Bioresource Technology 169 (2014) 768–772

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Lipid profiling and corresponding biodiesel quality of *Mortierella isabellina* using different drying and extraction methods

Javid Hussain [a], Zhenhua Ruan [b], Iracema Andrade Nascimento [a], Yan Liu [b], Wei Liao [b,*]

[a] LABIOMAR, Institute of Biology, Federal University of Bahia, Salvador, Brazil
[b] Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA

HIGHLIGHTS

- Bligh & Dyer method had the highest lipid yield among four extraction methods.
- Non-polar lipid was the most abundant one in the fungal lipid.
- Correlative models concluded that fungal biodiesel has a good fuel quality.

ARTICLE INFO

Article history:
Received 10 May 2014
Received in revised form 19 June 2014
Accepted 20 June 2014
Available online 1 July 2014

Keywords:
Biodiesel quality
Fatty acids
Fungal biomass
Lipid extraction and fractionation
Mortierella isabellina

ABSTRACT

Four lipid extraction methods (Bligh & Dyer, hexane & isopropanol, dichloromethane & methanol, and hexane) were evaluated to extract lipid from freeze- and oven-dried fungus *Mortierella isabellina* ATCC42613. The highest lipid yield (41.8%) was obtained from Bligh & Dyer extraction on the oven-dried fungal biomass with a methanol:chloroform:water ratio of 2:1:0.8. Other lipid extraction methods on both freeze- and oven-dried samples had lipid yields ranging from 20.7% to 35.9%. Non-polar lipid was the main lipid class (more than 90% of total lipid) in *M. isabellina*. Regarding fatty acid profile, there was no significant difference on fatty acid concentration between different drying and extraction methods. Estimation of biodiesel fuel properties using correlative models further demonstrated that the fungal biodiesel is a good alternative to fossil diesel.

1. Introduction

In response to rapid expansion of biodiesel demand, the current interest of biodiesel research and development has been diverted to heterotrophic oleaginous microbes such as bacteria, yeasts and filamentous fungi that have characteristics of fast growth rate, relatively simple production process, and easy scale-up (Dey et al., 2011). Our previous studies indicated that fungi have advantages of simultaneously utilizing multiple carbon sources (glucose and xylose) as well as better tolerance to inhibitors (furfural, hydroxymethylfurfural (HMF), and aromatic compounds) in lignocellulosic hydrolysates (Ruan et al., 2012, 2013). The lipid content in oleaginous fungi ranges from 21% to 74%, which is also comparable to most oleaginous yeasts and microalgae (Nascimento et al., 2014; Ruan et al., 2012). Therefore, the exploitation of fungal lipid is important for the development of microbial biodiesel production on lignocellulosic feedstocks. Since dewatering processes and extraction methods as key steps in microbial biodiesel conversion could have major impacts on lipid quality (de Boer et al., 2012), understanding their effects on fungal lipids could make a major contribution towards a fungi-based biodiesel production.

The industrial-scale transesterification is designed to convert acylglycerols (non-polar lipids) into biodiesel. An ideal lipid extraction technology for biodiesel production should have high level of non-polar lipid specificity, minimum reactivity with the target lipid, high extraction efficiency, and less negative environmental impacts (Medina et al., 1998). Several approaches have been intensively studied to extract and convert lipid into biodiesel, such as supercritical CO_2 lipid extraction and transesterification, direct transesterification without lipid extraction, and conventional approaches of using polar and non-polar solvent extraction and transesterification, etc. Supercritical CO_2 lipid extraction has high lipid extraction efficiency, while energy demand and pressure requirement make it difficult to be scaled up (Halim et al., 2012). Direct transesterification does not need the steps of drying and lipid extraction, though, the low lipid recovery and conversion

* Corresponding author. Address: 524 South Shaw Lane, Room 202, East Lansing, MI 48824, USA. Tel.: +1 517 432 7205.
E-mail address: liaow@msu.edu (W. Liao).

