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Assessment of Bio-oil Extraction from *Tetraselmis chui* Microalgae Comparing Supercritical CO_2, Solvent Extraction, and Thermal Processing

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ABSTRACT: Microalgae have significant capacity to fix CO_2 and to become a major industrial biomass resource. Examining ways to innovate the microalgae cultivation and processing value chain includes a focus on the most efficient and economical means to produce a liquid oil fraction from the microalgae species. This work compares the use of organic solvent, supercritical carbon dioxide ($SC\text{-}CO_2$), and pyrolysis to assess their relative capacity to derive oil from the marine microalgae *Tetraselmis chui*. The $SC\text{-}CO_2$ technique was shown to be the least effective in natural oil extraction from *T. chui*. The results reveal that pure solvent extraction produces the most complete extraction of natural oil at just under 15% by weight. Subsequent pyrolysis of the post-solvent extraction residue and examination of the byproduct suggest that extraction of natural lipids prior to thermal processing increases the total quantity of bio-oil yield production by more than 11%.

INTRODUCTION

There are many technical and commercial challenges associated with the processing of biomass resources on an industrial scale, requiring thorough investigation of the various trade-offs that come into play.[1] In the case of microalgae biomass, each link in the value chain offers scope for innovation and much work is currently being performed to optimize critical steps, such as cultivation, harvesting, dewatering, drying, transport, and/or processing.[2] Once the microalgae has been cultivated, it requires effective processing to derive the maximum value. Because the natural lipid fraction in many species of microalgae has high potential for direct conversion to liquid transport fuel, efficient removal of the liquid fraction from the algal cells must be achieved in a manner that maximizes production yields at acceptable costs.[3]

Use of organic solvents for the extraction of oils from biomass is well-known, and this has been successfully trialed in the past for the recovery of lipids from microalgae.[4] For instance, a variety of solvent techniques were trialed on *Botryococcus braunii* to extract fatty acid content, with up to 93.1% of the total fatty acid content in the $C_{16}-C_{18}$ range recovered.[5] Likewise, ethanol was successfully used to extract 75% of the fatty acid content from the algae *Porphyridium cruentum*.[6] Many of the solvents commonly used for this purpose are eco-toxic; however, they have the potential to be recovered and managed effectively to mitigate these risks. They also have the advantage of working with little additional process input, including energy, are relatively inexpensive, and can be highly effective. Drawbacks include the fact that a large volume of solvent is often required to achieve effective extraction and the solvent recovery process can be expensive in terms of both energy and cost.[3]

Supercritical CO_2 ($SC\text{-}CO_2$) is an oil extraction technique also used in high-volume, commercial biomass applications.[7] $SC\text{-}CO_2$ is commonly used in processes such as decaffeination or for isolating cooking oil from rapeseed and is acknowledged as a relatively benign means to achieve extraction of useful compounds from biomass, reducing or even eliminating the need for the use of highly toxic organic solvents.[8] For food applications and, thereby, human consumption, $SC\text{-}CO_2$ is emerging as a preferred technique because of substantially reduced contamination risk; however, in the context of liquid transport fuel substitutes, this prospect is of less concern because it is not an ingested product.

$SC\text{-}CO_2$ has previously been trialed on both micro- and macroalgae and has demonstrated the general viability of this extraction technique for select species. Mendes et al. found that, in comparison to organic solvents, such as hexane or acetone (extraction efficiency of 18.5 and 16.8%, respectively), $SC\text{-}CO_2$ provides a comparable yield of 13.3% by weight in lipid from a crushed sample of *Chlorella vulgaris*.[9] In a study of *Spirulina plantesis*, $SC\text{-}CO_2$ yielded 90% of extractable oils in only 15 min at 700 bar and 55 °C, as compared to almost 6 h to achieve the same using Soxhlet extraction with hexane.[10] Likewise, $SC\text{-}CO_2$ is able to efficiently extract the polyunsaturated fatty acid content from microalgae, providing selection sensitivity for additional compounds, such as chlorophyll, which are otherwise insoluble at lower temperatures and pressures.[11]

$SC\text{-}CO_2$ has several potential advantages over other oil extraction processes, such as solvent extraction or mechanical pressing, by providing higher selectivity of individual compounds, low toxicity, and relatively fast processing times.[12,13] Overall efficiency of extraction of fatty acid content appears to

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Table 1. Known Physical and Chemical Properties of *T. chui*[a]

	proximate (air-dried basis)				ultimate (dry and ash-free basis)				
CV (MJ/kg)	IM (%)	VM (%)	FC (%)	ash (%)	C (%)	H (%)	N (%)	O (%)	S (%)
16.1	3.2	68.9	12.9	15.0	46.3	7.8	4.8	40.5	0.6

[a] CV, calorific value; IM, inherent moisture; VM, volatile matter; FC, fixed carbon.

