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Evaluation of a Pilot-Scale Oil Extraction from Microalgae for Biodiesel Production

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***Abstract*— Biodiesel derived from microalgae is one of a suite of potential solutions to meet the increasing demand for a renewable, carbon-neutral energy source. However, there are numerous challenges that must be addressed before algae biodiesel can become commercially viable. These challenges include the economic feasibility of harvesting and dewatering the biomass and the extraction of lipids and their conversion into biodiesel. Therefore, it is essential to find a suitable extraction process given these processes presently contribute significantly to the total production costs which, at this stage, inhibit the ability of biodiesel to compete financially with petroleum diesel. This study focuses on pilot-scale (100 kg dried microalgae) solvent extraction of lipids from microalgae and subsequent transesterification to biodiesel. Three different solvents (hexane, isopropanol (IPA) and hexane + IPA (1:1)) were used with two different extraction methods (static and Soxhlet) at bench-scale to find the most suitable solvent extraction process for the pilot-scale. The Soxhlet method extracted only 4.2% more lipid compared to the static method. However, the fatty acid profiles of different extraction methods with different solvents are similar, suggesting that none of the solvents or extraction processes were biased for extraction of particular fatty acids. Considering the cost and availability of the solvents, hexane was chosen for pilot-scale extraction using static extraction. At pilot-scale the lipid yield was found to be 20.3% of total biomass which is 2.5% less than from bench scale. Extracted fatty acids were dominated by polyunsaturated fatty acids (PUFAs) (68.94±0.17%) including 47.7±0.43 and 17.86±0.42% being docosahexaenoic acid (DHA) (C22:6) and docosapentaenoic acid (DPA) (C22:5, ω-3), respectively. These high amounts of long chain poly unsaturated fatty acids are unique to some marine microalgae and protists and vary with environmental conditions, culture age and nutrient status, as well as with cultivation process. Calculated physical and chemical properties of density, viscosity of transesterified fatty acid methyl esters (FAMEs) were within the limits of the biodiesel standard specifications as per ASTM D6751-2012 and EN 14214. The calculated cetane number was, however, significantly lower (17.8~18.6) compared to ASTM D6751-2012 or EN 14214-specified minimal requirements. We conclude that the obtained microalgal biodiesel would likely only be suitable for blending with petroleum diesel to a maximum of 5 to 20%.**

Keywords*— microalgae, lipid/fatty acid extraction, biodiesel, fuel properties

I. INTRODUCTION

Commercial interest in microalgae-based products is mainly focussed on food supplements, beta-carotene and related pigments for neutraceuticals and the food market [1-3]. On the other hand, extraction of bio-oils/lipids from microalgae for biodiesel production is relatively new and immature at large scale. The downstream process technologies, including dewatering, extraction of oil/lipids and fractionation, remain critical steps to be addressed for commercial-scale biodiesel production from microalgae. Lipids are mainly classified on their chemical characteristics [4-6]. Polar lipids (phosphoglycerides and glycosylglycerides) function in membrane formation and fluidity, while non-polar lipids, such as sterols, hydrocarbons, waxes and triacylglycerides (TAGs, also known as neutral lipids) are storage lipids [5, 7]. TAGs are the most suitable compound for biodiesel production, if mechanical extraction is possible with the microalgal biomass [8]. At laboratory-scale, many different extraction processes have been trialled, for example mechanical disruption, supercritical fluid extraction and solvent extraction, but

implementation at pilot-scale for biodiesel production from microalgae remains a hurdle. Mechanical disruption methods such as homogenisation using for example bead milling or pressing, aim to break the cells walls (if present) and extract the intercellular materials [7, 9, 10]. A major advantage of mechanical disruption is the reduced risk of chemical (solvent) contamination of the extracted TAGs, while the presence of tough cells walls and extraction efficiency (e.g. incomplete extraction) can be disadvantages [1]. Supercritical fluid extraction (SPE) relies on the supercritical state of a gas which is achieved by keeping temperature and pressure above the critical point, where density (ρ) is between the gas and the liquid phase, which governs the dissolving power of the supercritical fluid, and viscosity (γ)is similar to the gas [1, 11]. The most commonly used solvent is carbon dioxide (CO_2) due to low critical temperature (31.1°C) and pressure (74 bar) and the added advantages of chemical inertness, low toxicity, relative pricing, availability and demonstrated capacity for implementation in pilot-scale extraction processes [9, 12], while infrastructure costs (at scale) and energy consumption are the main hurdles [1].

