Turnitin Originality Report

CEJ9873 by Suryadi Ismadji

From journal (Hippo-hippo)

Processed on 15-Feb-2018 21:55 WIB
ID: 916441967
Word Count: 5129

Similarity Index	Similarity by Source	
18%	Internet Sources:	10%
	Publications:	16%
	Student Papers:	11%

sources:

1 2% match (student papers from 28-Nov-2016)
Submitted to Central University of Gujarat on 2016-11-28

2 1% match (publications)
Ehimen, E.A.. "Variables affecting the in situ transesterification of microalgae lipids", Fuel, 201003

3 1% match (Internet from 03-Nov-2017)
http://tuceel.isc.tuc.gr/Publications/Journals/A75-CEJ-12.pdf

4 1% match (publications)
Li, Jingbo. "Ionic Liquids in Lipid Analysis", Ionic Liquids in Lipid Processing and Analysis, 2016.

5 1% match (student papers from 10-Aug-2015)
Submitted to Central Queensland University on 2015-08-10

6 1% match (Internet from 30-Apr-2016)
https://www.deepdyve.com/browse/journals/chemical-engineering-journal/2012/v213/idecember?page=4

7 1% match (Internet from 21-Jan-2014)
http://www.ijabe.org/index.php/ijabe/article/download/200/104

8 1% match (Internet from 05-Sep-2013)
http://rsce2012.its.ac.id/search.php

9 1% match (publications)
Gunawan, S.. "Design and operation of a modified silica gel column chromatography", Journal of the Chinese Institute of Chemical Engineers, 200811

10 1% match (publications)

Robert B. Levine. "Biodiesel Production from Wet Algal Biomass through in Situ Lipid Hydrolysis and Supercritical Transesterification", Energy & Fuels, 09/16/2010

11 1% match (Internet from 09-Sep-2017)
https://preview-biolres.biomedcentral.com/articles/10.1186/0717-6287-47-15

12 1% match (publications)
Ma, Zhen, Zi-Yang Shang, En-Jun Wang, Jun-Chen Xu, Qin-Qin Xu, and Jian-Zhong Yin. "Biodiesel Production via Transesterification of Soybean Oil Using Acid Catalyst in CO_2 Expanded Methanol Liquids", Industrial & Engineering Chemistry Research, 2012.

13 1% match (Internet from 10-Sep-2012)
http://www.freepatentsonline.com/y2012/0065416.html

14 1% match (student papers from 20-Feb-2016)
Submitted to Taipei Medical University on 2016-02-20

15 1% match (student papers from 18-Aug-2014)
Submitted to Cranfield University on 2014-08-18

16 1% match (publications)
Kasim, N.S.. "Recovery of @c-oryzanol from biodiesel residue", Journal of the Chinese Institute of Chemical Engineers, 200705

17 1% match (student papers from 31-Jan-2012)
Submitted to Institute of Graduate Studies, UiTM on 2012-01-31

18 < 1% match (Internet from 03-Mar-2017)
http://researchonline.jcu.edu.au/42312/1/42312-bainbridge-2015-thesis.pdf

19 < 1% match (Internet from 30-Jan-2012)
http://www.gls.co.jp/technique/inertsearch_gc/pdf/GA191.pdf

20 < 1% match (publications)
Wahidin, Suzana, Ani Idris, and Sitti Raehanah Muhamad Shaleh. "Ionic liquid as a promising biobased green solvent in combination with microwave irradiation for direct biodiesel production", Bioresource Technology, 2016.

21 < 1% match (student papers from 18-Nov-2015)
Submitted to Universiti Teknologi Petronas on 2015-11-18

22 < 1% match (Internet from 26-Apr-2012)
http://www.wirsas.com/pdf/Stand_Alim.pdf

23 < 1% match (publications)
Alptekin, E.. "Optimization of pretreatment reaction for methyl ester production from chicken fat", Fuel, 201012

24 < 1% match (Internet from 09-Sep-2017)
http://putnam.lib.udel.edu/bitstream/handle/19716/17754/2016_MaengMin-Ho_PhD.pdf?isAllowed=y&sequence=1

25 < 1% match (student papers from 30-Jul-2010)
Submitted to Ohio University on 2010-07-30

26 < 1% match (publications)
Surendhiran Duraiarasan, Sirajunnisa Abdul Razack, Anandan Manickam, Anbarasu Munusamy et al. "Direct conversion of lipids from marine microalga C. salina to biodiesel with immobilised enzymes using magnetic nanoparticle", Journal of Environmental Chemical Engineering, 2016

paper text:

3**Chemical Engineering Journal 213 (2012)** 104–108 **Contents lists available at SciVerse ScienceDirect Chemical Engineering Journal journal homepage: www.elsevier.com/locate/cej**

6**In situ biodiesel production from wet Chlorella vulgaris under subcritical condition**

1**Yeshitila Asteraye Tsigie** a, **Lien Huong Huynh** b, **Suryadi Ismadji** c, **Adam Mekonnen Engida**

a,

8**Yi-Hsu** Ju **a,⇑** a **Department of Chemical Engineering, National Taiwan University of Science and Technology, 43** Keelung Rd., **Sec. 4, Taipei 106-07, Taiwan b Department of Chemical Engineering, Can Tho University, Can Tho City,**

Viet Nam c Chemical Engineering Department, Widya Mandala Surabaya Catholic University, Kalijudan 37, Surabaya 60114, Indonesia

6**highlights**" The conventional biodiesel production process is not environmentally friendly. "**Biodiesel can be made directly from wet algal biomass and subcritical methanol. " Stirring shortens the reaction time to achieve high conversion yield of FAMEs.**

14**article info Article history: Received** 14 March **2012 Received in revised form 14 September 2012 Accepted 22 September 2012 Available online** 11 **October 2012 Keywords:**

Biodiesel FAME Subcritical water Chlorella vulgaris abstract The conventional base catalyzed biodiesel production process uses refined vegetable oil as feedstock oil and is not environmentally friendly. The supercritical methanol technology does not require the use of catalyst but it is energy intensive due to the high temperature and pressure required in the process. In this work, a process was developed for producing biodiesel

1**directly from wet Chlorella vulgaris biomass (80% moisture content) using subcritical water as catalyst.**

Under the following conditions:

1**The ratio of wet biomass to methanol** is **1/4 (g/mL),**

the reaction temperature is 175 °C and

1**after 4 h, the reaction product contained 89.71% fatty acid methyl esters (FAMEs). The yield** is **0.29 g FAME per g dry biomass.** This is considerably **higher than the yield of 0.20 g FAME per g dry biomass obtained when the neutral lipid of C. vulgaris biomass was extracted and converted into FAME.**

Ó 2012 Elsevier B.V. All rights reserved. 1. Introduction In order to satisfy the world's energy demand for fuel and de- crease the dependence on fossil fuels, research has been directed towards finding renewable, clean and environmentally-friendly alternative energy sources. Biofuel, especially biodiesel, is one such source that is receiving special attention. Oleaginous microalgae are being considered as potential feedstock for biodiesel produc- tion. Their rapid growth rate and high intracellular lipid content [1,2] make them a potential candidate for feedstock. Chlorella strains have been considered as promising candidates for commer- cial lipid production due to their fast growth and easy cultivation. In addition Chlorella strains are not contaminated by other strains of microalgae when cultivated in open ponds [3]. Although high biomass productivity, rapid lipid accumulation and ability to survive in saline water make microalgae

23**a promising feedstock for** industrial-scale **biodiesel production. The** high cost **of**

producing microalgae biomass and conventional biodiesel produc- tion processes make biodiesel production from microalgae biomass ⇑

16**Corresponding author. Tel.: +886 2 27376612; fax: +886 2 27376644. E-mail address: yhju@mail.ntust.edu.tw (Y.-H. Ju).**

3**1385-8947/$ - see front matter Ó 2012 Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.cej.2012.09.**

112 uneconomical [4]. The conventional method used for biodiesel preparation from microalgae is to first extract lipids. The

13**lipids are** then **converted into fatty acid** alkyl **esters. The**

extraction effi- ciency depends on factors such as microalgae species, method of cell wall disruption and solvent used for extraction [5–8]. Cell dis- ruption prior to extraction can increase the amount of extractable oil. The most commonly used physicochemical techniques for mic- roalgal cell disruption include grinding followed by ultrasonica- tion, microwave treatment, autoclaving, bead-beating and sonication [9,10]. However, the oil extraction step is considered uneconomical. Attention is now being focused on direct or in situ production of biodiesel from microalgae biomass. The conventional production of biodiesel uses refined oil (with free fatty acid (FFA) content less than 0.5%). This refined oil reacts with methanol and is catalyzed by alkali. The biodiesel production from microalgae uses alkali as a catalyst. This

