Bioresource Technology 156 (2014) 240–247

Contents lists available at ScienceDirect

Bioresource Technology

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

Extractive-transesterification of algal lipids under microwave irradiation with hexane as solvent

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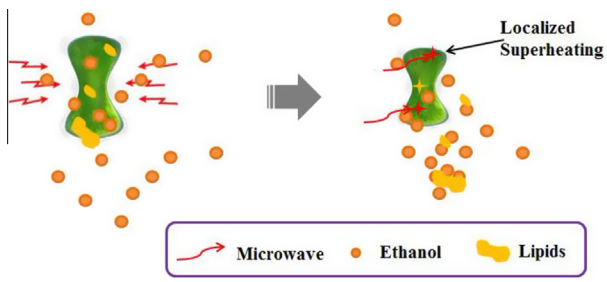
HIGHLIGHTS

- Two microwave-enhanced extractive-ethyl-transesterification reactions were studied.
- Hexane as co-reactant may enhance the lipid extraction from algae biomass.
- MW extractive-transesterification performs superior to Bligh and Dyer Method.
- Combined extraction and transesterification methods may save chemicals and energy.
- Process optimization and comparison of biodiesel conversion analysis were presented.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 November 2013
Received in revised form 4 January 2014
Accepted 6 January 2014
Available online 17 January 2014

Keywords:
Algal biodiesel
Chlorella sp.
Extractive-transesterification
Hexane
Microwave extraction

ABSTRACT

This study describes the use of microwaves (MW) for enhanced extractive-transesterification of algal lipids from dry algal biomass (*Chlorella* sp.). Two different single-step extractive-transesterification methods under MW irradiation were evaluated: (1) with ethanol as solvent/reactant and sodium hydroxide catalyst; and (2) with ethanol as reactant and hexane as solvent (sodium hydroxide catalyst). Biodiesel (fatty-acid-ethyl-esters, FAEE) yields from these two methods were compared with the conventional Bligh and Dyer (BD) method which followed a two-step extraction-transesterification process. The maximum lipid yields for MW, MW with hexane and BD methods were 20.1%, 20.1%, and 13.9%, respectively; while the FAEE conversion of the algal lipids were 96.2%, 94.3%, and 78.1%, respectively. The algae-biomass:ethanol molar ratio of 1:250–500 and 2.0–2.5% catalyst with reaction times around 6 min were determined as optimum conditions for both methods. This study confers that the single-step non-conventional methods can contribute to higher algal lipid and FAEE yields.

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1. Introduction

Microalgae are identified by many researchers as an ideal feedstock for biodiesel production due to their high-oil yielding capacity and environmental-friendly nature (Chisti, 2007; Gude et al., 2013a). Algal biodiesel production essentially involves two main steps: (1) extraction of oils from the biomass, and (2) conversion (transesterification) of oils (fatty acids) to biodiesel (alkyl esters). To date, biodiesel production from algae biomass is generally performed by one of the following three methods: (1) A two-step protocol in which algae oil is extracted with organic solvent and then converted to biodiesel using a catalyst, such as an acid, a base, or an enzyme; (2) direct production of biodiesel from algae biomass

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http://dx.doi.org/10.1016/j.biortech.2014.01.026

using an acid catalyst at atmospheric pressure and ambient temperature; and (3) one-step, single pot conversion to biodiesel at high pressure and high temperature in the absence of a catalyst (Chen et al., 2012). But combining the oil extraction step with transesterification improves the economics of overall biodiesel production. This process is also known as *in situ* transesterification (Haas and Wagner, 2011). The *in situ* transesterification method has the potential to simplify the conversion process, reducing the number of unit operations and consequently the overall process costs and final biodiesel product costs (Haas and Wagner, 2011). The *in situ* transesterification methods have been evaluated for biodiesel production from various raw materials, such as vegetable oil (Haas et al., 2004), wastewater sludges (Mondala et al., 2009), rice bran oil (Shiu et al., 2010), cotton seed oil (Qian et al., 2008) and microalgae (Li et al., 2011). Many researchers have recently evaluated acid or base catalyzed *in situ* transesterification of microalgae. To name a few, Johnson and Wen (2009) tested biodiesel production from algae *Schizochyrtium limacinum* SR21 using different solvents (methanol, chloroform, hexane and petroleum ether). They obtained a maximum 68% yield of FAMEs (Fatty-Acid-Methyl-Esters) when chloroform or hexane were added to methanol using 1.5 mol of sulfuric acid and 132:1 mol of methanol and solvent at 90 °C for 40 min. Ehimen et al. (2010) tested *Chlorella* sp. at different temperatures, alcohol molar ratios, reaction times and moisture contents in the production of biodiesel. Their study showed a maximum lipid to FAME conversion of around 88% after a reaction time of 2 h,using 0.04 mol of sulfuric acid, 500:1 mol of methanol and a temperature of 90 °C. Xu and Mi (2011) conducted alkaline *in situ* transesterification of *Spirulina* sp. in order to test different types of co-solvents. Similarly, Velasquez-Orta et al. (2012) reported on alkaline *in situ* transesterification of *Chlorella vulgaris* and the results were compared with an acid catalyst (sulfuric acid). All these studies utilized methanol as a solvent for extraction and transesterification steps. Currently, methanol is a commonly used reagent for transesterification, but ethanol is attractive for long term sustainability since it can be derived from renewable sources, is less toxic than methanol, and is generally considered safe (Demirbas, 2009). Fatty acid ethyl esters (FAEE) typically demonstrate slightly higher cetane numbers, improved low temperature operability, and greater oxidative stability when compared to fatty acid methyl esters (FAME) (Knothe, 2005; Demirbas, 2009). Apart from this, the majority of the world methanol production is based on natural gas. This is one of the reasons behind the dependence of the methanol cost on the price of crude oil. The higher toxicity of methanol relative to ethanol also raises additional concerns regarding its transportation and storage (Zanin et al., 2013).