increase with both the temperature and pressure, with optimum conditions for species such as *B. braunii*, *Dunaliella salina*, *S. platensis*, and *C. vulgaris* identified as lying between 40 and 55 °C and between 300 and 350 bar, depending upon the desired length of the hydrocarbon chain for extraction.[14]

The use of SC-CO_2 with a co-solvent can also assist with improving the efficiency and/or profile of extracts while substantially reducing the total volume of organic solvent required.[15] If a SC-CO_2 with co-solvent regime is adopted, temperature and pressure variations can have the effect of improving the selective removal of compounds in the oil matrix. Regardless of the polarity of the oil compounds that are sought or the co-solvent that is chosen accordingly, SC-CO_2 has been reported to raise the efficiency of oil extraction by enhancing the ability of the solvent to diffuse through a biomass sample.[16]

Another route to the production of liquid biofuels from microalgae biomass is through thermal degradation via pyrolysis. Slow pyrolysis of microalgae to a typical commercial operating temperature threshold of 500 °C offers an advantageous route to a broad spectrum of potentially useful commercial products, including biofuels and biochar.[17,18] The pyrolytic oil produced from microalgae has a distinctly different, typically lower grade, and more complex character than the natural lipid that it produces under cultivation. However, a pyrolysis processing regime enables the overall focus to be placed on highly productive microalgae species, where the biomass yield per hectare and, therefore, carbon cycling is prioritized,[19] as opposed to the natural oil yield alone.

The purpose of this work is to investigate direct extraction of the natural fraction of lipid in a fast-growing, highly productive microalgae species (*Tetraselmis chui*) using organic solvents, SC-CO_2, and oil production through pyrolysis. *T. chui* strains have been shown to produce 17% of dry weight in lipid and are traditionally used for culturing of fish and oysters in the aquaculture industry because of their high nutritional value.[20] Additionally, natural lipid extraction combined with pyrolysis of the biomass residue is also investigated here because it potentially presents a two-step process, in which a concentrated, high-value lipid might be directly extracted in the first instance, leaving a biomass residue from which a lower grade, higher volume pyrolysis oil fraction, in addition to biogas and biochar, could be derived.

EXPERIMENTAL SECTION

Microalgae samples of *T. chui* were selected as a reference species in this work. *T. chui* biomass was initially cultured indoors under controlled conditions at the NSW Department of Primary Industry (Fisheries) laboratory in Port Stephens, Australia, wherein the temperature, CO_2-enriched air flow (air + 2% CO_2), and light were controlled. A standard "f/2" nutrient load containing N, P, K, and minerals was introduced to each new volume during cultivation.[21] Bacterial infection during the transfer to progressively higher volume growth vessels was minimized by autoclaving all fittings and volumes.

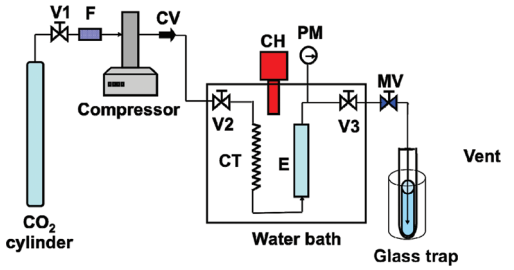

Figure 1. Schematic diagram of SC-CO_2 extraction: V1, V2, and V3, stopping valve; F, filter; CV, check valve; HC, heating coil; E, extraction vessel; CH, circulating heater; PM, pressure meter; and MV, micrometering valve.

Once the culture was stable in 20 L carboys, the solution was transferred to a 25 m^2 outdoor photobioreactor. Under these growth conditions, the microalgae culture was exposed to natural sunlight, weather, and fluctuations in operating conditions, such as temperature.

As soon as the culture matured and reached a stationary phase of growth in the photobioreactor, a sample was mechanically harvested by suspended solid centrifuge. The resulting slurry was then transferred to a cream separator to reduce the biomass to a thick paste and then initially dried in a conventional oven for at least 24 h at 50−55 °C. All samples were finally ground and dried at 70 °C for 3 h in a vacuum oven. Table 1 summarizes the chemical and physical properties of the strain of *T. chui* investigated in this work.

A Dionex accelerated solvent extractor (ASE) 300 apparatus was used to extract lipid from a finely ground and dried *T. chui* sample, using a solvent mixture of dichloromethane (DCM) and methanol (MeOH) in a 9:1 ratio.[22] The sample of microalgae was spaced within a stainless-steel extraction vessel using a quantity of inert baked sand. The extraction method incorporated 3 rolling cycles that involved a 5 min preheating stage, a 5 min heating stage, and a 5 min static stage, prior to a 70%/volume solvent flush over a 5 min purge period. The operating parameters were 100 °C at 103.4 bar (1500 psi) pressure, with 3 × 300 s purge cycles applied.

