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ORIGINAL PAPER

Optimization of an Oil Extraction Process for Algae from the Treatment of Manure Effluent

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Abstract Increasing interest in the coupling of biological wastewater treatment processes with the generation of value-added products (such as oil containing ω-3 fatty acids (FA)) has stimulated efforts in adapting extraction methods for treatment byproducts. This study's objective was to compare a high temperature/pressure extraction method (accelerated solvent extraction) (ASE) and a manual extraction method (modified Folch extraction) with regard to their ability to extract total oil from three algae samples from the treatment of dairy manure effluent. The efficiency of total oil and FA extraction with three solvents (chloroform/methanol, isopropanol/hexane, and hexane) was also evaluated using the ASE method. Results showed that the ASE method yielded higher values for total oil content compared to the Folch method but similar values for FA content and composition after four extraction cycles with chloroform/methanol. However, the ASE method yielded much higher amounts of FA in the first cycle (85–95% of total extracted) compared to the Folch method (44–55% of total extracted in the first cycle). As expected, the extraction efficiency of the ASE method for FA was dependent on the extraction solvent. FA content values using ASE with chloroform/methanol > isopropanol/hexane > hexane. FA content values using the Folch method or ASE with chloroform/methanol were not significantly influenced by sample particle size within the size range of 0.1–1 mm.

Keywords Accelerated solvent extraction · Algae · Manure · Bioenergy · Wastewater · Treatment

Introduction

There is considerable interest in the coupling of biological waste treatment processes to bio-energy production [1]. One possibility is to use an algae-based treatment process to treat agricultural wastewater or manure effluent and to extract fatty acids (FA) from the harvested algal biomass [2–5]. Although algae-based wastewater treatment processes have been in existence for over 30 years, the historically low price of crude oil has inhibited efforts to use this biomass as an energy feedstock [6]. However, increasing costs of crude oil and mandated targets for bio-based energy production have stimulated renewed interest in this strategy [7–9].

Direct extraction of FA from wastewater-grown algae is but one potential use for the algal biomass [10]. Other potential uses are an organic fertilizer, animal feed supplement, or chemical feedstock [11, 12]. With regard to the use of algae as an animal feed supplement, there has been significant research on the beneficial health effects of (ω-3) FA consumption. Determination of the FA composition of the oil extracted from algae is important because of the interest by poultry and dairy producers in using feeds to increase the ω-3 fat content of eggs and dairy products [13].

Our laboratory has conducted studies on the use of one algal based wastewater treatment technology, termed algal turf scrubbers, to remove nutrients from dairy manure effluents [14]. The total cost of such systems depends, in part, on the value of the resulting algal biomass [12]. In typical operations, algal biomass would be harvested from

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shallow raceways and air-dried prior to storage and subsequent use on- or off-farm. Thus, we have been especially interested in adapting extraction and analytical methods that would be amenable for fresh and air-dried material.

Determination of algal FA content and composition has been conducted by extraction with solvent mixtures (typically chloroform/methanol) as developed by Folch [15] and Bligh and Dyer [16]. Methods using supercritical CO_2 with have also been developed for extraction of algal FA, but are not yet in common use [17, 18]. Accelerated solvent extraction (ASE) has been used to determine total fat and FA composition in cereal, lyophilized egg yolk, lyophilized chicken muscle, corn, and soybean [19, 20]. The objective of this study was to compare ASE and a manual extraction method (modified Folch extraction) with regard to their ability to extract total oil from three algae samples from the treatment of dairy manure effluent. The efficiency of total oil and FA extraction with three solvent combinations (chloroform/methanol, isopropanol/hexane, and hexane) was also evaluated using the ASE method.

