased from Cambridge Isotopes Laboratories, Inc.

2.2 Algae culturing

Scenedesmus acutus obtained from the University of Texas Culture Collection (UTEX B72) was used for all experiments. Cultures were grown in a urea medium previously optimized for this *Scenedesmus* strain [26]. Details on the sequential culturing of this algae strain in 500 mL Erlenmeyer flasks, 7.5 liter airlift photobioreactors (PBRs) and a 1,200 L vertical tube PBR located outdoors at a coal-fired power plant (East Bend Station, KY) have previously been reported [22].

2.2.1 Crude lipid extraction from algae via a modified Bligh-Dyer method

Harvested algae (~20% solids) were dried under vacuum at 60 °C for 20 h prior to being pulverized using a coffee grinder and sieved to a maximum particle size of 1 mm. A 50 g sample of dry algae powder – henceforth designated as raw algae – was combined with a mixture of chloroform (200 mL) and methanol (100 mL), the resulting mixture being shaken on a ThermoScientific MaxQ 4450 Orbital Shaker for 5 minutes, which was followed by the addition

of methanol (100 mL) and 5 minutes of shaking and a final addition of water (100 mL) and 2 h of shaking. Vacuum filtration was then used to separate the soluble lipids from the filter cake, which is henceforth denoted as residual solids. The soluble lipids were then transferred to a separatory funnel where the mixture was given 2-6 days to completely separate into two distinct layers. The bottom chloroform layer was collected and the solvent was removed using a rotary evaporator to obtain the unpurified algae oil, which will henceforth be referred to as crude lipids.

2.2.2 Analysis of raw algae, crude lipids and residual solids

Lipid profiling was conducted in triplicate by means of direct transesterification of the raw algae, crude lipids and residual solids following a procedure previously described by Griffiths et al. [27]. Subsequent GC analysis of the transesterification reaction products was performed using an Agilent 6890 gas chromatograph equipped with an HP-88 column (J&W Scientific, 30 m × 0.25 mm × 0.2 µm) and a flame ionization detector. Helium was used as the carrier gas, the flow rate was set to 1 mL/min and a split of 20:1 was employed. The isothermal temperatures of the inlet and the detector were 250 and 300 °C, respectively. The oven temperature program included an initial temperature of 50 °C followed by a ramp of 20 °C/min to 140 °C and an ensuing ramp of 3 °C/min to a final temperature of 240 °C. The Supelco® 37-Component FAME Mix was used as an analytical standard.

ASTM methods were used for elemental analysis of the algae, extracted lipids and residual solid. Specifically, ASTM D6349 was used to determine Mg and P content, ASTM D7582 was used to determine moisture and ash content, ASTM D4239 was used to determine sulfur content and

ASTM D5373 was used to determine the carbon, hydrogen and nitrogen content, oxygen being determined by difference. ASTM D664 was used to determine Total Acid Number (TAN). All elemental analyses were performed in triplicate. UV-Vis measurements were performed by dissolving 7.5 μL of the sample of interest in 1992.5 μL of chloroform and analyzing the resulting solution using a Thermo Scientific Evolution 60 UV-Vis Spectrophotometer.

Quantitative phospholipid determination via ^{31}P NMR was undertaken using the method described by Sotirhos et al. [28], samples being dissolved in either chloroform-d or D_2O and examined by more than 15,000 transients on a 400MHz Varian Inova NMR spectrometer.

The protein content of the algae was calculated from the elemental N content using a literature conversion factor of 4.78 [29], while the carbohydrate content was determined according to a literature method using phenol and sulfuric acid [30].

2.3 Lipid purification

Crude algae lipids were purified via column chromatography utilizing K10 montmorillonite or Darco® KBG activated carbon as the column material. These low-cost adsorbents were dried overnight under vacuum at 120 °C prior to use. Glass chromatography columns of varying diameters equipped with a glass frit and stopcock were employed. The bottom of each column was first loaded with silica gel, which was packed on top of the aforementioned glass frit to a height of approximately 2 cm. Diethyl ether was then used to wet the silica gel prior to the addition of montmorillonite or activated carbon as the stationary phase, which was added to a depth of approximately 10 cm (ether being used to rinse down any material that adhered to the