http://dx.doi.org/10.1016/j.biortech.2014.06.074
0960-8524/

are the main obstacles for large-scale industrial applications of the direct transesterification methods (Montes D'Oca et al., 2011). Therefore, among these approaches, the conventional method of drying, extracting, and transesterification is still the preferred method to produce biodiesel. Various solvent extraction technologies have been developed and studied, such as Bligh & Dyer method (Bligh and Dyer, 1959), hexane & isopropanol method (Hara and Radin, 1978), and hexane extraction (Miao and Wu, 2006). Even though they are comparatively advantageous, each of them still has some disadvantages according to the requirements of ideal lipid extraction. Bligh & Dyer method using non-polar/polar organic solvent (chloroform:methanol) is able to completely extract both neutral and polar lipids from biomass. However, the high toxicity of the solvents limited its application in the large-scale biodiesel production. The hexane extraction methods (Hexane & isopropanol and n-Hexane) are less toxic and more selective towards non-polar lipids, which is good for biodiesel production (Lee et al., 1998). The main disadvantage of the hexane methods is the low lipid extraction efficiency.

Therefore, the objective of this study was to elucidate the effects of different extraction and drying methods on lipid yield and fungal biodiesel quality. Four extraction methods (Bligh & Dyer, hexane & isopropanol, dichloromethane & methanol, and hexane extraction) on freeze- and oven-dried fungus *Mortierella isabellina* ATCC 42613 were evaluated according to total lipid yield, selective extraction of different lipid components, and fatty acid profile for biodiesel production.

2. Methods

2.1. Fungal culture

M. isabellina ATCC 42613 fermentation was based on our previous study (Ruan et al., 2012), performed in a 7.5 L New Brunswick Bioflo 115 fermenter with a working volume of 5.0 L. Fermentation was carried on at 25 °C with an inoculum of 10% (V/V) seed. The culture pH was adjusted to 6.0 by pumping sterilized NaOH solution (20% w/w), and dissolved O_2 was maintained above 20% of air saturation by adjusting the agitation and aeration. The fermentation duration was 96 h. After fermentation, fungal biomass was collected by centrifugation, and washed twice with deionized water. Half of the fungal biomass was dried in a lyophilizer for 48 h, and another half was air-dried at 65 °C in an oven until constant weight.

2.2. Lipid extraction and fractionation

Four extraction methods were applied on the freeze- and oven-dried fungal biomass: (i) Bligh & Dyer method with a modified methanol:chloroform:water ratio of 2:1:0.8 (v/v/v); (ii) hexane & isopropanol (Halim et al., 2012); (iii) dichloromethane & methanol (Guckert et al., 1988); and (iv) hexane extraction (Halim et al., 2011). The 1 g dried fungal biomass was put in 50 mL centrifuge plastic tube with 18 mL organic solvent, and homogenized at 35,000 rpm for 10 min (LabGEN 7 Series Homogenizer, Cole-Parmer, USA) to rupture the cellular structure. 5 mL water was then used for the complete biphasic separation. The solvent was then volatized at 65 °C in fume hood until constant weight of lipid was achieved. The extracted lipid was re-suspended in 1 mL chloroform to prevent from oxidation, and stored at −20 °C for lipid fractionation and fatty acid analysis.

The extracted lipid was fractioned into neutral lipids, glycolipids and phospholipids using solid-phase extraction (Berry, 2004). Supelco Solid Phase (SPE™) column packed with 500 mg of silica gel (Sigma–Aldrich®) was used to do the fractionation. After flushing the column with 4 mL chloroform, 100 mg of total lipid was sequentially fractionated by three solvents of chloroform, acetone, and methanol to selectively collect non-polar lipids, glycolipids, and polar lipids, respectively. Each fraction was dried under anaerobic condition and weighed. The dried fractions were re-suspended in 0.1 mL of chloroform and stored at −20 °C.

2.3. Fatty acid profiling

Fatty acid profiling was conducted by fatty acid methyl ester (FAME) synthesis. A conventional transesterification method was applied to form FAMEs (Indarti et al., 2005). After phase separation, 100 μL of the lower phase (chloroform phase) sample containing FAMEs was transferred to a clean 10 mL glass tube, and diluted ten times with chloroform. The samples were stored at −20 °C for GC–MS analysis.