Conventional, thermochemical liquefaction and supercritical algal oil extraction processes require long extraction times and severe process conditions making biodiesel production an energy-intensive and expensive process (Aresta et al., 2005). For example, the well-established soxhlet process requires long extraction times in the range of 6–48 h. As described earlier, ethanol is a more sustainable reactant/solvent and has been proven to be a better solvent compared to methanol. Moreover, to date, lipid extraction and transesterification methods with ethanol are not well-explored especially for microalgae. Apart from this, utilizing novel extraction methods such as microwaves and ultrasound can benefit from shorter extraction and transesterification times and low solvent requirements (Gude et al., 2013b). In this study, the effect of microwave irradiation on extractive-transesterification of algal lipids directly from algal biomass using ethanol as reactant/solvent was evaluated. Additionally, the combined use of hexane as a solvent with ethanol as reactant to improve the algal lipid yields was evaluated. Finally, the two methods were compared with the conventional Bligh and Dyer method (Bligh and Dyer, 1959) which included two separate extraction and transesterification steps. The following sections present the experimental studies and optimized process conditions for microwave enhanced extractive-transesterification of algal lipids using ethanol with and without the solvent hexane.

2. Methods

2.1. Materials

Process steps involved in the extractive-transesterification process include preparation of dry algal biomass, addition of reactant/solvent and catalysts for extractive-transesterification under microwave irradiation, separation and purification of the reaction products and quality analysis. Dry algae biomass (*Chlorella* sp.) was purchased from a commercial producer. Ethanol (>99.5% purity), hexane (98% + extra pure) and sodium hydroxide (>99.5% purity) were purchased from Cole Parmer. All reagents and standards used in the GC and GC–FID analysis were purchased from Fisher Scientific.

2.2. Experimental Procedures

Four samples of algae powder were tested using the Bligh and Dyer method. 0.2 g of algae powder was washed with 5 mL of deionized water. Methanol, chloroform, and deionized water (12.5, 12.5, 6.25 mL, respectively) were added to the washed algae in series and the tubes were vortexed after the addition of each chemical. Methanol was added to change the polarity of the water layer which limits the polar lipids increment. The samples were centrifuged for 10 min at 3000 rotations per minute to separate the layers. The bottom layer containing lipophilic material was drawn off with a pasture pipette and run through a glass wool filled pipette into a preweighed 40 mL vial. 12.5 mL of chloroform were added to the content left in the test tube and then vortexed. The bottom layer containing the chloroform was again drawn off and run through the same glass wool filled pipette into the same vial until only the top layer which contained methanol and water was left in the tubes. The glass wool was rinsed with chloroform, and the 40 mL vial was placed on the TurboVap LV (Caliper Life Sciences, Hopkinton, MA, USA) for about an hour. The vials with lipid residue were weighed, and the percentage of lipids concentration was calculated. For the ease of chromatographic analysis, the triglycerides were broken down into fatty acids. Therefore, to identify the triglycerides, 2 mL of 2% sulfuric acid was added to methanol in a 40 mL vial containing the lipid residue and vortexed. After the addition of sulfuric acid, the samples were placed in a water bath maintained at 60 °C for 2 h and then cooled at room temperature. Once the mixture had reacted for 2 h, 5 mL of 3% $NaHCO_3$/5% NaCl and 2 mL of the standard (100 ppm butylated hydroxyl toluene (BHT) and 200 ppm 1,3-dichlorobenzene (1,3-DBC)) were added, and the sample was once again vortexed. 1,3-DBC was used as an internal standard and BHT was used as antioxidant. This process resulted in the production of fatty acid ethyl esters (FAEE) which were analyzed in the GC–FID.

The extractive-transesterification experiments were conducted in a MW reactor with temperature and power control functions manufactured by Columbia International Technologies®, USA. Microwave-transparent, three-neck custom-fabricated reaction vessels made of borosilicate glass (provided by Columbia International Technologies) were used as sample vessels. Averages of test results obtained for three repetitive tests were analyzed to evaluate the reproducibility of microwave effect. After each test, the microwave reactor was allowed to cool and return to original conditions with adequate cooling (by using a fan) and reaction interval. The MW reactor was equipped with a reflux condenser to provide for condensation of evaporating solvent mixture. A

magnetic stirrer was used to ensure uniform mixing with a constant mixing speed for all the experiments.

Four grams of dry algae powder were added to the premixed homogeneous solution of ethanol and sodium hydroxide catalyst. The mixture was then subjected to MW irradiation with exiting power of 700 W (at a power dissipation level of 50% = 350 W) under a matrix of conditions: reaction times of 3–7 min; catalyst concentrations in the range of 0.5–2.5 wt.% of dry biomass at 0.5% intervals; and dry algae to ethanol (wt./vol) ratios of 1:6–1:15 (24, 36, 48 and 60 mL of ethanol volumes to result in approximately algal oil to ethanol molar ratios of 1:250; 1:375; 1:500; and 1:625) and power output rates of 10–100%. The reflux condenser played an important role to condense, return and maintain the solvent volume in the reaction mixture throughout the experiments. Similar experiments were conducted with reaction mixture containing 24 mL of ethanol and 24 mL of hexane for the above mentioned process conditions to study the effect of hexane in the extractive-transesterification reaction. The samples were cooled using a fan and immediately placed in a freezer before the separation/purification steps and analysis by GC–FID.