column wall). Ether was added to the column until the solvent level was approximately 2 cm above the height of the stationary phase. At this point, 1-8 g of crude algal lipids (depending on the column diameter) were added to the top of the stationary phase with a syringe. A volume of ether approximately equal to the volume of the stationary phase was then added to establish the mobile phase, which was allowed to elute until the solvent level neared the top of the stationary phase. The eluent was collected in a pre-weighed flask and the solvent was removed via rotary evaporation. The resulting sample (labeled “Fraction 0”) was weighed prior to being analyzed using Simulated Distillation-GC and GC-MS methods described in a recent contribution [31] and summarized in section 2.3.1. Next, a 1:10 (v:v) mixture of chloroform and ether was added to the column. The procedure described above for Fraction 0 was followed 10 more times, increasing the concentration of chloroform by 10 vol% each time until a fraction eluted using pure chloroform (“Fraction 10”) was collected, weighed and analyzed.

2.3.1 Analysis of purified lipids

Purified lipids were analyzed using the same procedures described in section 2.2.2, albeit elemental analyses and TAN determination were confined to the AC-purified lipids due to the limited amount of K10-purified lipids available (see section 3.2). In addition, purified lipids were analyzed using a Simulated Distillation-GC method specifically developed to perform the quantitative analysis of feedstock and products in the deoxygenation of lipids to hydrocarbons, this method being described in a recent contribution (method B in [31]). Purified lipids were also analyzed using a previously described GC-MS method [16]. It is important to note that these two GC techniques are complementary: Simulated Distillation-GC was able to analyze the entirety of the samples in spite of lacking the ability to detect individual constituents, while GC-MS is a

powerful tool for compound identification but is not capable of detecting large molecules such as triglycerides. Therefore, the data stemming from these two GC techniques were combined – along with data resulting from lipid profiling and TAN measurements (see section 2.2.2) – in order to obtain a complete picture.

2.3.2 Analysis of spent column materials

Thermogravimetric analysis (TGA) of the spent column materials was performed – both before and after acetone washes – under flowing air (50 mL/min) on a TA instruments Discovery Series thermogravimetric analyzer. The temperature was ramped from room temperature to 800 °C at a rate of 10 °C/min. Acetone washes were conducted by exposing the spent material to 500 ml of acetone with vigorous stirring for 24 hours. The mixture was then filtered via vacuum filtration and the acetone was removed by rotary evaporation. The residue was dissolved in CHCl_3 and was analyzed using the GC-MS method described in section 2.3.1.

2.4 Catalytic upgrading of purified lipids via deCOx

Deoxygenation experiments in continuous mode were performed in a fixed bed stainless steel tubular reactor (1/2 in o.d.) equipped with an HPLC pump. The catalyst (0.5 g, particle size 150-300 μm , held in place using a stainless steel frit) was reduced under flowing H_2 at 400°C for 3 h. The system was then taken to the reaction temperature (260 °C) and pressurized with H_2 to 580 psi. A liquid solution of the crude algal lipids in dodecane (1.33 wt%) was introduced to the system at a rate of 0.2 mL/min along with a flow of H_2 (50 mL/min). A liquid gas separator (kept at 0 °C) placed downstream from the catalyst bed was used to collect liquid samples. The reactor

system and the reaction conditions employed were almost identical to those employed by Lercher and co-workers in a recent contribution [32]. For comparison purposes, a solution of phytol in dodecane (1.33 wt%) was also subjected to this testing procedure. Liquid products were analyzed by both the Simulated Distillation-GC and GC-MS methods described in sections 2.3.1, while spent catalysts were analyzed by TGA following the procedure described in section 2.3.2. A representative spent catalyst (as well as its fresh counterpart for comparison purposes) was analyzed by proton-induced X-ray emission (PIXE) at Elemental Analysis, Inc. (Lexington, KY) using a General Ionex Corp. Tandetron Model 4110A proton accelerator coupled with a custom X-ray Emission apparatus. Prior to PIXE analysis, solid samples were homogenized in an agate or cryogenic mixer mill and a pellet was pressed using ~300 mg of material.