This technique was repeated as long as an extract of material quantity could be detected, with the combined biomass and sand remixed between each run to ensure even distribution and maximum penetration of solvent within the vessel. The lipid from each run was accumulated in a glass bulb, and the excess solvent was reduced by rotary evaporation. The total lipid extract was quantified gravimetrically.

Three different SC-CO_2 extraction regimes were trialed in this study. In each instance, a 50 mL stainless-steel extraction column loaded with approximately 5 g of ground, dry *T. chui* was connected to the system shown schematically in Figure 1. Any remaining volume in the column was filled with glass spacing beads to distribute and pack the sample tightly and to prevent gravity from clogging the inlet pipe with algae prior to pressurization. The CO_2 pump (ISCO model 260D syringe pump) was cooled to 4 °C, and the pressurized CO_2 was delivered to the extraction vessel through a heating coil. The extraction column and heating coil were immersed in a water tank, the temperature of which was controlled by a circulating heater (Thermoline). The outlet of the

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extraction column was connected to a ball valve that was placed upstream of a micrometering needle valve. The extraction experiments were commenced when the system reached the predetermined pressure and temperature.

There were two stages of extraction: static and dynamic. The static stages were approximately 30 min for all experiments, during which time the system was effectively sealed and held at a constant pressure and temperature to allow for full saturation with SC-CO_2. The subsequent dynamic stages varied from 30 to 50 min and maintained temperature and pressure, while allowing the extract to be pushed through the line into a collection tube. Alternation between static and dynamic stages only occurred when the volume of SC-CO_2 in the system required replenishment. The flow rate of CO_2 was kept at 2 mL/min, measured at operating pressure and 4 °C for all experiments.

As SC-CO_2 was expanded across the micrometering valve during the dynamic stage, the extracted lipid was collected in a glass tube that was refrigerated between −20 and −5 °C in a cooling bath. After the experiments, residual extract that remained in the lines and valves was collected by flushing the line with the relevant solvent (methanol in the case of the "neat" SC-CO_2 run). The solvent flush was thus ultimately mixed with the extract collected in the glass tube. This mixture was later placed in a rotary evaporator to remove the solvent, so that the extracts could then be accurately weighed.

The first run involved "neat" SC-CO_2 only, pressurized at 250 bar and held at 60 °C. These relatively extreme SC-CO_2 parameters were selected for this regime because prior work with microalgae had suggested that extraction efficiency increases with both the temperature and pressure.[15,23−25] The second and third regimes involved use of supercritical CO_2 with the addition of methanol and then ethanol as co-solvents, respectively, at 180 bar and 40 °C. These lower parameters were adopted because it was assumed that the addition of co-solvent would improve extraction efficiency without the need for such relatively extreme conditions.

Pyrolysis oils were obtained by separately heating 100 mg samples of both raw microalgae biomass and post-extraction residues in an infrared pyrolysis furnace. The method incorporated a steady heating rate of 10 °C/min, rising from room temperature to a maximum of 500 °C (the typical threshold for industrial slow pyrolysis) and controlled by a thermocouple attached to the wall of the furnace cylinder. Oils were condensed at room temperature and dissolved using DCM, then immediately collected, and frozen for temporary storage to minimize degradation. Further details of the experimental technique are discussed elsewhere in the literature.[18,26]

Aliquots were derivatized for gas chromatography−mass spectrometry (GC−MS) analysis with *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (100 μL at 80 °C for 1 h). Samples were separated on a DB5-MS column using Agilent 7890A coupled to a Pegasus 4D time-of-flight mass spectrometer operating under the following program: isothermal heating at 40 °C for 2 min, then ramped at 4 °C/min to 310 °C, and isothermal heating at 310 °C for 90 min. Compounds were identified on the basis of library mass spectral data, comparison to known standards, and comparison to reported spectra from the literature.

Volatiles evolved during pyrolysis of microalgae and the post-solvent extraction residue were analyzed separately by a gas chromatograph. A M200 micro-gas chromatograph from MTI Analytical Instruments was connected to the gas outlet of the glass sample tube. A metallic molecular sieve 5A column (10 m in length and 0.32 mm in diameter) at 90 °C was used to separate H_2 and CO, while analysis of CO_2, CH_4, C_2H_4, and C_2H_6 was performed on a bonded polymer Poraplot U column (8 m in length and 0.32 mm in diameter) at 55 °C. Chromatograms were obtained every 90 s using a gas thermal conductivity detector. Carrier helium gas at a rate of 50 mL/min was passed through 50 mg of biomass while maintaining a continuous heating rate of 10 °C/min to a maximum temperature of 750 °C, to monitor compositional changes up to and beyond the industrial operating threshold of 500 °C.