Fatty acid analysis was performed using an Agilent 6890N gas chromatograph equipped with an Agilent DB-23 column (30 m × 250 μm × 0.25 μm) and a CTC PAL autosampler (Ruan et al., 2012). A mixture of C8-C24 FAMEs (Catalog No.: 18918-1 AMP) was used as the external standards for quantification. Nonadecanoic acid methyl ester (C19:0) derived from nonadecanoic acid (Sigma–Aldrich Catalog No: 646-30-0) was used as the internal standard (10 μg/mL).

2.4. Estimation of key fuel properties of biodiesel

Quality is an essential factor for the success of biodiesel industry. Several countries have established biodiesel quality standards and guidelines to regulate biodiesel production, such as EN 14214 in Europe, ASTM D 6751-10 in United States, RANP/2008 in Brazil, and similar guidelines for South Africa and Australia (Stansell et al., 2012). The fuel properties include cetane number (CN), iodine value (IV), saponification value (SV), cold filter plugging point (CFPP), and oxidation stability (OS) (Nascimento et al., 2013). It has been reported that these quality parameters of biodiesel are strongly related with structure properties (chain length and number of double bonds) of the fatty acids in TAG molecules (Mittelbach and Remschmidt, 2004). Correlative models have been developed to estimate the quality of biodiesel using fatty acid profile (Nascimento et al., 2014), which were adopted in this study to evaluate the quality of fungal biodiesel.

2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to compare and elucidate the effects of extraction and drying methods on lipid yield, lipid fraction, fatty acid profile and fuel quality of biodiesel. Statistica v 8.0 was used for all statistical analysis.

3. Results and discussion

3.1. Effect of drying and extraction methods on total lipid recovery and lipid fractionation

The statistical analysis demonstrated that the Bligh & Dyer extraction had the best lipid yields regardless drying and extraction methods (Fig. 1). The yields of 40.8% and 41.8% were obtained from freeze- and oven-dried fungal biomass, respectively, without significant difference ($P > 0.05$). The Bligh & Dyer extraction has been widely used as an efficient extraction method for polar and neutral lipid in algae (Halim et al., 2012). It is apparent that Bligh & Dyer extraction is also a good method for fungal lipid extraction. High toxicity of the solvent is a major disadvantage that limits its commercial applications.

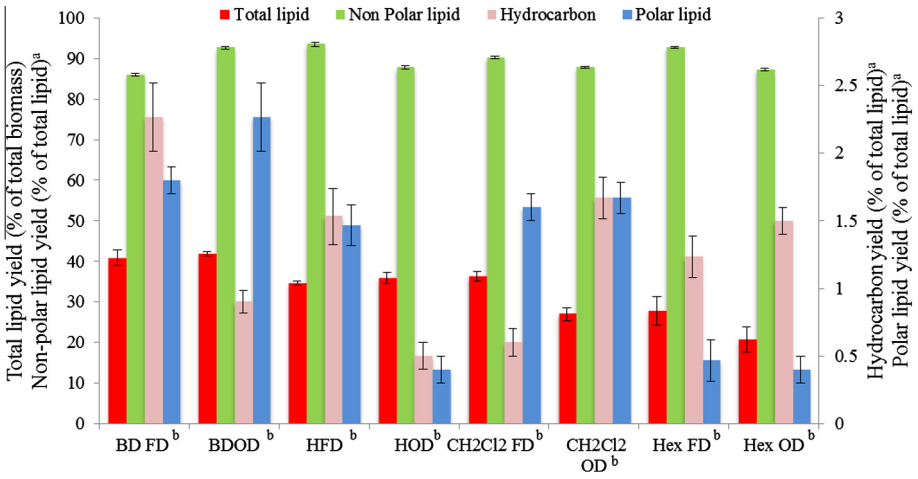

Fig. 1. Total lipid yield of four extraction methods on the freeze- and oven-dried fungal cell mass and their fractionation. (a) The percentages of non-polar lipid, polar and hydrocarbon in the total extracted lipid. (b) BDFD = Bligh & Dyer, freeze dried; BDOD = Bligh & Dyer, oven dried; HFD = hexane & isopropanol, freeze dried; HOD = hexane & isopropanol, oven dried, CH_2Cl_2 FD = CH_2Cl_2 & methanol, freeze dried; CH_2Cl_2 OD = CH_2Cl_2 & methanol, oven dried; Hex FD = hexane, freeze dried, Hex OD = hexane, oven dried. (c) Data are expressed as mean ± standard deviation of triplicates.