On the other hand algal lipids are most commonly extracted using solvents because of convenience and low setup cost [13]. Extraction efficiencies are enhanced when the solvent can penetrate algal cells and match the polarity of the crude lipids. As a result, non-polar solvents extract non-polar lipids, whereas polar lipids associated with the cell membrane, are extracted by polar solvents [14]. High lipid yields can be achieved using non-polar solvent along with a polar co-solvent [1, 15]. For example, using n-hexane and isopropanol for extraction produced higher lipid yields compare to n-hexane extraction [14] . In this case, the polar isopropanol disrupts the membrane-based lipid-protein interactions by forming hydrogen bonds with the polar lipids [16] allowing better access for the non-polar solvent. Solvent extraction, particularly in rural and remote areas, has a greater potential for pilot-scale biodiesel biodiesel production, as the solvents can be reused after extraction [9].

In this study, dry microalgae biomass was extracted at pilot-scale with the non-polar solvent n-hexane. Prior to, bench-scale extractions were performed to investigate the effect of extraction procedure (static vs. Soxhlet) and solvents (n-hexane, isopropanol and n-hexane/isopropanol mixtures) on lipid extraction efficiency and fatty acid methyl ester (FAME) profiles. Isopropanol yielded marginally (1~2%) more lipids than the other two solvents in both extraction procedures. However, n-hexane was chosen for pilot-scale extraction as it is inexpensive, readily available, and can be easily recovered from the mixture.

II. MATERIALS

Fatty acids were extracted from lyophilized samples in a single-step extraction and transesterification procedure modified from Rodriguez-Ruiz et al. [17] and Cohen et al. [18], followed by GC-MS analysis. Approximately 30 mg biodiesel was diluted 50x in hexane containing 0.01 % butylated-hydroxy-toluene (BHT) as an antioxidant. Following this, 2 mL freshly prepared methylation reagent (methanol:acetylchloride, 95:5 (v/v)) and 300 μL internal standard (nonadecanoic acid ($C_{19}H_{38}O_2$; >99%, Sigma Aldrich, Castle Hill, NSW, Australia), 0.2 mg L^{-1} in methanol) was added to 1 mL biodiesel containing hexane (~0.6 mg biodiesel mL^{-1}). Samples were heated at 100°C for 1h and allowed to cool, after which 1 mL de-ionized water was added to facilitate phase separation. The hexane phase containing the FAMEs was collected and filtered through a 0.2 μm PTFE syringe filter prior to injection on the GC column. All solvents were HPLC grade.

Fatty acid analysis was carried out in scan-mode on an Agilent 7890 GC (DB-23 capillary column with cyanopropyl stationary phase (60 m x 0.55 mm, inner diameter 0.15 μm) equipped with flame ionisation detector (FID) and connected to an Agilent 5975C electron ionisation (EI) turbo mass spectrometer (Agilent technologies), for identification of fatty acid methyl esters (FAMEs). Injector and FID inlet temperatures were 150 °C and 250 °C, respectively (split injection, 1/50). Column temperature was programmed following David et al. [19]; to ramp from 50 °C to 230 °C. Fatty acids were identified using external standards (Sigma Aldrich) and NIST08 Mass Spectral Library and the total fatty acid content was determined as the sum of all FAMEs corrected for recovery of internal standard (C19:0).

III. SOLVENT SELECTION

Selecting a solvent or co-solvent mixture yielding the highest lipid extraction efficiency and to determine the most efficient and economical extraction procedure for pilot-scale production of microalgal biodiesel. Based on published information [16, 20] on extraction efficiencies of microalgal lipids, n-hexane and isopropanol (IPA), as well as a co-solvent mixture of n-hexane/isopropanol were selected as solvents. In addition, 10 g dry microalgae biomass (DW) was extracted in the above solvents by either Soxhlet or static procedures for approximately 24 h.