2.3. Biodiesel analysis

2.3.1. Overall FAEE conversion

The FAEE (biodiesel) conversion analysis was carried out on a Varian Gas Chromatography (GC) with cool on column injection and FID detection as required by ASTM 6584 method for B100 to measure the FAEE conversion. The operating scheme of the biodiesel analysis using the gas chromatography is as follows: a sample size of 1 μL with initial temperature of 50 °C (hold 1 min) followed by rate 1 (15 °C/min to 180 °C); rate 2 (7 °C/min to 230 °C) and rate 3 (30 °C/min 380 °C) with flame ionization detector at 380 °C. Helium was used as a carrier gas and the internal standards were prepared by following the procedures described in ASTM-D 6584 B-100 Biodiesel method (ASTM-D 6584).

2.3.2. FAEE composition

The FAEE composition in ethanol and hexane phases (before separation) were analyzed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector (Agilent, Santa Clara, CA, USA). The column used was a 100 m × 0.25 mm × 0.2 μm Supelco SP 2380 capillary column (Supelco, Bellefonte, PA, USA) with stabilized poly (90% biscyanopropyl/10% cyanopropylphenyl) siloxane as the stationary phase. The column temperature was programmed to increase from 110 to 140 °C at 10 °C/min; maintained at 140 °C for 4 min; then ramped from 140 to 240 °C at 2 °C/min; and finally maintained at 240 °C for 40 min. The detector temperature was set at 260 °C. Helium was used as carrier gas (14 psi) at a flow rate of 45.0 mL/min, while the sample injection volume was 1.0 μL with a split ratio of 100:1. The internal standard used was 1,3-dichlorobenzene (Fisher Scientific, Pittsburgh, PA, USA) (Mondala et al., 2009). Calibration curves between peak area and concentration were established by injecting reference FAME samples of known concentrations into the GC–FID.

3. Results and discussion

3.1. Comparison with Bligh and Dyer method

A comparison between the conventional Bligh and Dyer (BD) method and the two single-step extractive-transesterification methods is shown in Fig. S1. Microalgae lipids have higher selectivity towards chloroform–methanol–water system, due to their polar nature and excellent solvent characteristics of chloroform (Araujo et al., 2013). Solvents used in BD method (especially chloroform) may cause cell wall disruption, thus contributing toward extraction of oil/lipids from the microalgae cells. Microwaves (MW) can contribute to both diffusive and disruptive extraction of algal lipids in the solvent. MW enhanced extraction process has performed consistently better than the BD method and the method using hexane as co-solvent in terms of both the lipid extraction (yields) and the fatty acid ethyl ester (FAEE) conversion. The lipid yield and FAEE conversion rates are shown in Fig. S1. The lipid yields were 13.88%, 20.13% and 20.1% for BD, MW and MW-Hexane methods, respectively while the FAEE conversions were 78.09%, 96.17%, and 94.31% for the three methods in the same order. Hexane addition reduced the requirement for higher ethanol concentrations and assisted further in extraction of lipids while resulting in higher FAEE conversion than BD method. The results show that the non-conventional microwave heating method is able to quickly extract the lipids and simultaneously convert them into FAEE. More importantly, the MW process requires one step simple extraction and transesterification compared to BD method which includes various extraction steps using different solvents requiring long reaction times. Hexane method (combined with MW) can be viewed superior to BD method considering the above factors. Lee et al. (2010) also reported that lipid extraction yield was higher for microwave method compared to autoclaving, bead-beating, sonication, and a 10% NaCl solution extraction methods.

3.2. Extractive-ethyl-transesterification of algal Lipids under microwave irradiation

3.2.1. Effect of algae oil to ethanol molar ratio (algal biomass to ethanol ratio)

Fig. 1 shows the effect of different process parameters namely algae oil to ethanol ratio, catalyst concentration expressed as wt.% of dry algae, reaction time (min), and the microwave power level on the lipid/fatty acid ethyl esters (FAEE) yield. The effect of ethanol on extractive-transesterification reaction is significant with increasing algae oil to ethanol molar ratios up to 1:500 at a fixed catalyst concentration (2% wt.). In this reaction, ethanol acts both as a solvent for extraction of the algal oils/lipids (Mulbry et al., 2009) as well as the reactant for transesterification of esters (Fig. 1a; Process Conditions: 4 g dry algae; 2% catalyst and 5 min reaction time). The lipid yield did not increase with higher molar ratios of oil to ethanol (algae biomass to ethanol) as shown in Fig. 1a. The FAEE conversion rates are shown in Fig. 2a (Process Conditions: 4 g dry algae; 2% catalyst and 5 min reaction time). Higher ratios of algae to ethanol could shift the reversible reaction forward (as observed) perhaps due to increased contact area between ethanol and oil/lipid, resulting in higher yield of FAEE. However, increased dry algae to ethanol ratios above 1:500 may not favor the extraction and transesterification since much of the microwave irradiation will be absorbed by the solvent, not affecting the algae cells which could result in inefficient extraction of algal oils. The MW method has performed consistently better than BD method in terms of both FAEE yield and conversion.