3. Results and Discussion

3.1. Crude lipid extraction from algae

Albeit many approaches to lipid extraction from algae have been investigated [1], solvent extractions represent some of the simplest strategies. In turn, the Bligh-Dyer method [5] is one of the best-established solvent-based methods for the extraction of lipids from biological matrices. In the original Bligh-Dyer method a miscible system is formed by homogenizing a wet biological substrate with a mixture of chloroform and methanol, further addition of chloroform and water separating this system into two layers: a chloroform layer containing most of the lipids and an aqueous methanol layer containing more polar compounds. While the Bligh-Dyer method has the noteworthy advantages of being efficient, reproducible and extremely simple – as it does not require sophisticated equipment or energy-demanding inputs (in the form of high temperatures and/or pressures) – this protocol is not free of drawbacks. Indeed, considerable quantities of

solvents are required, the original Bligh-Dyer method requiring ca. 55 mL of solvents to extract 1 g of sample (dry basis). Given that solvent use should be minimized for both laboratory and industrial applications, optimization work was undertaken in an effort to reduce the amount of solvents required, resulting in the modified Bligh-Dyer method described in section 2.2.1. Dry algae were employed in these experiments in order to control the amount of water in the system and through a number of iterations a protocol was developed requiring only 10 mL of solvent (total) per 1 g of sample (dry basis). This approach afforded lipid yields which were statistically identical to those of the original Bligh-Dyer method, although it extended the length of time necessary for the gravity separation of chloroform and methanolic layers; however, adequate separation could be achieved in 15 min by means of centrifuging.

3.1.1. Analysis of raw algae, crude lipids and residual solids

Table 1 shows the protein and carbohydrate content of the algae, along with the yields of crude lipids obtained using both the modified Bligh-Dyer method and for comparison purposes, the concentration of esterifiable lipids present in the algae determined by in situ transesterification. The latter was found to amount to 6.2 ± 0.8 wt%, while the crude lipid yield obtained by the modified Bligh-Dyer method used in this work was 12.6 ± 0.8 wt%, indicating that a significant amount of the extract is comprised of compounds other than esterifiable lipids. These other compounds most likely consist of proteins and pigments, the presence of chlorophyll being confirmed by UV-Vis spectroscopy (see below). Additionally, GC-MS analysis revealed the presence of sterols in low concentration (see Table S1 within the supplementary material). Parenthetically, the crude lipids were determined to have a TAN of 54.8 ± 2.9 mg KOH/g,

indicating that the extract contains free fatty acids (FFAs) in significant concentration, as confirmed by GC-MS analysis (Table S1).

The lipid profiles of the esterifiable fraction of these samples are included in Figure 1. Greater than 95% of the fatty acid chains in the lipids contained in all samples had a length of 14-18 carbon atoms (C14-C18). Given that diesel fuel typically consists of C10-C20 linear hydrocarbons [33], the lipids in this *Scenedesmus acutus* strain constitute a suitable feedstock for the production of diesel fuel via deoxygenation. Other compounds present such as chlorophyll, phospholipids and proteins have the potential of poisoning the catalysts used during upgrading and/or contaminating the resulting hydrocarbon products with heteroatoms such as nitrogen and sulfur, which is undesirable given that the latter can produce noxious pollutants (i.e., NO_x and SO_x) upon fuel combustion. Therefore, the raw algae, the crude lipids and the residual solids were analyzed in order to gain insights into the concentration of relevant species and their fate during extraction of the crude lipids from the algae.

Figure 2 includes the results of ultimate analyses performed to determine the ash, moisture, carbon, hydrogen, nitrogen, sulfur, and oxygen (calculated by difference) content of the raw algae, crude lipid extract and residual solids. According to these results the ash content of the raw algae is ~4.4 wt%, whereas the ash content in the crude algal lipids is ~1.4 wt%. When the same samples were specifically analyzed for Mg – as a proxy for chlorophyll – by means of ICP-OES (see Figure 3), the raw algae, the residual solids and the crude algal lipids were found to have magnesium contents of 0.25 wt%, 0.15 wt% and 0.24 wt%, respectively, indicating that the chlorophyll content of the crude lipids was practically the same as that of raw algae. This is in

agreement with the results of UV-Vis measurements that were performed on the crude algal lipids (see section 3.2), which showed that the crude algal lipids contained a significant amount of chlorophyll, clearly indicating the need for further purification prior to catalytic upgrading. Similarly, when the samples were analyzed for phosphorus – as a proxy for phospholipids – via ICP-OES, (see Figure 3), the raw algae, the residual solids and the crude lipids displayed phosphorus contents of 1.26 wt%, 0.93 wt% and 0.72 wt%, respectively, indicating that only ~6% of the phosphorus in the raw algae was extracted with the lipids. The remainder was present in the filter cake and in the water layer obtained from the modified Bligh-Dyer extraction, the latter being confirmed by ^{31}P NMR spectroscopy (see Figure S1 within the supplementary material).