Table 2. Comparison of Lipid Extraction Efficiency from *T. chui* Using Organic Solvent, Supercritical CO_2, Supercritical CO_2 + MeOH, Supercritical CO_2 + EtOH (wt %)

method	extract (wt %)
organic solvent (DCM/MeOH)	14.6
supercritical CO_2 (60 °C/250 bar)	0.01
supercritical CO_2 + MeOH (40 °C/180 bar)	4.3
supercritical CO_2 + EtOH (40 °C/180 bar)	3.8

The Fourier transform infrared (FTIR) spectra of the raw microalgae and the post-solvent extraction residues were recorded using a Nicolet 6700 FTIR spectrometer, applying an attenuated total reflectance (ATR) method with a diamond crystal. The total number of scans was 32, with spectral resolution of 4 cm^{-1}. Omnic Spectra software was used to assist with the interpretation of some of the spectra.

A Mettler Toledo thermogravimetric analysis (TGA) instrument (TGA/DSC 1 STARe system) operated with STARe software was employed to determine the weight loss of the unprocessed microalgae and post SC-CO_2 residue with the temperature. The samples (weighing approximately 30 mg) were placed in a circular aluminum crucible, with an additional empty crucible employed as a reference. All experiments were carried out using nitrogen as a carrier gas set at a flow rate of 20 mL/min, with a heating rate of 10 °C/min to a maximum temperature of 1000 °C. The buoyancy correction for TGA data was conducted using a blank experiment with no sample placed in either of the crucibles prior to each sample run.

RESULTS AND DISCUSSION

The total lipids extracted from the strain of *T. chui* used in this work under the ASE conditions were 14.6 wt %. This compares to the amount of 17% lipid recorded in the literature for the same species,[20] the variation of which may be accounted for by the differing cultivation and harvesting conditions, and genetic expression of individual strains, in addition to the efficiency of the solvent extraction method employed. Fractionation determined that the breakdown of oil fractions amounted to ∼0.5 wt % in aliphatic hydrocarbons, ∼0.2 wt % of aromatic compounds, with the majority of the balance (99.3 wt %) being polar in nature.

Derivatization of the extracts was essential because the polar fraction, specifically O−H groups, does not elute with the column used here and would not otherwise be observed by the mass spectrometer. Notably, H_2O is also a polar molecule; therefore, the ASE DCM/MeOH solvent mixture is likely to have removed residual moisture from the biomass in addition to lipids. Any moisture was later removed from the solvent mixture through the rotoevaporator and, hence, is not present in the derivatized, predominantly polar lipid sample.

The SC-CO_2 work with and without co-solvents was undertaken with varying degrees of success in relation to oil extraction rates, which are compared to the ASE method in Table 2. SC-CO_2 extraction at the pressure of 250 bar and temperature of 60 °C managed to extract only the smallest detectable amount of lipid (0.01 wt %). This is most likely due to the highly polar nature of the natural oil found in *T. chui*. SC-CO_2 is well-known to be most effective with the extraction and selectivity of nonpolar molecules, and the aliphatic fraction in this case represents only around 0.05 wt %.[27] Subsequent SC-CO_2 runs using methanol (4.3 wt %) and, later, ethanol (3.8 wt %) as co-

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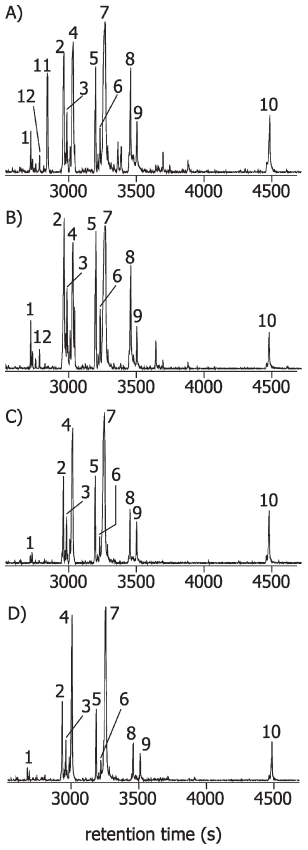

Figure 2. (a) Total ion chromatogram (TIC) of natural lipid extract from raw *T. chui* using solvent extraction (9:1 MeOH/DCM). (b) TIC of extract using pure SC-CO_2. (c) TIC of extract using SC-CO_2 and MeOH co-solvent. (d) TIC of extract using SC-CO_2 with EtOH co-solvent. Compounds detected (underivatized): 1, tetradecanoic acid; 2, polyunsaturated hexadecanoic acid; 3, monounsaturated hexadecanoic acid; 4, hexadecanoic acid; 5, phytol; 6, polyunsaturated octadecanoic acid; 7, monounsaturated octadecanoic acid; 8, polyunsaturated eicosanoic acid; 9, monounsaturated eicosanoic acid; 10, campesterol; 11, siloxane; and 12, phytol.