Experimental Procedures

Production of Algal Biomass

Algal biomass was produced using indoor and outdoor laboratory-scale algal turf scrubbers (ATS) with dairy manure effluent collected from the Dairy Research Unit of the USDA's Beltsville Agricultural Research Center in Beltsville, Maryland as described previously [14]. In addition, algal biomass was produced using an indoor laboratory-scale algal turf scrubber used to remove nutrients from a 3,000-L fresh water aquarium in Takoma Park, Maryland. Both indoor and outdoor systems were dominated by the filamentous green algae *Rhizoclonium hieroglyphicum*. Wet algal biomass was harvested weekly using a wet/dry vacuum, dewatered by sieving harvested material through 2-mm mesh nylon netting (Aquatic Ecosystems, Apopka, FL) to approximately 10% solids content, then air-dried for approximately 48 h using electric fans to approximately 90% solids content. Dried biomass was initially ground in a Wiley Mill to pass a 3-mm sieve and stored in sealed plastic bags at 20–25 °C. For oil and FA analyses, aliquots of dried biomass were ground to pass a 0.5-mm sieve and stored in sealed plastic bags as above. Characteristics of the three algae samples used in the study are shown in Table 1. For experiments designed to determine the effect of sample particle size on the extraction efficiency of FA, four aliquots of one algae sample were ground separately in a Wiley Mill to yield four particle size (<0.09, 0.09–0.18, 0.18–0.50, 0.50–1.0 mm) fractions.

Extraction of Oil from ATS Biomass

Accelerated Solvent Extraction

Oil content were extracted from algal samples (in triplicate) using an automated Dionex 200 accelerated solvent extraction (ASE) system (Dionex Corporation, Salt Lake City, UT, USA). Dried algal samples (2.5 g) were mixed with 1 g diatomaceous earth and 30 g Ottawa sand (20–30 mesh) prior to being loaded into 33-ml sample cells. After the sample mix was loaded, Ottawa sand was used to fill any extra space in the cell. Extraction conditions for the ASE were: 5 min preheat; pressure, 1,500 psi (10.3 MPa); temperature, 120 °C; heat time, 5 min; flush volume, 50% of cell volume; purge time, 60 s; static cycles, 4 [20, 21]. Solvents tested with the ASE included chloroform:methanol (2:1, v/v), isopropanol:hexane (2:1, v/v), and hexane [19, 20]. The resulting extracts (typically 30 ml per cycle) were stored at 4 °C prior to removing aliquots for oil and FA analysis. The percentage oil content was determined gravimetrically by transferring 12-ml aliquots of each extract to pre-weighed vials and evaporating the solvent under a stream of nitrogen. Results are reported on a dry weight (DW) basis.

Conventional Extraction Method

Oil content was manually extracted from algal samples using a modified Folch method [15]. Dried algal samples (1 g) were extracted in triplicate for 20 min with 20 ml of chloroform/methanol (2:1 v/v) at 25 °C in a 100 W sonication bath (Branson model 2510). After sonication, distilled water (2 ml) was added to each extract and the contents were mixed by vortexing (three pulses of 10 s each). Tubes were then incubated for 10 min at room temperature to allow the organic and aqueous layers to separate. After removing and saving the bottom (organic) layer, the aqueous layer was re-extracted by adding chloroform (6.6 ml), mixing by vortexing (three pulses of 10 s

Table 1 Characteristics of algal samples used for analysis

Algae sample no.	Source of algae	Nutrient input for algal growth	*N* (%)	*P* (5)	Moisture content (%)	Ash content (%)
1	Outdoor ATS	Dairy manure effluent	5.4	0.7	18	11
2	Indoor ATS	Dairy manure effluent	4.3	0.7	11	8
3	Indoor ATS	Freshwater aquarium	3.7	0.5	19	8

each) and allowing the layers to separate. The remaining aqueous layer was re-extracted twice more in this manner for a total of four extracts per sample. The resulting extracts (6–10 ml each) were stored at 4 °C prior to removing aliquots for oil and FA analysis. The percentage oil content was determined as described above.

FA Analysis

FA methyl esters (FAMEs) were prepared and analyzed using the MIDI system (Microbial ID, Inc., Newark, DE, USA). Aliquots of crude extracts (0.25–3 ml, containing approximately 200 µg FA) were dried under a stream of nitrogen, then saponified for 30 min at 100 °C using 1 ml of MIDI Reagent 1 (45 g NaOH, 150 ml H_2O, 150 ml methanol). After cooling, 2 ml of MIDI Reagent 2 (325 ml 6 N HCl, 275 ml methanol) were added and the solution heated at 80 °C for 10 min to methylate the FA. The FA methyl esters were extracted with 1.25 ml of MIDI Reagent 3 (1:1 hexane:methyl *tert*-butyl ether) for 10 min. The organic phase was washed with three ml of MIDI Reagent 4 (10.8 g NaOH, 900 ml H_2O) and then transferred to a gas chromatography vial.