2**would not be** suit- able **due to the high FFA content of microalgae lipids. The** high FFA concentration leads to soap formation and difficulties in bio- diesel purification [11]. Most studies on biodiesel production from microalgae were based on dry algal biomass. It was necessary to remove the water after harvesting biomass. Drying the biomass is energy intensive Y.A. Tsigie et al. / Chemical Engineering Journal 213 (2012) 104–108 105 and efforts are currently devoted to develop process for producing

26**biodiesel directly from wet algal biomass.**

A two-step process to produce biodiesel from wet biomass of C. vulgaris was proposed by Levine et al. [12]. In the first step, wet algal biomass (80% mois- ture) was hydrolyzed in subcritical water to release intracellular lipids. In the second step, the fatty acid-rich wet biomass was re- acted with ethanol under supercritical condition to produce bio- diesel. They reported a maximum fatty acid ethyl ester yield of 66% (w/w). During

7**acid catalyzed in situ production of biodiesel from**

biomass, inhibition occurred

7**when the biomass water content was greater than 115%**

[13]. One step in situ biodiesel production eliminates unnecessary and complex steps such as oil extraction. Velasquez-Orta et al. [14] reported that in situ alkali catalyzed transesterification of dry algal biomass can achieve high conversion (77.6%) in less time than that when using an acid catalyst. Xu and Mi [15] showed that addition of co-solvents such as mixture of toluene and methanol (2:1, v/v) resulted in the highest efficiency. They achieved a 86% yield from the in situ transesterification. It was

7**found that** in **the in situ transesterification** of **the** wet algae **biomass,**

neutral lipids such as triacylglycerols (TAGs), free fatty acids (FFAs) and phos- pholipids all contributed to the formation of fatty acid methyl es- ters (FAMEs) [16]. It is still a challenge to minimize the cost in biodiesel produc- tion related to the use of acid, base and biological catalysts. The catalyst can be eliminated if production is done under supercritical conditions. The supercritical methanol method is simpler, more environmentally friendly and can reach high conversion (>95%) in a very short time. The presence of high contents of FFAs and water has no effect on the efficiency of this method [17–20]. In this reaction,

21**a single homogenous phase is formed between methanol and oil. This** accelerates **the reaction by eliminating mass transfer** resistance **between phases.**

Pretreatment of feedstock is not re- quired [21]. A high alcohol to oil molar ratio (usually >40:1), a high temperature (300–350 °C) and a high pressure (20–50 MPa) makes this process energy intensive [22,23]. Reaction involving subcritical water (SCW) is considered as environmentally friendly. SCW can be used for extraction, hydroly- sis, and wet oxidation of organic compounds. SCW is water at tem- peratures

17**between 100 and 374 °C under high pressure to maintain** it **in liquid state.** The dielectric constant **is the** most **important** fac- tor **when using water as** solvent for **extraction.**

It decreases from 80 at room temperature to 27 at 250 °C [24–26]. In a previous work, it was reported that SCW pretreatment of the biomass of the oleagi- nous yeast Yarrowia lipolytica Po1 g can increase its extractable neutral lipids two folds [27]. SCW can also act as an effective cat- alyst for hydrolysis or biodegradation reactions and to increase the extractable neutral lipids from activated sludge [28,29]. Base catalyzed methanolysis of soybean oil under a subcritical condition of 160 °C was reported by Yin et al. [30]. A 98% yield of methyl es- ters can be obtained in 10 min. Without using a catalyst, only a 6% yield of methyl ester was obtained at 260 °C. The present work was focused on investigating the in situ prep- aration of fatty acid methyl esters (FAMEs) from wet C. vulgaris bio- mass without the need of traditional acid or base catalyst. The effects of reaction time, amount of methanol and stirring on the FAME yield were systematically investigated. 2. Materials and methods 2.1. Apparatus and chemicals Solvents and reagents used in the experiments are either gas chromatography (GC)