The hexane & isopropanol (3:2 v/v) mixture has been suggested as a low-toxicity substitute to chloroform:methanol solvent of Bligh & Dyer method (Halim et al., 2012). Lipid yields from freeze- and oven-dried biomass by this method were 34.5% and 35.9%, respectively, which were not significantly ($P > 0.05$) different from each other (Fig. 1). However, they were significantly ($P < 0.05$) lower than those from the Bligh & Dyer method. Comparison of the extraction on different biomass demonstrated that the hexane & isopropanol method functioned more efficiently on extracting fungal lipid than algal lipid (Lee et al., 1998). It has also been reported that hexane & isopropanol mixture is more selective towards non-polar lipid than other extraction solvents (Guckert et al., 1988; Lee et al., 1998), which could be beneficial for fungal biodiesel production.

Dichloromethane & methanol extraction had lipid yields of 36.3% and 27.0% for freeze- and oven-dried fungal biomass, respectively (Fig. 1), which showed a significant ($P < 0.05$) difference between two drying methods. Freeze-dried fungal biomass had much higher lipid yields than oven-dried biomass. Compared to algal lipid extraction (Lee et al., 1998), dichloromethane & methanol method was more efficient on fungal lipid extraction. Single solvent extraction of hexane using Soxhlet apparatus showed the similar lipid yield trend with dichloromethane & methanol extraction. Freeze-dried biomass had much higher yield than oven-dried one as well, except that the corresponding yields of 27.8% and 20.7% were much lower than those from dichloromethane & methanol extraction. It has also been reported that single solvent extraction has low extraction efficiency on algal lipid (Halim et al., 2012).

In order to further elucidate the effects of drying and extraction methods on lipid components, the extracted total lipid was fractionated into non-polar lipid, polar lipid, and hydrocarbon using solid-phase separation. The results showed that non-polar lipid was the main lipid class (more than 85% of the extracted total lipids) in *M. isabellina* from all eight combinations of drying and extraction methods (Fig. 1). Bligh & Dyer method obtained a lower non-polar lipid content of 86.0% from freeze-dried biomass than 92.6% from the oven-dried sample. While, other three extraction methods showed an opposite trend, in which the non-polar lipid fractions from freeze-dried biomass were significantly higher than those from oven-dried samples. Non-polar lipid contents from freeze-dried samples were 93.5%, 90.2%, and 92.7% for hexane & isopropanol, dichloromethane & methanol, and hexane extraction, respectively. Corresponding non-polar lipid contents of oven-dried samples were 87.7%, 87.8%, and 87.3%. There was no significant ($P > 0.05$) difference on non-polar lipid content between extraction methods regardless of different drying approaches.

Polar lipid and hydrocarbon contents were much smaller than non-polar lipid. There was no significant ($P > 0.05$) difference on polar lipid content between drying approaches for different extraction methods except hexane & isopropanol (Fig. 1). It had a much higher polar lipid content of 1.4% on the freeze-dried sample than 0.4% on the oven-dried sample. The highest (2.0%) and lowest (0.4%) polar lipid contents were obtained from the Bligh & Dyer method and hexane extraction, respectively (Fig. 1). As for hydrocarbon, the highest content (2.3%) was obtained from Bligh & Dyer extraction of the freeze-dried biomass, while the lowest content was 0.5% from hexane & isopropanol of oven-dried biomass. Significant difference ($P < 0.05$) on hydrocarbon content was observed between oven- and freeze-dried biomass for different extraction methods except hexane extraction (Fig. 1). Due to the fact that some non-polar lipids in fungus (i.e., ergosterol) require binary or trinary solvent systems, the fractionation method used in this study was not able to separate them even though they were extracted by the organic solvents as part of the lipid (Fried and Edwards, 1972). This was the reason that the sums of the fractionated lipids were slightly less than the total extracted lipid (Fig. 1).