Maximal total lipid extraction and FAME yields were achieved with IPA (total lipids 28% of DW and total FAME 19.7% of DW, respectively) using the Soxhlet process (Table I), suggesting that the IPA/Soxhlet procedure extracted slightly more polar cellular compounds. In contrast, total lipid yields (23.8% of DW) were identical for IPA and the n-hexane:IPA mixture, while the mixture yielded more total FAMEs, followed by single solvent extraction with n-hexane and IPA, respectively (Table I). In contrast, use of the co-solvent mixture in the Soxhlet extraction had the lowest total lipid yield but a comparable FAME yield to IPA/Soxhlet extraction (Table I). The overall lower total lipid yield in the IPA/Soxhlet procedure can be explained by back drainage of the lipid/hexane mixture (hexane boiling point: 68°C), reducing the overall temperature and preventing IPA to reach its boiling point (83°C). Only marginal differences in total FAME yields (~3% or less) were recorded independent of extraction procedure or solvent choice (Table I).

TABLE I. TOTAL LIPID YIELDS AND FATTY ACID METHYL ESTERS (FAME) FROM MICROALGAE USING DIFFERENT SOLVENTS AND EXTRACTION PROCEDURES.

Extraction Method	Solvent	Algae DW biomass (g)	Total lipid (% of DW biomass)	Total FAME (% of DW biomass)
Soxhlet	Hexane	10.0	27.0	16.6
	Isopropanol	10.0	28.0	19.7
	Hexane-IPA	10.0	26.0	19.6
Static	Hexane	10.1	22.8	17.3
	Isopropanol	10.1	23.8	16.9
	Hexane-IPA	10.1	23.8	18.2

Extracted FAME profiles are largely independent of solvent and/or extraction procedure used, except of the consistent extraction of alpha-Linolenic acid (C18:3 n-3) in the static extraction procedure, while this fatty acid was not extracted in the Soxhlet procedure (Table II). Therefore method and solvent choice for the pilot-scale extraction should only minimally influence fatty acid profiles and thus fuel properties.

TABLE II. FATTY ACID METHYL ESTER (FAME) PROFILES OF DIFFERENT EXTRACTION METHODS AND SOLVENTS

FAME	Soxhlet Method Extracted FAME (mg/g DW)			Static Method Extracted FAME (mg/g DW)		
	Solvent			Solvent		
	Hexane	IPA	Hexane-IPA	Hexane	IPA	Hexane-IPA
C14:0	64.1	65.2	67.8	63.3	66.5	67.2
C16:0	165.7	167.3	173.4	169.5	171.5	176.0
C18:0	4.5	4.2	4.6	4.5	4.7	4.3
C18:3 n-6	3.0	2.8	3.6	3.5	3.0	3.3
C18:3 n-3	0.0	0.0	0.0	3.9	2.7	3.5
C20:3	3.7	3.6	4.1	4.9	4.1	4.1
C20:4	4.3	4.8	5.4	6.8	5.2	5.4
C20:5 (EPA)	9.7	11.8	12.8	8.1	11.6	13.4
C22:5	103.7	115.0	119.2	134.6	125.4	137.8
C22:6 (DHA)	251.1	311.3	327.8	360.3	315.8	350.8

Therefore n-hexane was chosen together with static extraction, because hexane is cheap in relation to other solvents on the market, especially in regards to IPA; readily available; and generated only 1% less total lipid yields compare to IPA in both method.

IV. STATIC N-HEXANE EXTRACTION

Static extraction with n-hexane in 55 L pneumatically controlled rocking extraction vessels met all the design requirements as ~98% of n-hexane can be efficiently recycled for re-use, significantly reduced production costs. The simultaneous use of 3 vessels additionally decreased extraction time

For extraction, biomass was divided into 4 batches. The first batch (10 kg) was used for trial extraction to optimise the extraction process. A single extraction cycle was determined based on plateauing of lipid yields, as any additional cycles would negatively impact production costs. As such, dry microalgae biomass was incubated with hexane in 55 L pneumatic rocking vessels for 28 to 30 hours. The total lipid/hexane extracts were filtered through Whatman No.1 filter paper into a separate vessel for further downstream processing. Residual total lipid still contained in the extracted biomass was sacrificed to save on solvent, time and labour costs. As a consequence, some n-hexane was lost. The remaining hexane was recovered from the total lipid by distillation using a rotary evaporator. Recovered n-hexane was used in the subsequent extractions whereas the fatty acids contained in the total lipid extract were transesterified to FAMEs (biodiesel).