FAME analysis was performed using an Agilent 6890 gas chromatograph with autosampler, split-splitless inlet, flame ionization detector, and Ultra two column (25 m long × 0.2 mm I.D. × 0.33 µm film thickness) (Agilent Technologies, Palo Alto, CA, USA). The carrier gas was hydrogen at a constant pressure of 9.7 psi. A 100:1 split injection was used. The injector was held at 250 °C while the detector was kept at 300 °C. The oven was at 170 °C initially, then ramped to 300 °C at 5 °C/min, and held at 300 °C for 10 min. The system was controlled with Chemstation (Agilent) and Sherlock (MIDI) software. The MIDI EUKARY method was used to identify the FA methyl esters. Palmitic acid (16:0), heptadecanoic acid (17:0), and glyceryl triheptadecanoate (Sigma) were alternately used as external standards for FAME quantitation. The standards were derivitized using the MIDI protocol and analyzed by gas chromatography under the same conditions described above.

Results and Discussion

Extraction Efficiencies of Oil and FA Content

The results presented in Table 2 show that the ASE method consistently yielded higher oil content values as compared to those resulting from the classical manual extraction (Folch's) method. However, similar values for FA content were obtained after four extraction cycles by both ASE and Folch's methods using chloroform/methanol as extraction solvent (Table 1). The higher total oil value with ASE procedure may be attributed to extraction of additional phytochemicals at higher temperature (120 °C) with ASE as compared to ambient temperature with the Folch procedure. Use of ASE with two other solvent combinations (isopropanol/hexane or hexane alone) yielded significantly lower values for oil content and FA content compared to values from the manual and ASE methods with chloroform/methanol (Table 2).

Table 2 Effect of extraction method on oil and fatty acid content from three dried algal samples

Solvent	Manual extraction	ASE		
Sample no.	Chl/Me	Chl/Me	IPA/hex	Hexane
Oil content (% DW ± SD)				
Algae no. 1	6.7 ± 0.4	10.7 ± 0.2	5.9 ± 0.4	2.8 ± 0.2
Algae no. 2	7.0 ± 0.3	11.0 ± 0.7	6.5 ± 1.0	2.4 ± 0.1
Algae no. 3	4.8 ± 0.5	6.1 ± 0.1	5.3 ± 0.9	0.9 ± 0.6
Fatty acid content (% DW ± SD)				
Algae no. 1	1.64 ± 0.18	1.59 ± 0.14	1.07 ± 0.03	0.78 ± 0.16
Algae no. 2	1.53 ± 0.10	1.41 ± 0.03	0.92 ± 0.08	0.38 ± 0.04
Algae no. 3	1.41 ± 0.16	1.31 ± 0.18	1.03 ± 0.10	0.25 ± 0.03

Chl/Me chloroform/methanol (2:1, v/v), *IPA/hex* isopropanol/hexane (2:3, v/v)

Although the manual and ASE methods with chloroform/methanol yielded comparable amounts of FA after four cycles of extractions, the two methods showed very different efficiencies with each extraction cycle. When the oil and FA content results are detailed for each extraction cycle, results show that the manual method yielded 46–55% of total oil in first extraction, 23–26% in second cycle, 14–17% in third, and 7–11% in fourth. The FA extraction efficiency was nearly identical to that for oil (44–54%, 28–32%, 12–16%, and 6–8% of FA extracted in the sequential extractions, respectively) (Table 3). In comparison, ASE extractions with all of the three solvent mixtures yielded the majority of oil and FA in the first extraction cycle (Table 3). The ASE extractions with chloroform/methanol yielded 78–81% of total extractable oil and 92–95% of total extractable FA in first extraction, 9–11% of oil and 3–6% of FA in second extraction, and roughly 5% of oil and 1% of FA in each of third and fourth extractions. The ASE extractions with isopropanol/hexane yielded comparable results to those of ASE with chloroform/methanol (78–84% of oil and 83–92% of FA extracted in the first cycle, 8–9% of oil and 5–7% of FA extracted in the second cycle, and roughly 5% of oil and 4% of FA in each of the third and fourth extraction cycles. Oil extraction efficiencies of ASE with hexane were slightly higher in the first extraction compared to those from use of the other solvents (87–92%) but FA extraction