9**or analytical reagent grade obtained from**

different suppliers. For GC analysis, all standards of fatty acids and FAMEs were purchased from Acros Organics (New Jersey, USA) and Sigma–Aldrich (St. Louis, MO 63103, USA), respectively. Qualitative filter paper (grade No. 2, 0.26 mm thickness, 80% col- lection efficiency and grade No. 5C) was obtained from Advantec (Tokyo, Japan). A 37 components FAME mixture was supplied by Sigma–Aldrich (Bellefonte, USA). GC-2010 gas chromatograph equipped with a flame ionization detector (Shimadzu, Japan) and a polar column Rtx-2330 composed of 10% cyanopropylphenyl– 90% biscyanopropyl polysiloxane (30 m 0.25 mm, Restek, Belle- fonte, PA) were used for analyzing FAMEs in the reaction product. For analysis of lipid contents and fatty acid profile, a gas chromato- graph (GC-17A, Shimadzu, Japan) with a flame ionization detector and a DB5-HT capillary column (30 m 0.32 mm) were used. Mag- netic stirring was provided by using a Corning PC 320 hot plate magnetic stirrer model PC-320 (Lowell, USA). 2.2. Experimental setup All reactions were conducted in a stainless steel autoclave reac- tor. The reactor has a working volume of about 175 mL. The reactor is 2 cm thick and can withstand an estimated maximum operation pressure of 20 MPa. Temperature and pressure in the reactor was monitored by a thermocouple and a pressure gauge, respectively. Nitrogen gas (99.9% purity) was used to maintain pressure in the reactor required to keep water and methanol in liquid state. The experimental setup is shown in Fig. 1. 2.3. Production of biodiesel from C. vulgaris biomass The

23**objective of this study was to produce biodiesel from** wet microalgae biomass. The biomass was cultivated according to Yeh et al. [31]. Typically, 5 g of wet C. vulgaris biomass (moisture content adjusted to 80% by adding 4 mL deionized water to 1 g dry biomass) and a pre-determined amount of methanol were put into the reactor. Temperature in the reactor was raised to 175 °C, with a corresponding vapor pressure of about 22 bar, based on results from our previous works [27,28]. After a pre-determined time, reaction was stopped by releasing vapor in the reactor to re- duce the pressure to about 2 bar. The reactor was cooled to room temperature and its content was transferred to a separatory funnel. Hexane was then added and after shaking, the aqueous and organic phases were separated. After removing hexane in the organic phase, its composition was analyzed. Fig. 2 is the flowchart show- ing the producing of biodiesel from wet microalgae. Fig. 1. Schematic diagram of reactor set-up. (1) Nitrogen cylinder. (2) Needle valve. (3) Reactor. (4) Electric heater. (5) Magnetic stirrer plate. (6) Safety valve. (P) Pressure gauge. (T) Thermocouple. 106 Y.A. Tsigie et al. / Chemical Engineering Journal 213 (2012) 104–108 2.4. Analysis of reaction product FFA and acylglycerols (AGs) contents of the reaction products were analyzed by using a GC-17A gas chromatograph (Shimadzu, Japan) with a flame ionization detector, as described elsewhere [32]. Separations were carried out on a DB5-HT capillary column (30 m 0.32 mm; Agilent Technologies, USA).

5**Temperatures of the injector and the detector were both set at 370 °C. Temperature of the column was started at 80 °C,** then was **increased to 365 °C at a rate of 15 °C/min and maintained at 365 °C for** 10 **min. The** total run time **was**

29 min. The

9**split ratio was 1:50 using nitrogen as** the **carrier gas with a linear velocity of** 30 cm/s **at 80** °C. **A 20 mg** sam- ple **was dissolved in** 1 mL **ethyl acetate, and**

0.5 lL sample was ta- ken and injected into the GC. To identify the types of fatty acids in the products, 20 mg of a standard of fatty acid was dissolved in 1 mL ethyl acetate and 0.5 lL was injected into the GC. 2.5. Determination of FAME profile Chromatographic analysis of FAME profile in the product was performed using a GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector. The column used was Rtx-2330 10% cyanopropylphenyl–90% biscyanopropyl polysilox- ane

24**column 30 m 0.25 mm i.d.,**

(Restek, Bellefonte, PA). The oper- ating conditions were as follows. The

11**injector and detector temperatures were set at 250 °C. The column temperature was** held **at** 150 **°C for** 2 **min,** and **then** raised to 250 **°C** at 5 °C **/min and held for** 8 **min.**

15**Hydrogen flow, air flow and make up flow were set at** 50 **.0 mL/min,** 500 **.0 mL/min and** 30 **mL/min, respectively. The**

linear velocity and purge flow were 8.0 cm/s and 3.0 mL/min, respectively. Individual FAME was identified by comparing its retention time with the retention times of a

24**37-component FAME mix (Sigma–Aldrich, Bellefonte, USA).**

Total biodiesel yield was cal- culated by using the equation: Wet algal biomass (80 % water content) Addition of methanol Mixture of biomass, water and methanol Total weight of FAMEsðgÞ Total FAMEs yield ð%; w=wÞ ¼ Dry biomass ðgÞ 100% FAME conversion (%, peak area) was calculated based on peak area of the GC chromatogram of the reaction product. FAMEs conversion yield ð%; peak areaÞ ¼ Total peak area of FAMEs 100% Total peak area of the products 3. Results and discussion 3.1. Effect of reaction time and stirring Variables such as reaction time, type and amount of alcohol, catalysts, temperature and method of preparation play important roles in determining biodiesel yield from different feedstocks [13]. Tsigie et al. [27] showed that after SCW pretreatment, the extractable lipid of Yarrowia lipolytica biomass increased two times. It has been suggested that SCW