3.2.2. Effect of catalyst amount

The effect of catalyst concentration on the lipid yield and the FAEE conversion are shown in Fig. 1b (Process Conditions: 4 g dry algae; 48 mL ethanol and 5 min reaction time) and Fig. 2b (Process Conditions: 4 g dry algae; 48 mL ethanol and 5 min reaction time), respectively. At low concentrations the BD method performed better than MW method in terms of lipid extraction. Higher catalyst concentrations show a positive effect on the MW extractive-transesterification reaction while the lipid yield remained unaffected. As this is two phase reaction mixture, the oil/lipid concentration in the ethanol phase is low at the start of the reaction leading to mass transfer limitations. As the reaction continues,

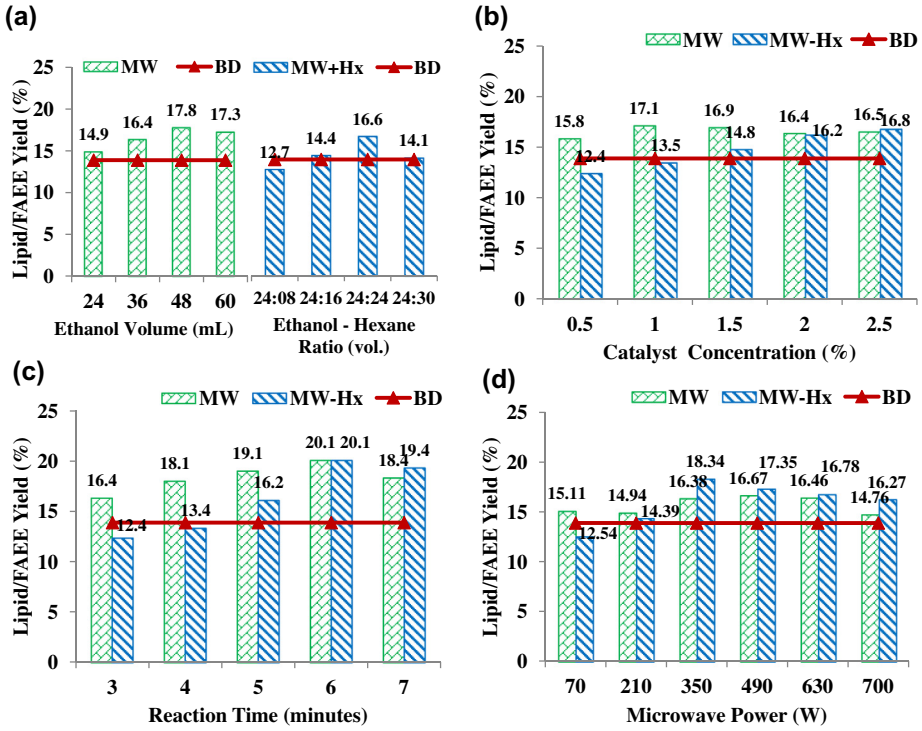

Fig. 1. Lipid yield for microwave and microwave-hexane extractive transesterification: (a) Effect of ethanol and hexane in lipid extraction; (b) effect of catalyst concentration; (c) effect of reaction time; and (d) effect of microwave power.

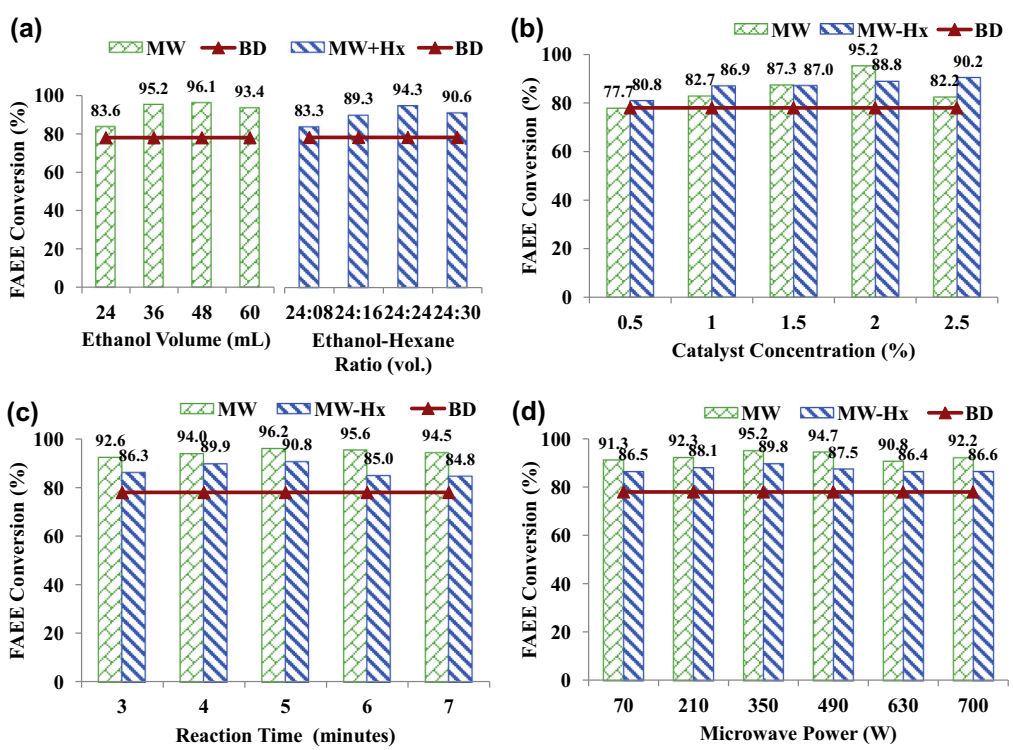

Fig. 2. FAEE conversion for extractive-ethyl-transesterification with microwave and microwave-hexane: (a) Effect of ethanol and hexane in lipid extraction; (b) effect of catalyst concentration; (c) effect of reaction time; and (d) effect of microwave power.

the concentration of oil/lipid in the ethanol phase increases leading to higher transesterification rates with increasing catalyst concentrations (Boocock et al., 1998). The homogeneous, solvent-catalyst ethoxide prepared using sodium hydroxide is more susceptible to microwave irradiation as compared to solid catalysts and yield high biodiesel conversion rates (Patil et al., 2011; Refaat, 2010). Lower concentrations of the catalyst may not efficiently advance the reaction as the catalyst effect can be hindered by the presence

of other organic compounds resulting from algal biomass extraction.