Table 3 Effect of number of extraction cycles and method on oil and fatty acid content from three algal samples

Solvent	Manual extraction Chloroform/methanol (2:1, v/v)	Accelerated solvent extraction		
		Chloroform/methanol (2:1, v/v)	*iso*-Propanol/hexane (2:1, v/v)	Hexane
Oil content (% of total extracted ± SD)				
Algae no. 1				
Cycle 1	55 ± 2	81 ± 2	84 ± 4	92 ± 3
Cycle 2	23 ± 1	10 ± 1	8 ± 2	4 ± 1
Cycle 3	14 ± 2	5 ± 1	4 ± 1	2 ± 2
Cycle 4	7 ± 2	4 ± 1	3 ± 1	1 ± 1
Algae no. 2				
Cycle 1	51 ± 2	78 ± 1	79 ± 11	87 ± 2
Cycle 2	25 ± 1	11 ± 1	9 ± 3	7 ± 2
Cycle 3	14 ± 1	5 ± 1	5 ± 2	3 ± 1
Cycle 4	10 ± 1	6 ± 1	6 ± 6	2 ± 1
Algae no. 3				
Cycle 1	46 ± 2	81 ± 5	78 ± 8	89 ± 4
Cycle 2	26 ± 1	9 ± 1	8 ± 2	6 ± 5
Cycle 3	17 ± 2	6 ± 1	9 ± 4	3 ± 3
Cycle 4	11 ± 4	4 ± 5	4 ± 3	3 ± 3
Fatty acid content (% of total extracted ± SD)				
Algae no. 1				
Cycle 1	51 ± 4	95 ± 1	92 ± 1	95 ± 1
Cycle 2	29 ± 3	3 ± 1	5 ± 1	3 ± 1
Cycle 3	14 ± 3	1 ± 1	2 ± 1	1 ± 1
Cycle 4	6 ± 1	1 ± 1	1 ± 1	1 ± 1
Algae no. 2				
Cycle 1	54 ± 2	92 ± 1	86 ± 7	91 ± 3
Cycle 2	28 ± 1	6 ± 1	7 ± 2	6 ± 2
Cycle 3	12 ± 1	1 ± 1	3 ± 1	2 ± 1
Cycle 4	6 ± 1	1 ± 1	5 ± 5	1 ± 1
Algae no. 3				
Cycle 1	44 ± 1	93 ± 5	83 ± 7	94 ± 4
Cycle 2	32 ± 2	4 ± 1	6 ± 2	4 ± 3
Cycle 3	16 ± 1	2 ± 1	8 ± 7	1 ± 1
Cycle 4	8 ± 2	1 ± 1	3 ± 2	1 ± 1

efficiencies were similar to the others (91–95% of FA extracted in the first extraction cycle) (Table 3). The above result illustrates the importance of evaluation of extraction cycles for quantitative extraction of oil or other phytochemicals. The optimization of number of extraction cycles is frequently overlooked during method development.

Effect of Extraction Methods on FA Composition of the Extracted Oil

There were no differences in FA composition of extracts from manual extraction method and ASE using chloroform/methanol as the solvent (Tables 4, 5, 6). Sequential manual extractions yielded extracts with consistent FAMEs compositions. In general, the compositions of sequential ASE extracts were also consistent. However, composition of third and fourth ASE extracts (that generally contained less than 5% of total FA) differed from first and second extracts because of the disappearance of minor components from the GC profiles. Unsaturated FA were preferentially extracted with decreasing polarity of the solvent used, with hexane extraction yielding the extracts containing the highest concentrations of unsaturated FA (Tables 4-6).