10**hydrolysis of vegetable oils is predominately a homogeneous reaction in the oil phase,** consist- ing **of three reversible stepwise** reactions **that convert** triacylglyc- erol (TAG) **into**

diacylglycerol (DAG), monoacylglycerol (MAG), and finally glycerol [33]. To study the effect of reaction time and stirring on FAMEs yield, the reaction mixture was allowed to react for a predetermined time (0, 2, 4, 6 or 8 h) with or without stirring. The results are shown in Fig. 3. A general trend was observed.

20**An increase in** reac- tion **time has a positive effect on the amount of FAMEs that can be produced from the** microalge **biomass.**

According to Ehimen et al. [13], during in situ acid catalyzed transesterification of Chlorella biomass, the FAME yield increased from 70% to 92% when reaction time was increased from 15 min to 1 h. These results are better than the results of this study. The maximum FAME yields were obtained at 4 h (88.65%, with stirring) and at 8 h (89.12%, without stirring). An acid catalyzed in situ FAMEs synthesis from Chaetoceros gracilis biomass indicated that increasing time from 25 min to 2.5 h using 2.5 mL methanol per 100 mg biomass, increased the yield of FAME from 7.4% to 22.6% [16]. A longer time (P4 h) is necessary for high FAME yield during in situ, uncatalyzed FAMEs synthesis from wet C. vulgaris. A longer time is necessary to break down cell wall, expose lipids in the cells Reaction under subcritical condition (175 oC, 35 bar) 100 90 80 70 Cool to room temperature FAME without stirring Peak area (%) 60 FAME with stirring Addition of hexane 50 FFA without stirring 40 FFA with stirring Separation of aqueous and organic phases AG without stirring 30 AG with stirring 20 10 Evaporation of hexane in organic phase 0 0 2 4 6 8 10 Reaction Time (h)

Analysis of products Fig. 3. Effect of reaction time and stirring on product composition. Reaction conditions: 5 g wet microalgae (80% moisture content), 30 mL methanol, 175 °C and Fig. 2. Flow chart for the preparation of biodiesel from wet microalgae. 22 bar. Data are average of at least two independent experiments. Y.A. Tsigie et al. / Chemical Engineering Journal 213 (2012) 104–108 and react lipid with methanol under subcritical condition without using acid catalyst. Stirring significantly increases the rate of biodiesel formation in the

7**acid-catalyzed in situ production of biodiesel from microalgae lipids**

[13]. In our

2**study, the effect of** stirring **on the in situ process was**

investigated and the results are shown in Fig. 3. During the first 2 h of reaction, no significant difference was observed in the FAME yields between reaction with and without stirring. The max- imum achievable FAME yield at 2 h was 69.07% without stirring and 71.05% with stirring. A maximum FAME yield of 88.65% can be obtained in 4 h with stirring. It took 8 h to reach a maximum FAME content of 89.12% without stirring. The effect of stirring on FAME content is most pronounced at 4 h. At 6 h and 8 h, the differ- ences in FAME contents between reactions with stirring and with- out stirring are insignificant. The purpose of stirring

2**was to prevent clumping and ensure that biomass** was **adequately exposed to**

methanol.

2**When the in situ transesterification was conducted without stirring, the** con- version **of microalgae oil to**

FAME was significantly lower than that with stirring. This phenomenon was also observed in the in situ acid catalyzed transesterification of microalgal biomass [13]. Ma et al. [39] reported that stirring had a significant effect on the transesterification of beef tallow and methanol. A catalyst free, two-step biodiesel production from wet C. vulga- ris was developed by Levine et al. [15]. Without stirring the reac- tion mixture, they showed that

10**hydrolysis of wet algal biomass** by SCW **at 250 °C**

followed by supercritical in situ transesterifica- tion with ethanol at 275 °C for 2 h produces crude biodiesel of which 79% (w/w) is fatty acid ethyl esters [15]. Our work suggested a maximum FAME yield (88.65%) through a simple one step SCW assisted reaction at 175 °C with continuous stirring. Better results can be achieved with