3.2.3. Effect of reaction time

The reaction time has significant effect on the FAEE content (Fig. 1c; Process Conditions: 4 g dry algae; 2% catalyst and 48 mL ethanol). Intuitively, extended reaction times are favored for better yields of extraction and biodiesel conversion since lower reaction times do not provide sufficient interaction of the reactant mixture with microwaves. The microwave effect is two-fold in the extractive-transesterification reaction: first, a thermal effect caused by the microwaves increases the extractive properties of ethanol to extract the oils from the algal biomass in suspension (diffusive extraction) and next, an extended microwave effect causes the penetration through the cell walls and forces out the oils (from the biological matrix) into the solvent mixture (disruptive extraction). It was noted that the reaction time around 6 min was required to extract the algal lipids while a reaction time around 5 min was adequate to complete extraction and transesterification reaction under microwave irradiation since higher reaction times did not necessarily result in improved lipid yields or FAEE conversion (Fig. 2c; Process Conditions: 4 g dry algae; 2% catalyst and 48 mL ethanol). Higher reaction times above 6 min did not result in higher FAEE yields due to overheating of the reaction mixture which results in by-product formation and solvent and energy losses. This can also be explained as increase in conversion but a reduction in selectivity of the solvent.

3.2.4. Effect of microwave power

Efficient utilization of microwave energy is critical to enhanced extractive transesterification of algal lipids. Tests at different power levels between 10% and 100% at 10% intervals were conducted. As shown in Fig. 1d (Process Conditions: 4 g dry algae; 2% catalyst; 48 mL ethanol and 5 min reaction time) and Fig. 2d (Process Conditions: 4 g dry algae; 2% catalyst; 48 mL ethanol and 5 min reaction time), lower levels of power supply (about 350 W) resulted in higher lipid yields and FAEE conversion rates indicating that the energy losses were minimized at low power supply and a balance between conversion and selectivity. Also, the temperature profiles (Fig. 3a) at different microwave power levels between 10% and 100% at 10% intervals show that the boiling temperatures of the bulk medium are not required for the enhanced extractive-transesterification by MW method. This is because the microwaves produce localized superheating at molecular levels which provide for enhanced heat and mass transfer in the extractive-transesterification reaction. Current commercial microwave ovens (both domestic and industrial) contain large reaction cavities (chambers) which absorb some of the microwave energy without affecting the reaction mixture. This means improving the microwave reactor design would enhance the energy performance which reflects as FAEE conversion efficiency.

3.3. Extractive-ethyl-transesterification with hexane under microwave irradiation

Extraction of lipids from microalgae is basically a mass transfer operation which depends on the nature of the solute and solvent, the selectivity of the solvent and the level of convection in the medium. Here, hexane was introduced as a solvent and as a medium to increase the mass transfer rate (extraction) of lipids into the reaction mixture to evaluate its effect on the extractive-transesterification reaction. The premise behind this concept is that hexane and ethanol are miscible and hexane as co-solvent may enhance the extractive ability for free fatty acids and eventually improve the transesterification reaction yields. The assumption is that if higher FAEE can be obtained by this method, cost reductions may

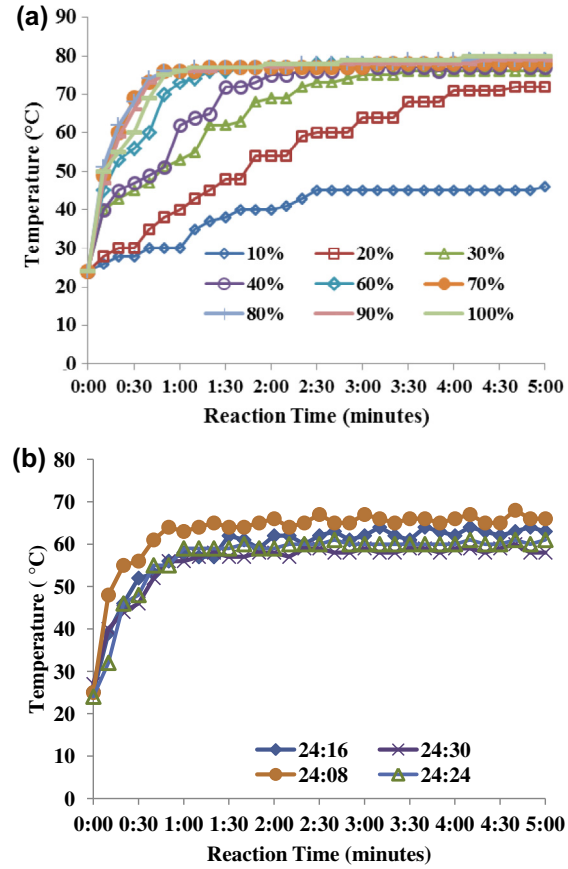

Fig. 3. (a) Temperature profiles for MW power effect (process conditions: 4 g dry algae; 2% catalyst and 5 min reaction time); and (b) temperature profiles for ethanol and hexane mixtures (vol:vol in mL) (process conditions: 4 g dry algae; 2% catalyst and 5 min reaction time).

be achieved with reduced ethanol usage and with hexane recycling to the reaction mixture for successive extractive-transesterification cycles.