Effect of Sample Particle Size on Extraction Efficiency

In order to determine the effect of sample particle size on the extraction efficiency of FA in air-dried algae samples,

Table 4 Fatty acid composition (% of total ± SD) as a function of extraction method and number of extraction cycles for algal sample no. 1

Solvent	Manual extraction				Accelerated solvent extraction											
	Chl/Me				Chl/Me				IPA/hex				Hexane			
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
14:0	3.6 (±0.2)	3.3 (±0.2)	3.5 (±0.2)	3.5 (±0.3)	3.7 (±0.2)		3.7 (±2.5)		4.9 (±0.3)	5.2 (±1.2)	4.3 (±0.2)	1.8 (±3.1)	6.0 (±1.8)	3.2 (±5.6)	8.3 (±1.9)	0.0
15:0	3.4 (±0.2)	3.1 (±0.0)	3.2 (±0.1)	2.9 (±0.4)	2.1 (±0.7)				1.8 (±0.4)							
16:0	39.4 (±1.1)	37.1 (±0.6)	37.9 (±1.5)	36.8 (±1.0)	39.7 (±1.7)	55.5 (±7.8)	49.1 (±2.7)	60.2 (±20)	45.5 (±2.4)	47.6 (±9.2)	43.8 (±3.0)	47.7 (±2.0)	56.7 (±10.6)	81.0 (±3.5)	72.9 (±13.3)	94.6 (±9.4)
16:1 ω9	5.7 (±0.4)	1.6 (±0.3)	3.3 (±2.6)	2.2 (±0.6)	4.6 (±2.0)				1.6 (±2.7)	1.3 (±2.3)		1.9 (±3.3)				
16:1 ω7	7.9 (±0.2)	7.6 (±0.2)	7.8 (±0.2)	8.2 (±0.5)	8.2 (±0.4)	15.2 (±2.0)	12.5 (±1.3)	8.8 (±7.6)	7.6 (±0.1)	8.6 (±1.7)	8.3 (±0.7)	10.5 (±3.9)	7.6 (±1.2)		3.8 (±6.5)	
16:2 ω6	1.6 (±0.1)	1.7 (±0.1)	1.9 (±0.0)	2.1 (±0.2)	2.1 (±0.1)											
18:0	4.7 (±0.2)	3.8 (±0.1)	4.1 (±0.3)	4.1 (±0.2)	4.5 (±0.3)				5.4 (±0.5)	3.8 (±3.3)	3.3 (±2.9)		8.0 (±1.4)	9.4 (±8.2)	7.1 (±6.1)	
18:1 ω9	22.2 (±0.2)	24.0 (±1.3)	24.8 (±1.2)	25.4 (±2.0)	25.4 (±1.8)	21.7 (±8.4)	27.1 (±1.8)	24.9 (±7.2)	22.2 (±0.7)	24.9 (±5.1)	28.5 (±1.8)	27.5 (±0.6)	15.4 (±13.3)	6.4 (±11.0)	7.8 (±13.5)	5.4 (±9.4)
18:2 ω6	5.8 (±0.0)	6.2 (±0.3)	6.7 (±0.2)	6.9 (±0.2)	6.7 (±0.1)	7.6 (±1.3)	8.4 (±0.6)	6.1 (±5.3)	5.4 (±0.3)	8.5 (±0.6)	7.8 (±0.6)	8.7 (±0.7)	3.4 (±2.9)			
Total sat	54	54	52	51	52	56	52	60	61	57	54	50	73	94	88	95
Sat/unsat	1.2	1.2	1.1	1.1	1.1	1.2	1.1	1.5	1.6	1.3	1.2	1.0	2.7	14.6	7.6	17.5

Table 5 Fatty acid composition (% of total ± SD) as a function of extraction method and extraction cycle for algal sample no. 2