2**in situ transesterification of** wet **microalgae** biomass with stirring **at**

considerably lower temperature. 3.2. Effect of methanol amount During in situ transesterification, alcohol acts both as solvent for extracting

13**lipids from biomass and as the reactant** for **converting lipids to fatty acid esters** [16]. In transesterification **of**

lipids and alcohol, alcohol amounts higher than the theoretical amount was used to favor the formation of fatty acid esters [34,35]. The effect of methanol amount on FAME content in the in situ methanolysis of wet C. vulgaris biomass is shown in Fig. 4. Fig. 4 shows that as the amount of methanol (mL) per gram of wet microalgae was raised from 2 to 4, the FAME content in the product increased from 79.45% to 89.71%. Further increase in methanol amount (6 or 8 mL) caused a slight decrease in FAME 107 100 90 80 70 Peak area (%) 60 FAME 50 FFA 40 AG 30 20 10 0 0 2 4 6 8 10 12 Amount of Methanol (mL/g biomass) Fig. 4. Effect of methanol amount on product composition. Reaction conditions: 5 g wet microalgae (80% moisture content), 175 °C and 22 bar, reaction time 4 h. The water amount was fixed at 4 g water per g dry microalgae to simulate the moisture content of wet algae after harvesting. In our previous study on producing FAMEs from soybean oil under subcritical condition, it was found that water amount did have significant effect on the yield of FAMEs [40]. 3.3. Composition of FAMEs FAME profile of the product was analyzed by using gas chroma- tography and the result is shown in Table 1. Palmitoleic acid methyl ester (C16:1) is the most abundant FAME present in the biodiesel produced from the in situ methanol- ysis of C. vulgaris under subcritical condition. Other FAMEs present in substantial amounts (15%) are palmitic

25**acid methyl ester (C16:0),** linolenic **acid methyl ester (C18:** 3n3), **and** linoleic **acid methyl ester (C18:** 2n6c). **The** fatty acids **composition**

of lipids from C. vulgaris was studied by Petkov and Garcia [37] and their results are similar to ours. Small quantities of methyl esters from fatty acids with less than 15 carbons and more than 19 carbons were also observed in this study, and was confirmed by a previous study [38]. The presence of substantial amounts of linolenic acid methyl ester (15.19%), and linoleic acid methyl ester (13.84%) will have negative effect on the oxidative stability of the biodiesel produced. Modifying fatty acid profile of C. vulgaris through genetic content. It has been suggested that the presence of excess metha- Table 1 nol in transesterification process is essential since it is responsible Composition of FAMEs produced from wet biomass of Chlorella vulgaris at 175 °C and for breaking the glycerin–fatty acid linkages [36]. A study on bio- 22 bar. Each value is the average of two independent experiments. diesel

12**preparation from Chlorella protothecoides showed that excess**

Type of FAME Amount

12**methanol in large quantities reduced the amount of products and**

(peak area (%)) slowed down

12**the separation of glycerol and FAME** [6]. **The**

conver- Capric acid methyl ester (C10:0) 0.85 sion of crude microalgal oil to biodiesel increased with increasing

4**Lauric acid methyl ester (C12:0)**

3.32 methanol to oil ratio. This reached a maximum and started to de- Tridecanoic acid methyl ester (C13:0) 1.41 crease when methanol to oil ratio was further increased [8]. A sim- Myristic

19**acid methyl ester** (C14 **:0)** 6.5 **cis-10-Pentadecenoic acid methyl ester (C15:1)**

3.17 ilar trend was observed in this study (Fig. 4).

4**Palmitic acid methyl ester (C16:0)**

15.66 According to Yin et al. [30], the advantage of using excess MeOH

4**Palmitoleic acid methyl ester (C16:1)**

27.73 is that the reaction can be carried out in one phase because oil be-

19**cis-10-Heptadecenoic acid methyl ester (C17:1)**

3.86 comes soluble in subcritical methanol. Both yield of FAME and

4**Stearic acid methyl ester (C18:0)**

0.21 reaction rate are enhanced due to homogeneous mixture of reac- Elaidic acid methyl ester (C18:1n9t) 6.08 Oleic acid methyl ester (C18:1n9c) 1.32 tants as well as higher concentration of methanol which favors

4**Linoleic acid methyl ester (C18:2n6c)**

13.84 FAME formation. We found that a wet microalgae biomass to

4**cis-11-Eicosenoic acid methyl ester (C20:1n9)**

0.33 methanol ratio of 1:4 (w/w) was necessary to achieve maximum

4**Linolenic acid methyl ester (C18:3n3)**

15.19 FAME yield because the presence of excess methanol is conducive

22**cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3) 0.** 32 **Nervonic acid methyl ester (C24:**