V. TRANSESTERIFICATION AND FAME PROFILE ANALYSES

Transesterification of the fatty acids was carried out in the presence of KOH as a catalyst. To optimize the amount of KOH, temperature and time, 3 different commercially available vegetable oils (canola oil, sunflower oil and peanut oil) with acid values of 0.2 were transesterified. 100 mL of vegetable oil transesterified with 0.79 g 85% KOH in 12.5 mL 99.8% methanol at 55 oC on a magnetic stirrer hot plate, yielded the best results.

A. Pilot-scale transesterification procedure

For practical reasons, total extracted lipids were divided into 2 L aliquots in a 5 L glass beaker on a magnetic stirrer hot plate.15.8 g of 85% KOH was dissolved in 250 mL of 99.8% methanol and slowly added to the oil at 55 oC under constant stirring. The colour of the mixture changed from mid-brown to almost black after approximately 5 minutes and a concomitant rise in temperature of ~3 oC was observed, confirming completion of the transesterification reaction (Fig. 1). Stirring at 55 oC was continued for 30 minutes before transfer to a glass separation funnel (Fig. 1). A black heavy layer settled at the bottom; while the top layer containing the FAMEs was sparged with approximately 100 mL min-1 of instrument air over 48 h before draining of the bottom layer. pH of the top layer was adjusted to ≥ 7and filtered through Whatman no.1 analytical filter paper. The filtered FAME was then left to settle for a minimum of 48 h before re-filtering through Whatman no.1 filter paper to remove precipitation and produce very clear FAME. Some FAME was lost through filtration and when draining off the glycerine. At this scale of work, recovery would not have been economical. The resulting biodiesel yields (Table III) and FAME profiles (Table IV) were very consistent in all different batches. The FAME profile was also found very consistent in all different batches (Table IV) with less than 0.3% standard deviation. Interestingly the α-Linolenic acid was not extracted in the pilot-scale static n-hexane extraction, while it was present in the bench-scale procedure.

TABLE III. CONVERSION PERFORMANCE OF MICROALGAE BIODIESEL FROM EXTRACTED RAW OIL

Batch	Total lipid (kg)	Process (L)	Biodiesel (L)	Biodiesel (kg)
0	2.2	1×2	2	1.8
1	5.9	3×2	6	5.4
2	5.9	3×2	6	5.4
3	6.3	4×2	7.8	7
Total	20.3		21.8	19.6

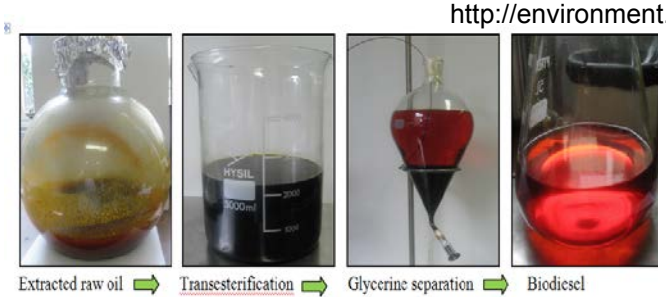

Fig.1. Chronological view of microalgae biodiesel production through transesterification

TABLE IV. FATTY ACID METHYL ESTER (FAME) CONTENT (MG G^{-1} BIODIESEL) OF THREE DIFFERENT BATCHES OF BIODIESEL USING STATIC HEXANE EXTRACTION AND ALKALI-CATALYSED TRANSESTERIFICATION