Solvent	Manual extraction				Accelerated solvent extraction											
	Chl/Me				Chl/Me				IPA/hex				Hexane			
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
14:0	5.6 (±0.1)	5.2 (±0.2)	5.0 (±0.2)	5.7 (±0.2)	6.7 (±0.1)	6.0 (±0.2)	7.8 (±1.3)	9.9 (±1.0)	4.9 (±0.9)	6.8 (±0.7)	6.2 (±0.1)	6.4 (±2.0)	11.5 (±1.4)	12.9 (±3.8)	15.5 (±2.8)	16.5 (±2.7)
15:0	3.3 (±0.2)	3.3 (±0.3)	2.8 (±0.5)	3.5 (±0.2)	2.1 (±0.8)				1.1 (±1.2)			0.8 (±1.4)				
16:0	31.4 (±0.1)	31.2 (±0.9)	32.7 (±0.8)	33.4 (±1.7)	35.2 (±0.9)	45.4 (±6.9)	57.6 (±0.4)	69.1 (±0.5)	31.4 (±5.1)	40.5 (±2.0)	40.7 (±0.8)	42.2 (±3.0)	48.6 (±3.9)	63.6 (±16.6)	67.4 (±13.6)	74.2 (±13.7)
16:1 ω9	7.1 (±0.2)	6.9 (±0.5)	7.7 (±0.5)	7.4 (±0.1)	7.5 (±0.3)	4.1 (±0.2)			6.9 (±1.3)	7.2 (±0.5)	7.8 (±0.4)	6.9 (±1.3)				
16:1 ω7	12.5 (±0.1)	11.6 (±0.8)	13.2 (±0.6)	12.4 (±0.3)	13.5 (±1.0)	10.0 (±1.0)	8.6 (±0.7)	3.4 (±6.0)	13.9 (±3.4)	13.7 (±0.7)	12.1 (±0.3)	10.5 (±1.4)	17.4 (±3.3)	12.3 (±10.7)	11.0 (±9.7)	4.0 (±7.0)
16:1 ω5	1.1 (±0.1)	1.1 (±0.1)	1.2 (±0.1)	1.2 (±0.1)	0.4 (±0.7)											
16:2 ω6	1.4 (±0.1)	1.4 (±0.1)	1.6 (±0.1)	1.4 (±0.1)	1.6 (±0.1)											
18:0	3.3 (±0.1)	2.9 (±0.1)	3.2 (±0.3)	3.3 (±0.2)	3.9 (±0.3)	3.6 (±0.8)			4.5 (±1.1)	2.8 (±2.4)	3.4 (±0.1)	0.9 (±1.5)	8.7 (±0.5)	5.0 (±4.3)		
18:1 ω9	12.1 (±0.1)	9.9 (±2.5)	7.4 (±6.5)	10.9 (±6.9)	23.1 (±1.7)	15.1 (±6.7)	19.1 (±0.6)	17.5 (±5.9)	18.7 (±5.0)	14.4 (±12.5)	18.6 (±7.7)	8.0 (±13.9)	12.5 (±3.4)	6.3 (±5.4)	6.0 (±10.5)	5.2 (±9.1)
18:2 ω6	4.5 (±0.1)	4.5 (±0.1)	4.7 (±0.2)	4.5 (±0.2)	4.9 (±0.2)	6.2 (±0.8)	5.7 (±0.7)		5.3 (±2.2)	5.9 (±1.4)	5.9 (±0.2)	6.0 (±0.8)				
18:3 ω3	10.4 (±0.1)	10.5 (±0.7)	14.0 (±4.7)	9.7 (±9.2)		3.0 (±5.3)				7.9 (±13.6)	4.3 (±7.4)	15.7 (±13.6)				
Total sat	47	48	47	49	49	59	66	79	48	50	51	52	70	81	83	91
Sat/unsat	0.9	0.9	0.9	1.0	0.9	1.4	2.0	3.8	0.9	1.0	1.0	1.1	2.3	4.4	4.9	9.9

Table 6 Fatty acid composition (% of total ± SD) as a function of extraction method and extraction cycle for algal sample no. 3