3.3.1. Effect of hexane as co-reactant and solvent

Further, the effect of solvent n-hexane along with ethanol on extractive-transesterification of algal lipids was investigated. Fig. 3b shows the temperature profiles for this method where different volumetric ratios (mL) of ethanol and hexane were added. As it can be seen the reaction temperatures are in the range of 60–70 °C which is lower than the MW method with ethanol alone (70–80 °C). This is due to the non-polarity of n-hexane which does not allow n-hexane to heat up under the microwave effect. As a result, the overall temperature of the reaction mixture is maintained low during the reaction. This suggests that addition of n-hexane as co-solvent will reduce the reaction condition severities under microwave effect. Fig. 1a (Process Conditions: 4 g dry algae; 2% catalyst and 5 min reaction time) shows the effect of ethanol to hexane ratio on the lipid/FAEE yield and Fig. 2a (Process Conditions: 4 g dry algae; 2% catalyst and 5 min reaction time) shows the FAEE conversion rates. 24 ml of ethanol (approximately 1:250 oil to ethanol molar ratio) was mixed with different volumes of hexane to create solvent mixtures of 3:1, 1.5:1, 1:1 and 1:1.25 ratios (24:08; 24:16; 24:24; and 24:30 ethanol:hexane volumetric ratios, respectively). It was observed that 1:1 ratio of ethanol and hexane resulted in higher FAEE yields and conversion. This will result in 50% ethanol savings since hexane can be recycled for repetitive use when separated by distillation method. Also, the effect of hexane on FAEE yield was

not significant at lower volumetric ratios; however, its effect appears to be significant at higher volumetric ratios.

3.3.2. Effect of hexane on catalyst amount

Similar to the MW extractive transesterification reaction, 2.5% catalyst concentration resulted in the highest lipid/FAEE yield and FAEE conversion shown in Fig. 1b (Process Conditions: 4 g dry algae; 24–24 mL ethanol–hexane and 5 min reaction time) and Fig. 2b (Process Conditions: 4 g dry algae; 24–24 mL ethanol–hexane and 5 min reaction time). The yield and conversion rates are higher than the BD method but slightly lower than the MW extractive transesterification reaction with ethanol alone. In MW extractive-transesterification reaction, ethanol served as the solvent for lipid extraction while in this method hexane acted as mediator to provide for improved mass transfer between the catalyst and ethanol which enhances the transesterification reaction. In another study, the presence of hexane as a solvent in transesterification reaction was reported to have no effect on the *in situ* transesterification reaction of marine macroalgae lipids. Optimum reaction conditions of 300:1 methanol-to-oil molar ratio, 1% catalyst concentration, 60 °C reaction temperature and 11 h reaction time, resulting in a methyl esters yield of 17.1% were reported in that study. While hexane may not affect the transesterification reaction, its presence may provide for improved mass transfer between the oils and ethanol due to its miscibility with ethanol (Sanchez et al., 2012).

3.3.3. Effect of hexane on reaction time

The two described (in Section 3.2.3) extractive mechanisms take place in this reaction but dominated by diffusive extraction due to the presence of hexane, a solvent. Similar to the MW extractive transesterification reaction, a reaction time of 6 min was found to be sufficient for this reaction (Fig. 1c; Process Conditions: 4 g dry algae; 2% catalyst and 24–24 mL ethanol–hexane and Fig. 2c; Process Conditions: 4 g dry algae; 2% catalyst and 24–24 mL ethanol–hexane). The presence of hexane did show a significant effect on the lipid/FAEE yields at lower reaction times. The lipid/FAEE yield was lower than BD method at lower reaction times. This can be attributed to the lower available heat transfer as well as mass transfer rates at low solvent temperatures. Unlike the ethanol only reaction, the reaction temperature was maintained below 70 °C in this reaction (Fig. 3b). It is important to select a solvent with high extracting power and strong interaction with the microwaves and the analyte (oils). Polar molecules and ionic solutions (typically acids) strongly absorb microwave energy because of the permanent dipole moment. On the other hand, when exposed to microwaves, non-polar solvents such as hexane will not heat up but they will contribute to mass transfer of analytes. Solvents that are transparent to microwaves do not heat up under irradiation. Hexane is an example of microwave-transparent solvent whereas ethanol is an excellent microwave-absorbing solvent. Therefore a combination of these two (polar and non-polar solvents) can be used in microwave assisted extraction if mass/heat transfer controlled reactions need to be achieved. This is the main concern currently when using microwave irradiation as a heat source due to rapid heating of solvent mixtures which may cause product degradation and byproduct formation. However, it is yet to be verified if the presence of hexane has caused any undesired product formation. It can be noted from Figs. 1c and 2c that the lipid/FAEE yield and conversion rate for MW-hexane method are slightly lower than MW only method, however, the amount of ethanol used in this method was only 50% of MW only method.

3.3.4. Effect of hexane on microwave power

The microwave power effect was significant on the FAEE conversion since lower microwave power was not able to overcome the effect of non-polarity of hexane and complete the transesterification reaction. A portion of the microwave power is utilized to raise the temperature of solvent hexane which is actually caused by convective heat transfer from ethanol. The lipid yield was affected by the power levels but the FAEE conversion rates were not affected similar to microwave only method (Fig. 1d; Process Conditions: 4 g dry algae; 2% catalyst; 24–24 mL ethanol–hexane and 5 min reaction time and Fig. 2d; Process Conditions: 4 g dry algae; 2% catalyst; 24–24 mL ethanol–hexane and 5 min reaction time).