FAME	Batch-1	Batch-2	Batch-3	STDEV (%)
C14:0	68.45	63.70	64.63	0.07
C16:0	181.73	170.92	173.48	0.16
C18:0	4.96	4.23	4.15	0.04
C18:3 n-6	3.27	3.09	3.43	0.03
C20:3	4.11	4.14	3.91	0.02
C20:4	5.75	5.55	5.64	0.01
C20:5 (EPA)	13.67	13.35	13.85	0.06
C22:5	146.05	138.27	138.74	0.04
C22:6 (DHA)	392.46	371.06	367.16	0.29

B. Fuel properties

The obtained biodiesel was characterised for its fuel properties by measuring density (ρ), kinematic viscosity (γ) and higher heating values and estimating cetane number and oxidation stability based on the FAME profile (Table 5). Fuel properties were compared to relevant standards ASTM D6751-2012 and EN 14214 to determine whether it could be used in a diesel engine. Kinematic viscosity (γ) was calculated from the measured dynamic viscosity/ measured density (ρ) of the fuel. Dynamic viscosity and density (ρ) were measured using a Brookfield DV-III rheometer and KSV Sigma 702 tensiometer, respectively. Various limitations during testing of density (ρ) led to the inability to heat the fuel to 40°C which was required for kinematic viscosity (γ) by the standards. Nevertheless, density (ρ), oxidation stability and calorific value all fit within the desired range.

TABLE V. MICROALGAE BIODIESEL FUEL PROPERTIES

Properties	Density (ρ)	Kinematic viscosity (γ)	Cetane number	HHV	Oxidation stability
	(g/cm^3)	@40 °C (mm^2/s)		(MJ/kg)	(Hour)
ASTM D6751-	-	1.9-6.0	47	-	≥3
EN 14214	0.86-0.90	3.5-5.0	51	-	≥6
Biodiesel_Batc	0.89	5.97	17.8[a]	39.8	3.09[b]
Biodiesel_Batc	0.89	5.43	18.3[a]	39.9	3.17[b]
Biodiesel_Batc	0.89	5.52	18.6[a]	39.9	3.19[b]

[a] Estimated using algorithm on [21]; [b]estimated using algorithm on [22] .

VI. CONCLUSION

An investigation into the extraction of total lipids from dry microalgae biomass on a pilot-scale was performed in order to determine whether the processes and equipment could be used at an industrial level. Static extraction using n-hexane as a solvent produced a total lipid yield of 20.3 wt% of dry biomass from an initial supply of 100 kg of microalgae. Transesterified fatty acids (FAMEs), which are the biodiesel portion of the total lipid fraction, were recovered with 97% efficiency. The remaining 3% of the biodiesel was lost through partitioning of the glycerol and filtration, but it was not recoverable in this set up due to economic constraints. Given the consistencies of biomass extraction and FAME yields and profiles, it would be beneficial to trial the extraction procedure at industrial-scale using at scale professional processing equipment to ascertain whether consistencies can be reproduced at scale and solvent recovery can be further improved and FAME losses can be minimised The recycling efficiency of 97% of the solvent n-hexane is encouraging despite the low technical equipment configuration, suggesting that solvent re-use would make industrial-scale transesterification of microalgal total lipids very cost competitive. It can be anticipated that de-watering and drying of the microalgal biomass would be the most energy demanding and costly production steps [23]. It would therefore be desirable to extract total lipids from microalgal wet biomass and research into this topic could further improve microalgal biodiesel economics.

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REFERENCES

[1] M. Cooney, G. Young, and N. Nagle, "Extraction of bio oils from microalgae," *Separation & Purification Reviews*, vol. 38, pp. 291-325, 2009.

[2] M. Olaizola, "Commercial development of microalgal biotechnology: from the test tube to the marketplace," *Biomolecular Engineering*, vol. 20, pp. 459-466, 2003.

[3] F. Shahidi, *Nutraceutical and specialty lipids and their co-products*: CRC Press, 2006.

[4] M. A. Borowitzka and N. R. Moheimani, *Algae for Biofuels and Energy* vol. 5: Springer, 2013.

[5] N. Singh and D. Dhar, "Microalgae as second generation biofuel. A review," *Agronomy for Sustainable Development*, vol. 31, pp. 605-629, 2011/10/01 2011.