It is worth noting that the residual solids (“defatted” algae) consist of mainly protein and carbohydrate. As such, a number of potential uses can be envisaged, such as animal feed, as a source of fermentable sugars for bio-ethanol production, or as a functional filler for bioplastics [34]. Of these applications, the latter is particularly attractive as the algae (after spray drying) command a price similar to that of the raw plastic.

The nitrogen content of the crude algal lipid extracted via the modified Bligh-Dyer method was 2.26 wt%. GC-MS analysis indicated a trace amount of nonadecanitrile was present according to the NIST MS Spectral identification library, although in an insufficient amount to account for the measured N content. A portion of the N was clearly associated with the chlorophyll present, the measured Mg concentration of the crude lipid (0.24 wt%) implying a nitrogen concentration of 0.55 wt%. The remainder is associated with compounds that are not amenable to analysis by GC-MS, most likely hydrophobic proteins. As mentioned above, the presence of sulfur is also problematic given that any sulfur reaching the fuel would result in the formation of SO_x during

fuel use. However, the sulfur content of raw algae and crude lipids were found to be low and practically identical (0.88 and 0.9 wt%, respectively) as illustrated in Figure 2. Attempts to identify the S-containing compounds present from GC-MS data proved unsuccessful, which is unsurprising given the low S-content.

As shown in Table S1, the crude extract also contained 16.4% of unidentified oxygenates. It is worth noting that the vast majority of these unidentified compounds elute late in the chromatograms suggesting that they are large molecules. These results are consistent with the fact that most phosphorous and nitrogen compounds have been observed to partition towards the water fraction during the hydrothermal liquefaction of algae [35, 36], which suggests that the compounds containing these two heteroatoms show a preferential affinity for the water layer in systems comprising an aqueous and an organic phase, thereby providing an opportunity to recycle these nutrients [36-38]. Notably, Figure 2 also shows that albeit the oxygen content of the lipids extracted using the modified Bligh-Dyer method was 7.42 wt% – the main components of which were triglycerides and free fatty acids (~52%), phytol-like compounds (~15%), other oxygenates including alcohols and esters (~27%), as well as small amounts of sterols (~3%) and hydrocarbons (~2%) according to GC-MS results (see Table S1 and Figure S2) – this oxygen content is still too high for most fuel applications, clearly illustrating the need for upgrading via catalytic deoxygenation.

3.2. Lipid purification

In order to rid the crude algae lipids of chlorophyll prior to catalytic upgrading, a simple and inexpensive approach was devised based on column chromatography using low-cost adsorbents

as the stationary phase. Given that chlorophyll is known to interact chemically with acid sites – higher acidity resulting in improved chlorophyll adsorption [39-41] – the use of K10 montmorillonite and Darco KB-G activated carbon (henceforth to be referred to as K10 and AC, respectively) as high surface area, acidic adsorbents was assayed following the procedure described in section 2.3. Based on a series of screening tests, a gradient of ethyl ether and chloroform was selected as the best mobile phase to separate chlorophyll and lipids, as shown by the UV-Vis spectra included in Figure 4. Comparison of the UV-vis spectra of a chlorophyll standard and the crude algal lipids clearly shows that the latter contain a considerable amount of chlorophyll, which is in agreement with both the results of ICP-OES analyses for Mg (see section 3.1.1 and Figure 3) and the dark green color of the algae extract. However, each of the 11 fractions collected – which correspond to a change in the mobile phase employed (Fraction 0 being obtained with pure diethyl ether, Fraction 10 with pure chloroform and Fractions 1 to 9 with mixtures of these two solvents in which the chloroform concentration was gradually augmented in increments of 10 vol%) – showed a complete absence of chlorophyll irrespective of the adsorbent employed (see Figure 4). For completeness, the spent adsorbent materials were extracted with acetone in an effort to gain insights into the compounds that remained adsorbed on the columns. While some chlorophyll was extracted from the spent K10, none was recovered from the spent AC judging from the color of the extract solution, suggesting that chlorophyll is adsorbed more strongly on AC. Nevertheless, it is important to note that irrespective of the adsorbent, acetone extraction resulted in only a small amount of extracted material (ca. 0.33 wt% of the spent adsorbent, corresponding to 5.5% of the initial crude lipid mixture), GC-MS analysis of the extracts revealing only trace amounts of lipids. In addition, the spent adsorbent materials were subjected to TGA both before and after acetone extraction and albeit the AC showed a