1n9) 0.21 to extracting microalgae oil and transforming the oil into FAMEs. 108 Y.A. Tsigie et al. / Chemical Engineering Journal 213 (2012) 104–108 manipulation and/or optimizing of culturing conditions on this species is required if it is to be considered as a potential candidate as feedstock for biodiesel production. 4. Conclusion A catalyst free method

26**to produce biodiesel directly from wet biomass of C.**

vulgaris under subcritical condition was studied. Stir- ring has positive effect on the reaction by shortening reaction time required to achieve high conversion. The maximum biodiesel yield from C. vulgaris using this method was 0.29 g/g dry biomass. This was obtained under the following conditions: 5 g wet biomass (80% moisture content), 20 mL methanol, 175 °C, with stirring for 4 h. This compares favorably with the theoretically yield of 0.20 g FAME/g dry biomass which can be obtained by firstly extracting all neutral lipids from dry biomass of C. vulgaris and then transform all neutral lipids into FAMEs. Acknowledgements This work was supported by National Science Council of Taiwan through the grant NSC100-2623-E-011 -001–ET. We are also thankful to Professor Jo-Shu Chang, National Chung Kung Univer- sity, for his generous gift of the Chlorella vulgaris biomass. References [1] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, Plant J. 54 (2008) 621–639. [2] C. Yusuf, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306. [3] M. Huntley, D. Redalje, CO2 mitigation and renewable oil from photosynthetic microbes: a new appraisal, Mitig. Adapt. Strat. Global Change 12 (2007) 573– 608. [4] R. Halim, M.K. Danquah, P.A. Webley, Extraction of oil from microalgae for biodiesel production: a review, Biotech. Adv. (2012), http://dx.doi.org/ 10.1016/j.biotechadv.2012.01.001. [5] J.Y. Lee, C. Yoo, S.Y. Jun, C.Y. Ahn, H.M. Oh, Comparison of several methods for effective lipid extraction from microalgae, Bioresour. Technol. 101 (2010) S75– S77. [6] X. Miao, Q. Wu, Biodiesel production from heterotrophic microalgal oil, Bioresour. Technol. 97 (2006) 841–846. [7] N. Nagle, P. Lemke, Production of methyl ester fuel from microalgae, Appl. Biochem. Biotechnol. 24–25 (1990)