[6] Y. Gong and M. Jiang, "Biodiesel production with microalgae as feedstock: from strains to biodiesel," *Biotechnology letters*, vol. 33, pp. 1269-1284, 2011/07/01 2011.

[7] H. Greenwell, L. Laurens, R. Shields, R. Lovitt, and K. Flynn, "Placing microalgae on the biofuels priority list: a review of the technological challenges," *Journal of the Royal Society Interface*, vol. 7, pp. 703-726, 2010.

[8] J. Sheehan, T. Dunahay, J. Benemann, and P. Roessler, "A Look Back at the US Department of Energy's Aquatic Species," *Close-Out Report National Renewable Energy Laboratory, US Department of Energy's Office of Fuels Development, Golden, Colorado, USA*, 1998.

[9] P. Mercer and R. E. Armenta, "Developments in oil extraction from microalgae," *European Journal of Lipid Science and Technology*, vol. 113, pp. 539-547, 2011.

[10] Y. Chisti, Moo-Young, Murray, "Disruption of microbial cells for intracellular products," *Enzyme and Microbial Technology*, vol. 8, pp. 194-204, 1986.

[11] M. Herrero, A. Cifuentes, and E. Ibanez, "Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review," *Food Chemistry*, vol. 98, pp. 136-148, 2006.

[12] M. D. Nodar, A. M. Gómez, and E. M. de la Ossa, "Characterisation and process development of supercritical fluid extraction of soybean oil," *Food science and technology international*, vol. 8, pp. 337-342, 2002.

[13] M. A. Islam, M. Magnusson, R. J. Brown, G. A. Ayoko, N. Nabi, and K. Heimann, "Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles," *Energies*, vol. 6, pp. 5676-5702, 2013.

[14] R. Halim, M. K. Danquah, and P. A. Webley, "Extraction of oil from microalgae for biodiesel production: A review," *Biotechnology Advances*, 2012.

[15] S. F. Sing, A. Isdepsky, M. A. Borowitzka, and N. R. Moheimani, "Production of biofuels from microalgae," *Mitigation and Adaptation Strategies for Global Change*, vol. 18, pp. 47-72, 2013.

[16] R. Halim, B. Gladman, M. K. Danquah, and P. A. Webley, "Oil extraction from microalgae for biodiesel production," *Bioresource Technology*, vol. 102, pp. 178-185, 2011.

[17] J. Rodriguez-Ruiz, E. H. Belarbi, J. L. G. Sanchez, and D. L. Alonso, "Rapid simultaneous lipid extraction and transesterification for fatty acid analyses," *Biotechnology Techniques*, vol. 12, pp. 689-691, Sep 1998.

[18] Z. Cohen, A. Vonshak, and A. Richmond, "Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate," *Journal of Phycology*, vol. 24, pp. 328-332, Sep 1988.

[19] F. David, P. Sandra, and P. L. Wylie, "Improving the analysis of fatty acid methyl esters using retention time locked methods and retention time databases.," in *Agilent Technologies application note 5988-5871EN*, A. T. a. n. 5988-5871EN, Ed., ed, 2002.

[20] J. Doucha and K. Lívanský, "Influence of processing parameters on disintegration of Chlorella cells in various types of homogenizers," *Applied microbiology and biotechnology*, vol. 81, pp. 431-440, 2008/12/01 2008.

[21] K. Krisnangkura, "A Simple Method For Estimation of Cetane Index of Vegetable Oil Methyl Esters," *JAOCS,*, vol. VoL 63, no . 4 1986.

[22] L.-b. Wang, H.-y. Yu, X.-h. He, and R.-y. Liu, "Influence of fatty acid composition of woody biodiesel plants on the fuel properties," *Journal of Fuel Chemistry and Technology*, vol. 40, pp. 397-404, 2012.

[23] D. Klein‐Marcuschamer, C. Turner, M. Allen, P. Gray, R. G. Dietzgen, P. M. Gresshoff, *et al.*, "Technoeconomic analysis of renewable aviation fuel from microalgae, Pongamia pinnata, and sugarcane," *Biofuels, Bioproducts and Biorefining*, 2013.