higher weight loss (~16 wt%) relative to K10 (~9 wt%) – which can be attributed to a higher amount of thermally labile surface functional groups on AC – the TGA plots of the adsorbents before and after acetone extraction look extremely similar (see Figure S3) which is consistent with the small amount of acetone-extracted material.

According to GC-MS analysis of the fractions collected, whereas nearly all of the lipids recovered from the K10 column were found to be concentrated in Fraction 0, the lipids recovered from the AC column were distributed across all of the fractions. In terms of yield, the material recovered from Fraction 0 using the K10 column represented ~33 wt% of the crude algae extract, the total yield of recovered material representing ~58 wt% of the crude extract. For the AC column, the yield of recovered material amounted to ~46 wt% of the crude algae extract. Expressed as a percentage of the initial weight of algae, the yields of purified material amounted to ~7 wt% and ~6 wt% for the K10 and AC adsorbents, respectively. These values are close to the 6.2% yield of esterifiable lipids measured by the in situ transesterification method, suggesting that the adsorbents were effective in removing the non-esterifiable material present in the crude extract.

Notably, the lipid-containing material recovered from each column – i.e., Fraction 0 from the K10 column and the combination of all fractions from the AC column – showed a different composition according to GC-MS results (see columns labeled “Feed” in Tables S2 and S3 and Figure S2). For the K10-purified material an FFA content of ~40% was determined (48.2% total lipids according to lipid profile data, i.e., ~40% FFAs and ~8% glycerides), hydrocarbons (21.4%) and phytol-like compounds (8.6%) also being detected. The AC-purified material

exhibited a higher FFA content of ~50% (64.9% total lipids according to the lipid profile data, i.e., ~50% FFAs and ~14.9% glycerides), together with hydrocarbons (7.1%) and phytol-like compounds (9.3%). In both cases the balance was comprised of other oxygenates. Tellingly, AC-purified lipids were determined to have a TAN of 85.6 ± 0.8 mg KOH/g, which confirms that FFAs were concentrated during the lipid purification process (as stated in section 3.1.1, the crude lipids were determined to have a TAN of 54.8 ± 2.9 mg KOH/g). This is in apparent contrast with the GC-MS results in Tables S1 and S3, which show an almost identical FFA content for both crude and AC-purified lipids; however, this can be attributed to the limitations of GC-MS vis-à-vis the analysis of the non-volatile fraction of these samples (see section 2.3.1). Moreover, the sterols detected in the crude algae extract (see Table S1) were absent altogether from the purified lipids regardless of the adsorbent employed, which suggests that this purification approach has the potential of allowing the separation and recovery of these potentially valuable compounds (sterols have been shown to block cholesterol adsorption in humans [42] and thus, they are used as nutritional supplements and food additives).

Notably, the profile of the lipids recovered from both the K10 and the AC column were very similar to each other and to the lipid profile of the crude algae extract (see Figure 1). Indeed, as for the latter, almost the entirety of the fatty acid chains in the purified lipids had a length of 14-18 carbon atoms (C14-C18) irrespective of the adsorbent employed, confirming the potential of these feeds in the production of diesel fuel via deoxygenation. Moreover, the hydrocarbons detected in the purified materials consisted of linear C10-C20 (see columns labeled “Feed” in Tables S2 and S3), i.e. diesel-like hydrocarbons.

As mentioned above, in addition to chlorophyll other algae components including phospholipids and N-containing compounds have the potential of poisoning the catalysts used during upgrading and/or contaminating the products with undesirable heteroatoms such as nitrogen and sulfur. According to the results of elemental analysis the lipid purification strategy employed was also successful in removing phospholipids (see Figure 3) while at the same time reducing the content of nitrogen, sulfur and even oxygen (see Figure 2), thereby affording an excellent feedstock for the production of fuel-like hydrocarbons.