Different lipid extraction methods have advantages and disadvantages regarding solvent toxicity and extraction efficiency of individual lipid components. The experimental results elucidated that Bligh & Dyer method had the best performance among four extraction methods in terms of total lipid yield, non-polar lipid content, polar lipid content, and hydrocarbon content. The main problem of Bligh & Dyer method was solvent toxicity to the environment. Hexane and dichloromethane & methanol methods are less toxic extraction approaches, though their extraction efficiencies are relatively low. Thus, considering solvent toxicity and lipid extraction efficiency, hexane & isopropanol extraction method with less toxicity, comparable lipid yield, and higher non-polar lipids content is a preferred method to extract fungal lipid.

3.2. Effect of different drying and extraction methods on fatty acid profile

Table 1 showed the average values of the representative fatty acid in *M. isabellina* using different drying and extraction methods. There was no significant difference ($P < 0.05$) on fatty acid profile between different drying and extraction approaches, except dichloromethane & methanol extraction that promoted a higher

Table 1
Fatty acid profiles of *M. isabellina* lipid by different drying and extraction methods.*

Fatty acids	BD FD (%)	BD OD (%)	H FD (%)	H OD (%)	CH_2Cl_2 FD (%)	CH_2Cl_2 OD (%)	Hex FD (%)	Hex OD (%)
C14:0	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0	0.9 ± 0	1.1 ± 0.3	1.3 ± 0.3	0.9 ± 0.1	0.9 ± 0
C16:0	23.2 ± 2.1	22.8 ± 1.5	28.2 ± 0.8	27.6 ± 0.7	28.7 ± 0.7	28.9 ± 1.5	22.4 ± 2.3	24.0 ± 1.1
C16:1	3.3 ± 0.4	3.4 ± 0.3	2.8 ± 0	2.9 ± 0.1	2.9 ± 0.2	3.0 ± 0.2	3.4 ± 0.4	3.3 ± 0.2
C18:0	12.1 ± 0.2	12.1 ± 0.1	11.2 ± 0.2	11.3 ± 0.1	11.1 ± 0.2	11.0 ± 0.4	12.1 ± 0.4	12.0 ± 0.2
C18:1	52.1 ± 0.5	52.3 ± 0.4	49.2 ± 0.7	49.6 ± 0.3	48.0 ± 1.4	47.3 ± 0.9	52.6 ± 0.6	51.7 ± 0.4
C18:2	7.1 ± 0.7	7.2 ± 0.5	6.2 ± 0.1	6.3 ± 0.2	6.5 ± 0.3	6.6 ± 0.3	7.4 ± 0.7	7.0 ± 0.3
C20:0	0.8 ± 0.12	0.8 ± 0.1	0.9 ± 0	0.9 ± 0	1.1 ± 0.2	1.2 ± 0.3	0.9 ± 0.1	0.8 ± 0
C22:0	0.2 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0	0.2 ± 0
C24:0	0.2 ± 0.1	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0	0.2 ± 0

* Data are expressed as mean ± standard deviation of triplicates.

Table 2
Influence of different drying and extraction methods on fungal biodiesel quality.

Fuel properties of biodiesel from fungal biomass							FAME composition according to number of double bonds (%)*		
Source of biodiesel	CN	SV mg $KOHg^{-1}$	IV g I_2 100 g^{-1}	DU	LCSF	CFPP (°C)	S-FAME	MU-FAME	PU-FAME
BD FD	58.8	203.2	63.6	70.3	10.1	15.1	37.0	55.7	7.3
BD OD	58.8	203.2	63.8	70.5	10.1	15.1	36.8	55.9	7.3
H FD	59.9	204.2	58.3	64.4	10.2	15.6	41.8	52.0	6.2
H OD	59.8	204.1	59.0	65.2	10.1	15.4	41.1	52.5	6.4
CH2Cl2 FD	60.0	204.3	57.7	63.8	10.6	16.8	42.7	50.8	6.5
CH2Cl2 OD	60.1	204.2	57.6	63.6	10.9	17.8	43.1	50.3	6.6
Hex FD	58.6	203.1	64.6	71.3	9.9	14.8	36.2	56.2	7.5
Hex OD	59.1	203.5	62.5	69.1	10.0	14.8	38.0	55.0	7.0