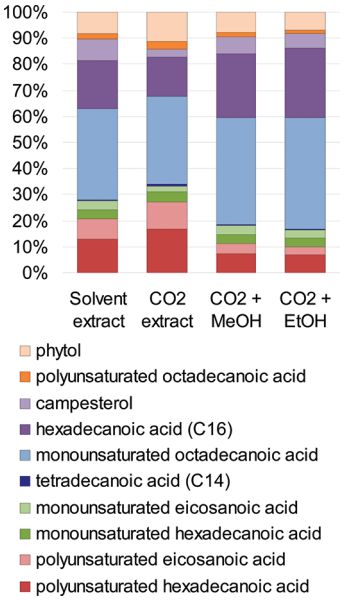

Figure 3. Comparison of extraction techniques and the oil species eluted, as a percentage of the total extract weight (*T. chui*).

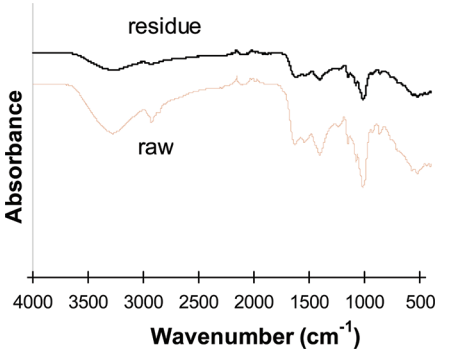

Figure 4. FTIR results of raw algae (*T. chui*) superimposed over post-solvent extraction residue (*T. chui*).

solvents improved the bio-oil extraction ratio compared to pure SC-CO_2; however, these extraction rates were lower than the extraction efficiency of the ASE method.

This study reveals that the SC-CO_2 method does not appear well-suited to oil extraction from *T. chui* using the conditions studied in this work. Variations in the temperature and pressure, in addition to the prospect of pretreatment, such as cellular disruption of the microalgae, may improve SC-CO_2 extraction efficiency; however, this is only likely to be of value where a co-solvent is used, given the polarity of the lipids in this species. Considering the polar nature of water, it is also possible that an increase in the moisture content of the sample could aid SC-CO_2 extraction of commensurately polar oil molecules to achieve the desired outcome.

Of particular note in this study was characterization of the extracts derived in each of the four experimental regimes. The compounds detected by GC−MS in the pure SC-CO_2 solute were close to identical to those extracted by the solvent and SC-CO_2/co-solvent methods, despite differences in extraction volume overall (Figure 2). The most common compounds detected include a selection of free fatty acids (FFAs) extracted in varying concentrations, including both the mono- and polyunsaturated form of eicosanoic acid (peaks 7 and 8). This substance is common to peanut oil and other fatty substances, such as butter. Peak 5 in all samples corresponds to phytol, with a chemical structure of $C_{20}H_{40}O$. Phytol is an ester-linked side chain of chlorophyll *a* and a biogeochemical marker in petroleum sediments.[28] As a material feedstock in the pharmaceutical industry, it is used to synthesize vitamin E and K_1 and potentially has direct commercial applications.[29] Peaks 2, 3, and 4 are related to hexadecanoic acid ($C_{16}H_{32}O_2$), otherwise known as palmitic acid. All fatty acids detected can have direct commercial application in the production of a petrodiesel liquid transport fuel substitute through the process of transesterification that forms biodiesel.

A major difference between the extracts is that pure SC-CO_2 was unable to remove some of the lightweight oil molecules from the microalgae biomass (not presented in Figure 2 because they elute substantially earlier), namely, glycerol, methyl 1*H*-indole, and pyrimidine, which are otherwise extracted in all cases where organic solvent is present. The solvent method is also able to extract additional phytol. This extra phytol (peak 12) is detected in the pure SC-CO_2 regime also, however, not in the SC-CO_2

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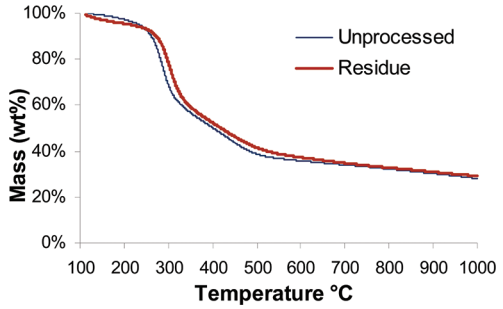

Figure 5. Mass loss from raw *T. chui* compared to mass loss from post-solvent extraction *T. chui* residue determined by TGA (wt %).

co-solvent runs. In each extraction regime, the major lipids (FFAs) are detected in similar ratios of abundance (Figure 3). As such, this suggests that there is no obvious selectivity of compounds taking place in relation to SC-CO_2 extraction compared to the organic solvent.

A sample of raw *T. chui* was compared to the post-solvent extracted residue derived from the same species, given the relative success of the organic solvent extraction approach in eluting most of the known natural lipids contained in this species. As shown in Figure 4, the FTIR spectra of each of these two samples are similar and present bonds that are typically found in samples of biological origin. In particular, absorption bands detected in the FTIR spectra of the examined samples indicate the presence of lipids, proteins, peptides, and sugars.