3.3. Catalytic upgrading of purified algal lipids via deCO_x

The development of non-precious metal catalysts showing high selectivity towards diesel-like hydrocarbons at mild operating conditions and low hydrogen usage has been identified as a challenge that needs to be met in order to cost-efficiently convert algal lipids to liquid transportation fuels [12]. To date, most research on the catalytic conversion of algae oil to liquid fuels has focused on the use of simple model compounds in lieu of the more complex lipids that constitute algae oil [12]. Indeed, the few contributions utilizing real algae oil samples are limited by the fact that deoxygenation has been accomplished via hydrogen-intensive HDO [11], and/or using precious metal catalysts [43]. Moreover, most studies have been conducted in batch (or semi-batch) mode [19, 20, 43-45], these tests providing no information about the stability of the catalyst under the reaction conditions. Therefore, the catalytic upgrading of the algal lipids extracted and purified by the aforementioned methods was performed via hydrogen-efficient deCO_x using an inexpensive Ni-based catalyst [16, 23], a fixed-bed reactor and relatively mild operating conditions, this approach having afforded promising diesel yields in recent work [22-24]. The fact that the catalyst employed does not involve a carbon-containing active phase (e.g.,

a metal carbide) or support is also noteworthy, given that deoxygenation catalysts may deactivate through the accumulation of carbonaceous deposits during reaction and the use of a carbon-based active phase or support would preclude the facile regeneration of the spent catalyst by simply combusting these deposits [16, 24].

The results of these tests are summarized in Figure 5, which includes the boiling point distribution plots (BPDPs) of the K10- and AC-purified algal lipids along with the products of upgrading. Interestingly, the plots corresponding to the algae oil purified using K10 and AC look fairly similar, meaning that the constituents of these samples coincidentally show an analogous boiling point distribution profile in spite of having rather distinct compositions according to the GC-MS analysis (see the columns labeled “Feed” in Tables S2 and S3 and Figure S2).

Upon upgrading the feed purified using K10 (see Figure 5 left), ~90 wt% of the product mixture was found to boil within the diesel range at all reaction times sampled (the amount of diesel obtained representing ~6.6 wt% of the raw algae). However, progressively higher amounts of unconverted fatty acids such as stearic, palmitic, oleic and palmitoleic acids were observed after one hour of time on stream according to the GC-MS analysis of the product mixtures as shown in Figure 6 (see also Table S2 and Figure S2), small amounts of esters and fatty alcohols being observed after 3 h. From this, it can be concluded that the Ni-Al LDH catalyst used in the upgrading of the K10 purified feed was subject to deactivation, a phenomenon that has been reported previously [23] and is discussed below in more detail. Interestingly, no phytol-like compounds were detected in the reaction products, which is in agreement with the fact that these compounds are mostly deoxygenated to phytane (2,6,10,14-tetramethylhexadecane) under the

reaction conditions (vide infra). Notably, the BPDs of the reaction products obtained using the material purified with AC (see Figure 5 right) look conspicuously different from those obtained from the K10-purified material. Indeed, only 83 wt% of the reaction products boiled within the diesel range at t=1 h this value dropping to 65 wt% at t=4 h, these diesel amounts being equivalent to ~4.8 and ~3.8 wt% of the raw algae, respectively. Additionally, the selectivity to lower boiling point products within the diesel range was found to be higher, particularly at shorter reaction times. As with the experiment involving the K10-purified feed, GC-MS results show that progressively higher amounts of unconverted fatty acids (e.g. oleic, palmitic and palmitoleic acids) and esters were observed in the product mixtures with increasing time on stream (see Figure 6), no phytol-like compounds being detected as these were mostly converted to phytane (see Table S3 and Figure S2). In this regard it is important to note that since both the K10- and the AC-purified feeds contained a similar and non-negligible amount of phytol-like compounds (~9%), an upgrading experiment was performed using a dilute solution of phytol in dodecane as the feed in an attempt to gain insights on the reaction pathways followed by phytol-like compounds during catalytic upgrading. The results of this experiment, which are summarized in Figure S4, confirmed that ~84 wt% of the reaction products were hydrocarbons boiling within the diesel range at t=1 h, this value dropping to ~77 wt% at t=4 h. According to the GC-MS analysis of the reaction products phytol is mostly upgraded to phytane and phytene, albeit some C35 was also formed, along with C16 and small amounts of C34, 2,6,10-trimethylpentadecane and pristane (2,6,10,14-tetramethylpentadecane) (see Table S4).