Solvent	Manual extraction				Accelerated solvent extraction											
	Chl/Me				Chl/Me				IPA/hex				Hexane			
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
14:0	3.4 (±0.1)	2.9 (±0.1)	3.0 (±0.1)	5.8 (±0.2)	3.5 (±0.4)	5.0 (±0.2)	5.2 (±0.4)	1.8 (±3.1)	4.4 (±0.4)	5.5 (±2.1)	3.5 (±0.3)	3.8 (±0.8)	7.1 (±0.8)		3.7 (±6.3)	
15:0	2.3 (±0.4)	2.3 (±0.1)	2.0 (±0.1)	3.5 (±0.2)												
16:0	35.4 (±0.6)	34.7 (±0.2)	34.8 (±0.1)	33.8 (±1.7)	36.6 (±0.3)	52.8 (±2.8)	55.5 (±1.4)	57.7 (±1.0)	36.4 (±1.3)	45.2 (±2.1)	42.7 (±2.8)	45.1 (±3.2)	49.5 (±3.8)	89.1 (±18.9)	96.3 (±6.3)	100.0 (±0.1)
16:1 *ω*9	5.7 (±0.1)	5.7 (±0.4)	6.0 (±0.3)	7.5 (±0.1)	5.6 (±1.3)	1.4 (±2.5)	1.1 (±1.9)		4.8 (±0.4)	3.8 (±3.3)	5.3 (±0.7)	5.7 (±0.2)				
16:1 *ω*7	9.6 (±0.2)	9.3 (±0.2)	9.9 (±0.2)	12.6 (±0.3)	9.9 (±0.8)	10.5 (±0.2)	9.4 (±0.3)	13.2 (±1.5)	10.3 (±1.2)	10.6 (±0.6)	9.4 (±0.8)	9.7 (±0.9)	12.3 (±2.5)	4.5 (±7.7)		
16:1 *ω*5	2.8 (±0.2)	3.0 (±0.1)	3.1 (±0.1)	1.2 (±0.1)	3.1 (±0.2)				3.0 (±0.2)	2.3 (±2.0)	3.5 (±0.4)	2.3 (±2.0)				
16:2 *ω*6	1.7 (±0.1)	1.7 (±0.1)	1.9 (±0.1)	1.4 (±0.1)	2.1 (±0.1)				1.4 (±1.2)		1.3 (±1.2)	0.7 (±1.3)				
18:0	2.0 (±0.1)	1.5 (±0.1)	1.8 (±0.1)	3.3 (±0.1)	2.0 (±0.1)				2.0 (±0.1)		0.6 (±1.0)	0.7 (±1.2)				
18:1 *ω*9	3.5 (±3.1)	4.3 (±0.1)	4.3 (±0.1)	11.1 (±7.0)	4.8 (±0.6)			18.9 (±1.4)	4.8 (±0.3)		2.0 (±1.8)	1.0 (±1.8)	22.1 (±1.3)	6.4 (±11.1)		
18:2 *ω*6	8.6 (±0.1)	8.0 (±0.1)	8.1 (±0.1)	4.6 (±0.2)	9.0 (±0.4)	8.7 (±0.5)	8.3 (±0.5)	8.4 (±1.5)	8.9 (±0.1)	9.2 (±0.9)	7.9 (±0.4)	8.5 (±0.5)	8.9 (±0.8)			
18:3 *ω*6	1.3 (±0.1)	0.9 (±0.1)	1.0 (±0.3)													
18:3 *ω*3	20.8 (±0.4)	20.2 (±0.3)	20.9 (±0.2)	9.8 (±9.3)	22.1 (±0.5)	21.5 (±0.9)	20.4 (±1.7)		21.1 (±0.8)	23.3 (±1.0)	21.6 (±0.9)	22.4 (±0.7)				
18:4 *ω*3	1.9 (±1.7)	2.2 (±0.1)	2.3 (±0.1)	0.3 (±0.5)	0.9 (±1.5)				2.5 (±0.2)		0.4 (±0.7)					
Total sat	45	44	44	50	44	58	61	60	44	52	50	51	57	89	100	100
Sat/unsat	0.8	0.8	0.8	1.0	0.8	1.4	1.5	1.5	0.8	1.1	1.0	1.0	1.3	8.2	>100	>100

Table 7 Effect of sample particle size on extraction of oil and fatty acid content using algal sample no. 1

	Oil content (% DW)	Fatty acid content (% DW)
Particle size range (mm)		
0.5–1.0	11 ± 0.4	1.57 ± 0.04
0.18–0.5	12 ± 0.2	1.59 ± 0.14
0.09–0.18	13 ± 0.1	1.58 ± 0.05
<0.09	14 ± 0.5	1.62 ± 0.10

[a] The solvent mixture was chloroform/methanol (2:1, v/v)

one sample, four aliquots of one algae sample were ground separately to yield four particle size ranges (<0.09, 0.09–0.18, 0.18–0.50, 0.50–1.0 mm). Results from manual extractions (4 cycles in triplicate) and ASE with chloroform/methanol (four cycles in triplicate) showed that oil and FA content values using the manual method or ASE with chloroform/methanol were not significantly influenced by sample particle size within the size range of 0.1–1 mm (Table 7).

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