* S-, MU-, PU-FAME represents saturated FAME, mono-unsaturated FAME, and poly-unsaturated FAME.

percentage of myristic acid (14:0) than other methods. Oleic acid (C18:1) was the dominant fatty acid, its content ranged from 47.3% to 52.7%. The second most abundant fatty acid was palmitic acid (C16:0) ranging from 21.7% to 28.7%. Stearic acid (C18:0) was the third abundant fatty acid with an average content of 11.1%. The least fatty acids in the extracted lipids were docosanoic (C22:0) and tetracosanoic (C24:0) acids, which have average contents of 0.24% and 0.25%, respectively. The linolenic acid, one of major acids in vegetable oils and algal lipids, was a minor acid in this fungal species (Ruan et al., 2012). It was not detectable in the extracted lipids in this study.

The fatty acids profile of *M. isabellina* using different drying and extraction approaches were consistent with other studies of fungal lipids composition (Ruan et al., 2012). The results demonstrated that different drying and extraction methods did not significantly ($P > 0.05$) influence the distribution of the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in the extracted fungal lipids (Table 1).

3.3. Fungal biodiesel quality

It has been reported that the ratios of SFA, MUFA and PUFA, along with the length of fatty acid carbon chain have major impacts on biodiesel quality (Nascimento et al., 2013; Stansell et al., 2012). Fatty acids with short carbon chains and more double bonds give the resultant biodiesel a good cold flow property (lower CFPP). With long carbon chains and more saturated bonds, fatty acids tend to generate biodiesel with good ignition quality (high CN value and good combustion), but poor performance in cold temperatures (high CFPP) and issues of plugging fuel lines and filters (high viscosity) (Nascimento et al., 2014, 2013). In addition, DU value is also an important parameter to evaluate oxidation stability of biodiesel. The lower DU is, the better oxidation stability biodiesel has. Therefore, it is apparent that a balanced fatty acid profile is critical to promote lower CFPP, higher CN, and lower DU, and consequently improve biodiesel quality (See Table 2).

In the current study, there was no significant ($P > 0.05$) difference on the biodiesel fuel properties of CN, CFPP, and DU between different drying and extraction methods. The estimated CNs of fungal biodiesel using different drying and extraction methods varied from 58.6 to 60.1, which complied with all international standards for high ignition property and combustion quality. Meanwhile, the estimated DUs of the fungal biodiesel were from 63.6 to 71.3, which are much lower than most of oilseed and algal biodiesels (122 of rapeseed biodiesel, 158 of grapeseed biodiesel, and 105 of a green alga *Chalmydocapsa bacillus* biodiesel) (Nascimento et al., 2014). As aforementioned, low DU value contributes to better oxidation stability of biodiesel, which means the fungal lipid is one of the preferred sources to produce biodiesel with good oxidation stability. In addition, some biodiesel standards such as European standard limit the content of linoleic ester (less than 12% mol/mol) and polyunsaturated (more than 4 double bonds) ester (less than 1% mol/mol) in biodiesel to further improve biodiesel quality (Stansell et al., 2012). The fungal lipid profile demonstrated that fungal biodiesel could satisfy such requirement, while most of the land crop biodiesels failed (Nascimento et al., 2014). Despite these advantages, the only drawback of the fungal biodiesel is poor cold flow property (higher CFPPs from 14.7 to 17.7 °C) due to a large amount of long chain fatty acids in the fungal lipid. Mixing the fungal lipids with other low-CFPP lipid sources for biodiesel production could be a strategy to take full advantage of superior fuel properties that fungal lipids possess.