The change in conversion and selectivity observed with time on stream during the upgrading of both the K10- and AC-purified feeds suggests that catalyst sites responsible for deoxygenation

and cracking reactions were gradually poisoned as the reaction progressed. Since saturated hydrocarbons have been found to be unreactive under the reaction conditions employed [23], it can be concluded that the aforementioned poisoning is due to other species present in relatively high concentration in the K10- and AC-purified feeds. Therefore, phytol-like compounds can be identified as potential poisons, given that the concentration of these compounds was almost identical in the K10- and the AC-purified feeds (~9%) and the fact that some deactivation was observed during the phytol upgrading experiment (see Figure S4). Although a high concentration of FFAs in feeds containing both triglycerides and FFAs has been reported to lead to lower diesel yields and heightened catalyst deactivation (due to the formation of heavy hydrocarbons [46] resulting from FFAs undergoing sequential ketonization [47] and deoxygenation), FFAs can be ruled out as poisons based on our previous experiments in which a high concentration of fatty acids was not observed to result in lower yields of diesel range hydrocarbons [23]. Rather, the observed deactivation is likely due to the high degree of unsaturation of the feed (and particularly the presence of polyunsaturated species such as linolenic acid chains), previous studies having shown that unsaturated species can act as poisons by adsorbing strongly to the catalyst surface and/or acting as precursors to coke formation [16, 48-51]. However, attempts to confirm this by TGA of the spent catalysts were inconclusive. The weight losses associated with the combustion of coke and other organic deposits on the catalyst surface were small enough as to be confounded by the weight gain associated with the oxidation of Ni to NiO and the weight loss associated with the structural changes characteristic of LDHs, as evinced by the TGAs performed under air and nitrogen, respectively (see Figure S5). Parenthetically, the corresponding TGAs of the catalyst spent in the upgrading of phytol (not shown) are practically identical to the TGA plots in Figure S5.

In an effort to further elucidate the source of the decrease in catalyst activity, the spent catalyst utilized in the AC purified lipid upgrading was analyzed for inorganic deposits via PIXE (the fresh catalyst also being analyzed as a reference). While accumulation of Na, Ca, Mg and S on the spent catalyst was either non-existent or negligible, 1.50 wt% Cl was detected on the spent catalyst. This is attributed to the presence of trace amounts of chloroform in the algae oil (used for its extraction), supported Ni being an excellent dehydrohalogenation catalyst. It is widely known that electronegative elements such as Cl can act as catalyst poisons by reducing electron density at the metal surface; for example, Cl has been reported to poison the activity of Raney nickel in room temperature hydrogenation reactions [52]. However, electronegative elements can also exhibit promotion effects. Indeed, the use of Cl as a promoter for Ni catalysts has been reported as a means of reducing catalyst coking [53, 54]. Therefore, the effect of Cl on catalyst behavior can be complex.

In short, albeit it is known that impurities in the feed can poison deCO_x sites [46] and the work discussed above has allowed to both identify and rule out a few potential poisons – in contrast with previous studies [23] deactivation cannot be attributed to the presence of phospholipids or sterols since these compounds were absent from the feeds employed in this study (vide supra) as was also the case with chlorophyll – additional work proved necessary to improve the diesel yield. To this end, an additional catalytic upgrading experiment was performed at 300 °C using the algal lipids purified with the AC column as the feed, results of this run being summarized in Figure 7 and Table S5. A comparison between the BPDP in Figure 7 and the corresponding plot in Figure 5 readily shows that an increase in temperature results an increase in conversion and

selectivity to lighter hydrocarbons, which is unsurprising given that higher temperatures favor both deoxygenation and cracking reactions [55]. Interestingly, an increase in temperature also resulted in increased catalyst stability, 88 wt% of the reaction products boiling within the diesel range at $t=1$ h, this value dropping to 76 wt% at $t=4$. According to GC-MS data (Table S5), this decrease can be attributed mainly to the formation of heavy hydrocarbons, C25-C31 (which boil in the range 401-458 °C), at the longer run times. However, ~99 wt% of the products were hydrocarbons irrespective of the time on stream. Thus, the GC-MS results in Figure 7 suggest that when AC-purified algal lipids are catalytically upgraded at 300 °C, the products are almost completely deoxygenated. Finally, the TGAs of the catalyst spent in the catalytic upgrading reaction performed at 300 °C (not shown) were found to be similar to the TGA plots in Figure S5, i.e., significant accumulation of carbonaceous deposits was not observed.