3.4. Summary

The significance of various reaction parameters can be explained as follows. As shown in Figs. 1 and 2, the microwave irradiation process produced higher lipid yields (17.8%) at algae biomass to methanol ratio of 1:12 (wt.:vol); 1% catalyst (17.1%); 6 min reaction time (20.1%); and 350 W power (18.34%) individually when other reaction parameters were held constant. Similarly, maximum FAEE conversions were 96.1%, 95.2%, 96.2% and 95.2% at algae biomass to methanol ratio of 1:12 (wt.:vol); 2% catalyst, 5 min reaction time and at 350 W microwave power, respectively when other reaction parameters were held constant. The above results indicate that optimum reaction conditions need to be established for maximum lipid recovery and biodiesel conversion. For example, it can be noticed that 1% catalyst test results in 17.1% lipid yield but the FAEE conversion was significantly lower (82.7%). Therefore, an increase in catalyst percentage (2%) results in higher FAEE conversion (95.2%). When the algae biomass to methanol ratio is considered, 1:12 resulted in both higher lipid recovery (17.8%) and FAEE conversion (96.1%). Prolonged reaction times over 5–6 min did not positively affect the lipid yields or FAEE conversions and the same was true for power levels above 350 W. the amount of power to be supplied will be limited by the volume of reaction mixture.

From these experimental studies, it can be concluded that the optimum process conditions for microwave enhanced extractive-transesterification reaction are: dry algae to ethanol ratio of 1:500 (oil to ethanol molar ratio), NaOH concentration of 2.0% (wt.) and the reaction time of 6 min at a reaction temperature around 78 °C. For the method with hexane as solvent, the optimum conditions are: dry algae to ethanol ratio of 1:250 (oil to ethanol molar ratio) and 1:1 ethanol to hexane volumetric ratios, NaOH concentration of 2.5% (wt.) and the reaction time of 6 min at a reaction temperature of 60 °C.

3.5. FAEE analysis

FAEE analysis for algal biodiesel has shown the following major long chain fatty acid compounds: Lauric (C12:0); Myristic (C14:0); Palmitic (C16:0); Palmitoleic (C16:1); Stearic (C18:0); Oleic (C18:1); Linoleic (C18:2); Linolenic (C18:3); Archidic (C20:0); Behenic (C22:0); Erucic (C22:1); and Lignoceric (C24:0). The composition of these compounds as percentages for different reaction conditions are shown in Tables S1 and S2. Fig. 4a and b show the compositions of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Microwave extraction without hexane has extracted more polyunsaturated fatty acids (PUFAs) in the range of 45–75% whereas the extraction with hexane has shown a considerable portion (50–68%) of saturated fatty acids (SFAs). The fatty acid composition has a high percentage of C20, C21 and C22 carbon chain compounds which are typically found in algal lipids and not in other vegetable and seed oils (Chisti, 2007). High percentages of polyunsaturated compounds with microwave irradiation can be attributed to higher reaction temperatures (70–80 °C) compared to the

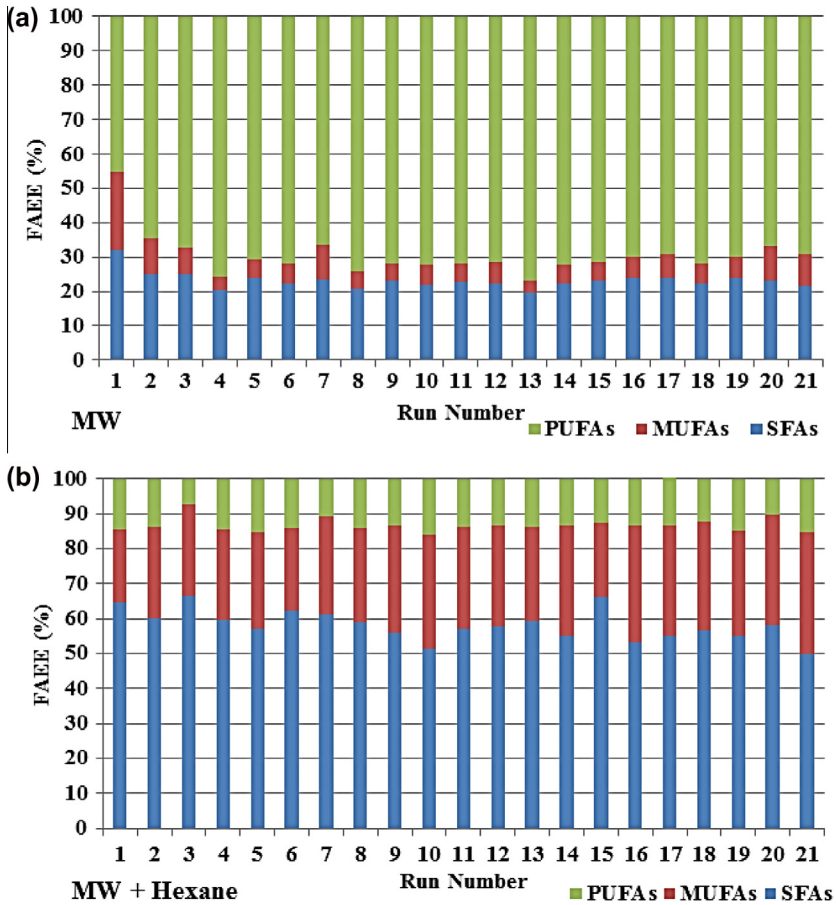

Fig. 4. Comparison of FAEE composition for: (a) Microwave; and (b) microwave-hexane extractions.

reaction mixture with hexane as solvent (57–65 °C). Although the measured temperatures (bulk reaction mixture) are not that high, the localized superheating at microscales (molecular levels) with ethanol cause the reaction products to have higher composition of monounsaturated and polyunsaturated fatty acids. The lipids/FAEEs contain the pigments and other impurities from algal biomass which can be washed with KCl solution to remove the non-lipid components and pigments as explained elsewhere (Makri et al., 2011; Bellou and Aggelis, 2012).