355–361. [8] D.T. Tran, K.L. Yeh, C.L. Chen, J.S. Chang, Enzymatic transesterification of microalgal oil from Chlorella vulgaris ESP-31 for biodiesel synthesis using immobilized Burkholderia lipase, Bioresour. Technol. (2012), http://dx.doi.org/ 10.1016/j.biortech.2011.12.145. [9] F. Pernet, R. Tremblay, Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters, Lipids 38 (2003) 1191–1195. [10] K.H. Wiltshire, M. Boersma, A. Möller, H. Buhtz, Extraction of pigments and fatty acids from the green alga Scenedesmus obliquus (Chlorophyceae), Aquat. Ecol. 34 (2000) 119–126. [11] S. Al-Zuhair, Production of biodiesel: possibilities and challenges, Biofuels Bioprod Biorefin 1 (2007) 57–66. [12] R.B. Levine, T. Pinnarat, P.E. Savage, Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification, Energy Fuels 24 (2010) 5235–5243. [13] E.A. Ehimen, Z.F. Sun, C.G. Carrington, Variables affecting the in situ transesterification of microalgae lipids, Fuel 89 (2010) 677–684. [14] S.B. Velasquez-Orta, J.G.M. Lee, A. Harvey, Alkaline in situ transesterification of Chlorella vulgaris, Fuel 94 (2012) 544–550. [15] R. Xu, Y. Mi, Simplifying the process of microalgal biodiesel production through in situ transesterification technology, J. Am. Oil Chem. Soc. 88 (2011) 91–99. [16] B.D. Wahlen, R.M. Willis, L.C. Seefeldt, Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures, Bioresour. Technol. 102 (2011) 2724–2730. [17] D. Ayhan, Biodiesel from waste cooking oil via base-catalytic and supercritical methanol transesterification, Energy Convers. Manage. 50 (2009) 923–927. [18] H. He, T. Wang, S. Zhu, Continuous production of biodiesel fuel from vegetable oil using supercritical methanol process, Fuel 86 (2007) 442–447. [19] V.F. Marulanda, G. Anitescu, L.L. Tavlarides, Investigations on supercritical transesterification of chicken fat for biodiesel production from low-cost lipid feedstocks, J. Supercrit. Fluids 54 (2010) 53–60. [20] J.Z. Yin, M. Xiao, J.-B. Song, Biodiesel from soybean oil in supercritical methanol with co-solvent, Energy Convers. Manage. 49 (2008) 908–912. [21] D. Kusdiana, S. Saka, Effects of water on biodiesel fuel production by supercritical methanol treatment, Bioresour. Technol. 91 (2004) 289–295. [22] D. Ayhan, Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics, Energy Convers. Manage. 47 (2006) 2271–2282. [23] E.S. Song, J.W. Lim, H.S. Lee, Y.W. Lee, Transesterification of RBD palm oil using supercritical methanol, J. Supercrit. Fluids 44 (2008) 356–363. [24] R.L. Holliday, J.W. King, G.R. List, Hydrolysis of vegetable oils in sub- and supercritical water, Ind. Eng. Chem. Res. 36 (1997) 932–935. [25] P. Khuwijitjaru, T. Fujii, S. Adachi, Y. Kimura, R. Matsuno, Kinetics on the hydrolysis of fatty acid esters in subcritical water, Chem. Eng. J. 99 (2004) 1–4. [26] A.T. Quitain, M. Faisal, K. Kang, H. Daimon, K. Fujie, Low-molecular-weight carboxylic acids produced from hydrothermal treatment of organic wastes, J. Hazard. Mater. 93 (2002) 209–220. [27] Y.A. Tsigie, L.H. Huynh, I.N. Ahmed, Y.-H. Ju, Maximizing biodiesel production from Yarrowia lipolytica Po1g biomass using subcritical water pretreatment, Bioresour. Technol. 111 (2012) 201–207. [28] L.H. Huynh, N.S. Kasim, Y.H. Ju, Extraction and analysis of neutral lipids from activated sludge with and without sub-critical water pre-treatment, Bioresour. Technol. 101 (2010) 8891–8896. [29] T. Rogalinski, S. Herrmann, G. Brunner, Production of amino acids from bovine serum albumin by continuous sub-critical water hydrolysis, J. Supercrit. Fluids 36 (2005) 49–58. [30] J.Z. Yin, Z. Ma, D.-P. Hu, Z.L. Xiu, T.H. Wang, Biodiesel production from subcritical methanol transesterification of soybean oil with sodium silicate, Energy Fuels 24 (2010) 3179–3182. [31] K.L. Yeh, J.S. Chang, W.M. Chen, Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga Chlorella vulgaris ESP-31, Eng. Life Sci. 10 (2010) 201–208. [32] Y.A. Tsigie, C.Y. Wang, C.-T. Truong, Y.H. Ju, Lipid production from Yarrowia lipolytica Po1g grown in sugarcane bagasse hydrolysate, Bioresour. Technol. 102 (2011) 9216–9222. [33] L. Lascaray, Mechanism of fat splitting, Ind. Eng. Chem. 41 (1949) 786–790. [34] K. de Boer, P.A. Bahri, Supercritical methanol for fatty acid methyl ester production: a review, Biomass Bioenergy 35 (2011) 983–991. [35] D. Kusdiana, S. Saka, Kinetics of transesterification in rapeseed oil to biodiesel fuel as treated in supercritical methanol, Fuel 80 (2001) 693–698. [36] M.I. Al-Widyan, A.O. Al-Shyoukh, Experimental evaluation of the transesterification of waste palm oil into biodiesel, Bioresour.

Technol. 85 (2002) 253–256. [37] G. Petkov, G. Garcia, Which are fatty acids of the green alga Chlorella?, Biochem Syst. Ecol. 35 (2007) 281–285. [38] T. Heredia-Arroyo, W. Wei, R. Ruan, B. Hu, Mixotrophic cultivation of Chlorella vulgaris and its potential application for the oil accumulation from non-sugar materials, Biomass Bioenergy 35 (2011) 2245–2253. [39] F. Ma, L.D. Clements, M.A. Hanna, The effect of mixing on transesterification of beef tallow, Bioresour. Technol. 69 (1999) 289–293. [40] Y.H. Ju, L.H. Huynh, Y.A. Tsigie, Q.P. Ho, Synthesis of Biodiesel in Subcritical Water and Methanol, Fuel, http://doi:10.1016/j.fuel.2012.05.061.

18**Author's personal copy Author's personal copy Author's personal copy Author's personal copy Author's personal copy**