4. Conclusions

Fractionation is an advantageous approach for algae utilization, due to the fact that it allows for the recovery and monetization of valuable algae components that could be destroyed in approaches that process algal biomass whole, such as HTL. In this work a modified Bligh-Dyer method was used as a convenient means to obtain crude algae lipids; however, for commercial application the Bligh-Dyer approach is unlikely to be economically viable and therefore alternative processes are to be preferred, i.e., processes that do not require the use of multiple – or indeed chlorinated – solvents. This notwithstanding, both activated carbon and K10 montmorillonite were found to function as efficient adsorbents for the removal of chlorophyll, phospholipids and sterols from crude algae oil extracted from *Scenedesmus acutus* algae. The lipid yield obtained using this approach was similar to that obtained by in situ transesterification

of the lipids in *Scenedesmus acutus*, confirming that adsorption is an effective method for the removal of non-esterifiable lipids.

During the deCOx of the purified algae oil at 260 °C, catalyst deactivation was observed. In the absence of chlorophyll, phospholipids and sterols in the oil, the observed deactivation can be attributed to the presence of highly unsaturated fatty acid chains in the feed, in addition to phytols. However, upgrading at 300 °C gave better results, the liquid product consisting of ~99 wt% hydrocarbons, diesel-like (C10-C20) hydrocarbons constituting 76 wt% of the liquid after 4 h on stream according to simulated-distillation GC analysis.

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731

732 Table 1. Protein, carbohydrate and lipid content of the algae used in this study^a

Protein (wt%)	Carbohydrate (wt%)	Extractable lipids via modified Bligh-Dyer method ^b (wt%)	Esterifiable lipids via in situ transesterification ^c (wt%)
35.3	20.12±9.6	12.6±0.8	6.2±0.8

733 ^a Lipid and carbohydrate yields represent the average of three replicates (± st. dev.).

734 ^b Lipid yield (wt%) = (Weight of crude extract/Weight of raw algae extracted) × 100

735 ^c Lipid yield (wt%) = (Weight of esterifiable lipids in crude extract/Weight of raw algae
736 extracted) × 100

737

738

772 **Figure captions**

773 Figure 1. Lipid profile of raw algae, crude algae lipids, residual solids, AC-purified lipids and
774 K10-purified lipids (error bars represent the st. dev. of three replicates).

775 Figure 2. Results of elemental analysis showing the ash, moisture, C, H, N, S, and O content of
776 raw algae, residual solids, crude algae lipids and AC-purified lipids (error bars represent the st.
777 dev. of three replicates).

778 Figure 3. Results of elemental analysis showing the Mg and P content of raw algae, residual
779 solids, crude algae lipids and AC-purified lipids (error bars represent the st. dev. of three
780 replicates).

781 Figure 4. UV/Vis spectra of chlorophyll, crude algae lipids and the different fractions recovered
782 during algae oil purification using a K10 montmorillonite column (left) and an activated carbon
783 column (right).

784 Figure 5. Boiling point distribution plots of the first fraction recovered from the purification of
785 algae oil using a K10 montmorillonite column (left), of all fractions recovered from the
786 purification of algae oil using an activated carbon column (right) and of their corresponding
787 catalytic upgrading products after 1, 2, 3 and 4 hours of time on stream in a continuous reactor at
788 260 °C.

789 Figure 6. Graphic representation of GC-MS data showing the evolution of different types of
790 compounds during the catalytic upgrading of K10-purified lipids (left) and AC-purified lipids
791 (right) at 260 °C.

792 Figure 7. Boiling point distribution plots of AC-purified lipids, the products collected after 1, 2,
793 3 and 4 hours of time on stream in a continuous reactor (right) and a graphic representation of
794 GC-MS data showing the evolution of different types of compounds (right) during catalytic
795 upgrading at 300 °C.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 6

— Diesel-like (C10-C20) hydrocarbons

— Other (>C20) hydrocarbons

— Alcohols

— Phytols

— Fatty acids

— Other oxygenates