4. Conclusion

Freeze-dried fungal biomass demonstrated slightly better overall extraction performance than oven-dried fungal biomass. Bligh & Dyer extraction had the highest lipid yield. Considering both solvent toxicity and fatty acid profile for biodiesel production, hexane & isopropanol extraction is a relatively low-toxicity and high-efficiency method to extract fungal lipid. Non-polar lipid was the most abundant one (93%) in the extracted lipid. Estimation of biodiesel fuel properties further demonstrated that fungal biodiesel has a good fuel quality except cold temperature performance. Therefore, this study concluded that *M. isabellina* is a potential microbial lipid source for high-quality biodiesel production.

Acknowledgements

The authors thank the International TWAS fellowship Program and CNPq – Brazil for the financial support of this study (process no. 190398/20011-6). Acknowledgements are also directed to Mass Spectrometry Facility at Michigan State University providing instrument for fatty acid analysis.

References

Berry, S., 2004. Lipid analysis: isolation, separation, identification and structural analysis of lipids. Nutr. Bull. 29 (1), 72–73.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37 (8), 911–917.

de Boer, K., Moheimani, N.R., Borowitzka, M.A., Bahri, P.A., 2012. Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption. J. Appl. Phycol. 24 (6), 1681–1698.

Dey, P., Banerjee, J., Maiti, M.K., 2011. Comparative lipid profiling of two endophytic fungal isolates *Colletotrichum* sp. and *Alternaria* sp. having potential utilities as biodiesel feedstock. Bioresour. Technol. 102 (10), 5815–5823.

Fried, J., Edwards, J.A., 1972. Organic Reactions in Steroid Chemistry. Van Nostrand Reinhold.

Guckert, J.B., Cooksey, K.E., Jackson, L.L., 1988. Lipid solvent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, *Chlorella*. J. Microbiol. Methods 8 (3), 139–149.

Halim, R., Danquah, M.K., Webley, P.A., 2012. Extraction of oil from microalgae for biodiesel production: a review. Biotechnol. Adv. 30 (3), 709–732.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. Oil extraction from microalgae for biodiesel production. Bioresour. Technol. 102 (1), 178–185.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90 (1), 420–426.

Indarti, E., Majid, M.I.A., Hashim, R., Chong, A., 2005. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. J. Food Compos. Anal. 18 (2), 161–170.

Lee, S.J., Yoon, B.-D., Oh, H.-M., 1998. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. Biotechnol. Tech. 12 (7), 553–556.

Medina, A.R., Grima, E.M., Giménez, A.G., Gonzalez, M., 1998. Downstream processing of algal polyunsaturated fatty acids. Biotechnol. Adv. 16 (3), 517–580.

Miao, X., Wu, Q., 2006. Biodiesel production from heterotrophic microalgal oil. Bioresour. Technol. 97 (6), 841–846.

Mittelbach, M., Remschmidt, C., 2004. Biodiesel: The Comprehensive Handbook. Martin Mittelbach.

Montes D'Oca, M.G., Viegas, C.V., Lemoes, J.S., Miyasaki, E.K., Moron-Villarreyes, J.A., Primel, E.G., Abreu, P.C., 2011. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenergy 35 (4), 1533–1538.

Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., de Carvalho, G.C., Nascimento, M.A., de Souza, C.O., Druzian, J.I., Hussain, J., Liao, W., 2014. Microalgae versus land crops as feedstock for biodiesel: productivity, quality, and standard compliance. BioEnergy Res., 1–12.

Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., Pereira, S.A., Druzian, J.I., de Souza, C.O., Vich, D.V., de Carvalho, G.C., Nascimento, M.A., 2013. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. BioEnergy Res. 6 (1), 1–13.

Ruan, Z., Zanotti, M., Wang, X., Ducey, C., Liu, Y., 2012. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. Bioresour. Technol. 110, 198–205.

Ruan, Z., Zanotti, M., Zhong, Y., Liao, W., Ducey, C., Liu, Y., 2013. Co-hydrolysis of lignocellulosic biomass for microbial lipid accumulation. Biotechnol. Bioeng. 110 (4), 1039–1049.

Stansell, G.R., Gray, V.M., Sym, S.D., 2012. Microalgal fatty acid composition: implications for biodiesel quality. J. Appl. Phycol. 24 (4), 791–801.