Protein absorption bands are associated with characteristic amide groups. A strong bond around 1628 cm^{-1} in both samples represents C═O stretching coupled with C−N stretching and also N−H bending vibrations. C−N and N−H also contribute to the bond detected around 1545 cm^{-1}, with further N−H bending exhibited as a weak peak around 760 cm^{-1}. Several of the bonds in the region between 1000 and 1500 cm^{-1} are likely absorption markers for nucleic acids. For example, the bonds between 1250 and 1500 cm^{-1} are due to vibration coupling between a base and a sugar, while in the range of 1000−1250 cm^{-1}, sugar−phosphate chains are observed.

Other bonds appearing in the region between 900 and 1450 cm^{-1} are mostly due to molecular groups containing oxygen, carbon, and hydrogen atoms. A peak at 930 cm^{-1} corresponds to C−O−H out-of-plane bending vibrations. A peak at 1077 cm^{-1} corresponds to C−O in alcohols and phenols. A peak at 1237 cm^{-1} corresponds to C−O stretching in esters and carboxylic acids. A peak at 861 cm^{-1} corresponds to C═S stretching in thioamides. The band detected at 3273 cm^{-1} in the raw sample corresponds to stretching vibrations in an O−H group, and because of strong hydrogen bonding, the band is broad, an indicator of moisture in the sample. The presence of water in the raw microalgae is further supported by a very weak corresponding signal evident in the residue, because the solvent elutes much of this.

Indicators of the lipid include CH_2 asymmetric stretching bonds at 2930 cm^{-1} and symmetric stretching vibrations at 2850 cm^{-1}. A CH_2 bending vibration is evident at around 1405 cm^{-1}. Ester groups are also represented by a weak C═O stretching vibration, which is markedly more visible in the raw algae sample, a logical finding given removal of some of the lipid content as fatty acids during solvent extraction.

The absorption bands identified by FTIR between 400 and around 750 cm^{-1} correspond to mineral matter (a metal−halogen stretching vibration), specifically a Si−O vibration. A double metal−oxygen bond (M═O) and Si−O also contribute to a strong peak at around 1016 cm^{-1} in both samples.

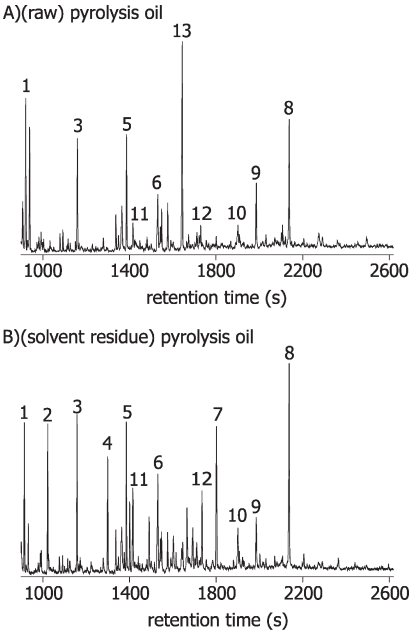

Figure 6. GC−MS spectra comparing (a) oil derived by slow pyrolysis of raw algae and (b) liquid derived by slow pyrolysis of post-solvent extraction microalgae residue (*T. chui*): 1, derivatizing agent; 2, silane, (2-furanylmethoxy)trimethyl-; 3, phenol; 4, 1,2-bis(trimethylsiloxy-2-(3′-trimethly silyoxyphenyl))ethanone; 5, methyl phenol; 6, hymexazole; 7, oxooctanoic acid; 8, unsaturated alkanoic acid; 9, tetramethyl quinolone; 10, methyl 1*H*-indole; 11, propanoic acid; 12, 1,3-cyclopentadiene, 5,5-dimethyl-1-trimethylsilyl-; and 13, glycerol.

Further, in this work, the raw algae and the post-solvent extraction algae residue were pyrolyzed independently at a heating rate of 10 °C/min and the pyrolysis properties were compared between the two samples. Analysis by TGA, shown in Figure 5, indicates that the *T. chui* post-solvent extracted residue initially loses weight more readily than the raw biomass sample, most likely because of the absence of the inherent moisture that has been removed by organic solvent. This has the effect of reducing the amount of process energy required to initiate decomposition, at least at low temperatures. However, at approximately 250 °C, the decomposition pathways crossover (expressed as a percentage of the weight), because the lipid content retained by the raw sample begins to decompose and devolatilize quickly. This behavior is consistent with the second stage of devolatilization that occurs when organic molecules in microalgae are decomposed.[30] Weight in the raw sample rapidly decreases with an increasing temperature at this point and quickly exceeds that of the residue. At around 300 °C, the residue lags the raw sample by as much as 10% of the total sample weight, before narrowing this gap to around 3% at around 340 °C. As the temperature increases to 500 °C, this gap is approximately maintained; however, the weight loss trajectories then begin to steadily converge toward 700 °C as all residual volatile matter is driven off.

The pyrolytic oils evolved during pyrolysis of the two samples were collected and analyzed by GC−MS, while the evolved volatile gases were analyzed with a micro-gas chromatograph. The pyrolysis oils produced from the raw and residue samples

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Table 3. Natural Lipid Extract Compared to Oil Species Obtained from the Slow Pyrolysis of Post-solvent Extract Residue (*T. chui*)[a]

raw	residue	peak number	oil species	retention time (s)
!!	!!	1	derivatizing agent	915
⊥	!!	2	silane, (2-furanylmethoxy)trimethyl-	1023
!!	!!	3	phenol	1161
⊥	!!	4	1,2-bis(trimethylsiloxy-2-(3′-trimethly silyoxyphenyl))ethanone[b]	1303
!!	!!	5	methyl phenol	1392
!!	!!	6	hymexazole	1535
⊥	!!	7	oxooctanoic acid	1809
!!	!!	8	unsaturated alkanoic acid	2146
!!	!!	9	tetramethyl quinolone	1995
!!	!!	10	methyl 1*H*-indole	1907
!!	!!	11	propanoic acid	1420
!!	!!	12	1,3-cyclopentadiene, 5,5-dimethyl-1-trimethylsilyl-	1742
!!	⊥	13	glycerol	1649
!!	⊥	14	monounsaturated octadecanoic acid	3220
!!	⊥	15	campesterol	4443

[a] !!, present; ⊥, absent. [b] Inconclusive match from the database.

are dominated by low-molecular-weight, cyclic, aromatic, and branched molecules, with some short-chain fatty acids also present. The more significant peaks detected in the GC−MS chromatograms (Figure 6) for each sample are numbered and presented in Table 3, indicating those that are shared and those that are unique to each. Compound identification was again made on the basis of mass spectral matching with libraries and a comparison to the literature.

The two pyrolysis oil samples exhibit some similarities in composition, albeit at varying degress of relative intensity. Methyl 1*H*-indole carries over from the natural lipid fraction to the pyrolyzed raw sample and is likewise found in the pyrolysis liquid derived from the residue, suggesting that it is not decomposed at temperatures up to 500 °C. Phenol and methyl phenol are present in both pyrolysis liquids (peaks III and V), a useful chemical building block of which there are already well-established markets. Hymexazole (VI) was detected in both pyrolysis bio-oil samples. This compound is of interest as an agrochemical and is commonly used as a pesticide.

Significant differences between the two pyrolysis oils include the presence of glycerol and campesterol in the raw pyrolysis oil, largely indicative of the natural lipid component of the algae. In contrast, an unidentified unsaturated alkanoic acid forms in a large quantity through pyrolysis of both samples. This compound is characterized by a carboxyl group that is readily converted to an ester. A large peak most closely identified as 1,2-bis(trimethylsiloxy-2-(3′-trimethly silyoxyphenyl))ethanone is detected at a retention time of 1303 s in the residue bio-oil only.

Analysis of the primary volatile gases evolved in each sample show differences in the pyrolytic behavior between the raw and post-solvent extracted residue samples, as shown in Figure 7. The retention of lipid in the raw sample gives rise to a greater amount of CO_2 at a peak rate of evolution of around 300 °C as the light oils in this fraction are gasified. The release of CO_2 from the post-solvent extraction residue occurs at approximately the same temperature, albeit at a lesser intensity, reflecting an absence of lipid. Because the evolution of CO_2 and CO is of a similar pattern in both samples, this suggests that a significant proportion of these gases are the result of decomposition of other components

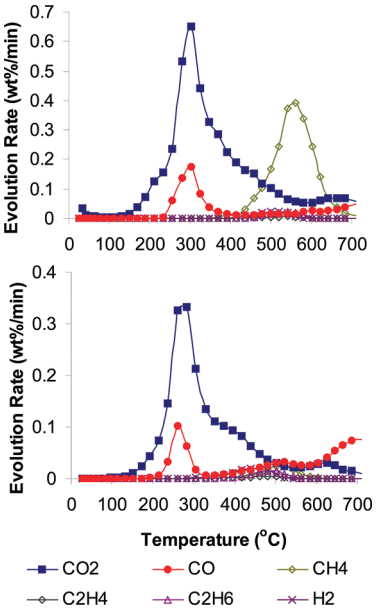

Figure 7. Evolution of volatile gases from (a) raw *T. chui* during slow pyrolysis compared to (b) slow pyrolysis of post-solvent extraction *T. chui* residue (wt %/min).

of the biomass sample, such as proteins, carbohydrates, and amino acids.

The other notable difference in biogas evolution relates to the emergence of methane in the raw biomass that begins at around 420 °C and expands to a rate of weight loss that is approximately 20 times the rate of evolution of methane from the post-solvent extraction sample at the peak of 570 °C. The differences in gas composition during pyrolysis of raw algae and post-solvent extraction residue relates to the cracking and gasification of the long-chain FFAs that were isolated during solvent extraction. The break-up of these acids releases CO_2 from 300 °C and, later, breaks up the terminal methyl group that forms the end of the lipid chains. It is also possible that these methyl groups react

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Table 4. Comparison of Evolved Liquid, Gas, and Char Ratios Derived from Slow Pyrolysis of Unprocessed *T. chui* and Post-solvent Extracted *T. chui* Residue (500 °C)

species	char (wt %)	gas (wt %)	liquid (wt %)
T. chui unprocessed	38.8	9.8	51.4
T. chui post-solvent extracted residue	38.7	4.9	56.4

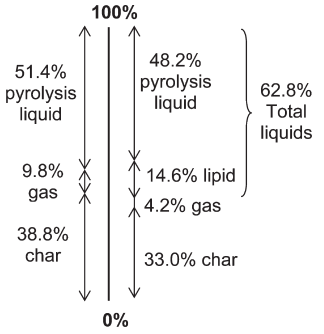

Figure 8. Comparison of evolved pyrolysis products and their ratios from raw *T. chui* versus post-solvent extraction *T. chui* residue (wt %).

further with some of the available H_2 to form methane (CH_4). The net result of the absence of FFAs in the post-solvent extraction residue is that the biogas fraction has a lower calorific value overall, because less CH_4 is produced.

The ratio of oil, gas, and char products observed at a temperature of 500 °C also reflects the aforementioned differences in behavior observed during slow pyrolysis (Table 4). The gas yield released to 500 °C by the raw sample was 9.8% by weight, considerably higher than the gas yield derived from the post-solvent extracted residue (4.9 wt %). The char production observed between the two samples was the same, with around 38.8 wt % in each. Finally, the amount of pyrolytic liquid was found to be 5.0% higher in the residue compared to pyrolysis of the raw sample, at 56.4 wt %.

Notably, in the case of the solvent residue byproduct, these percentages should be adjusted to reflect the percentage of the upstream biomass feedstock for proper comparison of evolved product ratios to be made. As shown in Figure 8, the ratio of gas, char, and oil derived from the residue equates to 4.2, 33.0, and 48.2%, of the starting weight, respectively. Furthermore, the pyrolysis liquid fraction derived from the residue is likely to contain less water because of the dehydrating effect of the prior solvent extraction (although water can also be reformed as a product of secondary reactions). Hence, the bio-oil component and energy density is likely to be higher again as a proportion of the liquid fraction, in addition to being greater in volume than the total liquid fraction derived from direct pyrolysis of the raw biomass.

Overall, a two-step combination of solvent extraction of natural lipids from microalgae biomass (which may in time be replaced by an alternative, equally efficient, and optimized process) combined with slow pyrolysis of the residue could yield more than 11% more oil product overall on a dry weight basis, as compared to pyrolysis of raw algae, because of the nature of the thermochemical decomposition process (Figure 5). The nature of the solvent extraction process is such that it has the added benefit of removing moisture from the biomass and extracts high-value chemical compounds. While industrial use of organic solvents raises concerns about environmental impact and toxicity, these can theoretically be captured, recycled, and reused as part of a stewardship system and, thus, their impacts can be managed.

CONCLUSION

Extraction of the natural lipid fraction found in *T. chui* presents an opportunity to recover a high-value product directly from a dried microalgae biomass sample. This work found that, for *T. chui*, in which the natural lipid fraction is polar in nature and consists primarily of fatty acids, use of neat SC-CO_2 for lipid removal is likely to be ineffectual. Co-solvent extraction with SC-CO_2 produces an improved result; however, further refinement and testing of SC-CO_2 processing parameters and methods with this species is required. ASE using DCM/MeOH in a ratio of 9:1 showed the largest natural lipid extraction from the studied microalgae species at almost 15 wt %. The bio-oil production yields could be further maximized by pyrolysis of the post-extraction residue. The oils resulting from pyrolysis of the post-solvent extraction microalgae residue were found to be of similar composition to those produced through pyrolysis of raw microalgae, albeit with minor differences that appear to reflect the presence or absence of quantities of natural lipid in the raw microalgae sample. This study demonstrates that the combination of a two-step lipid extraction and slow pyrolysis processing regime can yield an oil product high in valuable fatty acids in the first instance, in addition to increasing the total amount of oil yield produced overall when combined with slow pyrolysis processing. This can be achieved without greatly affecting the char yield, although the calorific content of the equivalent biogas fraction is reduced, commensurate to the preservation of the natural lipid. Subject to the techno-economic feasibility and life-cycle profile of a scalable system, a two-step lipid extraction and pyrolysis regime may further support the commercial viability of microalgae cultivation and processing through diversification of the product value chain. Ultimately, this maximizes retention and production of the bulk oil product while maintaining higher rates of unit area biomass productivity through cultivation of select microalgae species.

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