3.6. SEM analysis

The residual wet algae from the extracted samples were analyzed under scanning electron microscope (SEM). The samples were air dried for a week and then mounted on aluminum stubs using a conductive adhesive and coated with 20 nm Pt. The samples were examined with JEOL JSM-6500F equipment operated at 5 kv. Fig. S2 shows the SEM images of the raw algae powder cell before extraction and after microwave extraction with and without hexane, respectively. It can be noted that the algal cell became smaller, exhausted and notorious cracks are noticed after extraction due to release of lipids stored in the biological matrix (Chen et al., 2012).

4. Conclusions

This study demonstrated that extractive-transesterification of dry algal biomass can be performed in lieu of using separate extraction and transesterification steps. The yields of the microwave enhanced extractive-transesterification were higher compared to the conventional Bligh and Dyer method which indicates the significant chemical and energy savings that can be achieved by the novel methods. Hexane as a solvent reduced the reaction condition severities but produced comparable lipid and FAEE yields. This study concludes that extractive-transesterification with microwave irradiation may provide a sustainable alternative to the existing two-step extraction and transesterification reactions due to reduced chemical and energy consumption.

Acknowledgements

This research project was supported in part by The United States Environmental Protection Agency (USEPA) under the Grant number SU835519. This research was also supported by the Office of Research and Economic Development (ORED), the Bagley College of Engineering (BCoE), and the Department of Civil and Environmental Engineering (CEE) at Mississippi State University. Authors thank Linda McFarland and Amanda Lawrence for their assistance with Bligh and Dyer method and SEM sample analysis, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.01.026.

References

Araujo, G.S., Matos, L.J., Fernandes, J.O., Cartaxo, S.J., Gonçalves, L.R., Fernandes, F.A., Farias, W.R., 2013. Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. Ultrason. Sonochem. 20, 95–98.

Aresta, M., Dibenedetto, A., Carone, M., Colonna, T., Fragale, C., 2005. Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. Environ. Chem. Lett. 3, 136–139.
ASTM-D 6584, 2000. Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography. ASTM International, West Conshohocken, PA, United States.
Bellou, S., Aggelis, G., 2012. Biochemical activities in *Chlorella* sp. and *Nannochloropsis salina* during lipid and sugar synthesis in a lab-scale open pond simulating reactor. J. Biotechnol. 164, 318–329.
Bligh, E.G., Dyer, W.M., 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
Boocock, G.B., Konar, S.-K., Mao, V., Lee, C., Buligan, S., 1998. Fast formation of high-purity methyl esters from vegetable oils. J. Am. Oil Chem. Soc. 75, 1167–1172.
Chen, L., Liu, T., Zhang, W., Chen, X., Wang, J., 2012. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour. Technol. 111, 208–214.
Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 26, 126–131.
Demirbas, A., 2009. Production of biodiesel fuels from linseed oil using methanol and ethanol in non-catalytic SCF conditions. Biomass Bioenergy 33, 113–118.
Ehimen, E.A., Sun, Z.F., Carrington, C.G., 2010. Variables affecting the in situ transesterification of microalgae lipids. Fuel 89, 677–684.
Gude, V.G., Grant, G.E., Patil, P.D., Deng, S., 2013a. Biodiesel production from low cost and renewable feedstock. Cent. Eur. J. Eng. 3 (4), 595–605.
Gude, V.G., Patil, P., Martinez-Guerra, E., Deng, S., Nirmalakhandan, N., 2013b. Microwave energy potential for biodiesel production. Sustainable Chem. Processes 1 (5), 1–31.
Haas, M.J., Wagner, K., 2011. Simplifying biodiesel production: the direct or in situ transesterification of algal biomass. Eur. J. Lipid Sci. Technol. 113, 1219–1229.
Haas, M.J., Scott, K.M., Marmer, W.N., Foglia, T.A., 2004. In situ alkaline transesterification: an effective method for the production of fatty acid esters from vegetable oils. J. Am. Oil Chem. Soc. 81 (1), 83–89.
Johnson, M.B., Wen, Z., 2009. Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. Energy Fuels 23, 5179–5183.
Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol. 86, 1059–1070.
Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y., Oh, H.M., 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 101, S75–S77.
Li, P., Miao, X., Li, R., Zhong, J., 2011. In situ biodiesel production from fast-growing and high oil content chlorella pyrenoidosa in rice straw hydrolysate. J. Biomed. Biotechnol. 2011. Article ID 141207, 8 pages.
Makri, A., Bellou, S., Birkou, M., Papatrehas, K., Dolapsakis, N.P., Bokas, D., Papanikolaou, S., Aggelis, G., 2011. Lipid synthesized by micro-algae grown in laboratory- and industrial-scale bioreactors. Eng. Life Sci. 11 (1), 52–58.
Mondala, A., Liang, K., Toghiani, H., Hernandez, R., French, T., 2009. Biodiesel production by in situ transesterification of municipal primary and secondary sludges. Bioresour. Technol. 100, 1203–1210.
Mulbry, W., Kondrad, S., Buyer, J., Luthria, D., 2009. Optimization of an oil extraction process for algae from the treatment of manure effluent. J. Am. Oil Chem. Soc. 86, 909–915.
Patil, P.D., Gude, V.G., Mannarswamy, A., Deng, S.G., Cooke, P., Munson-McGee, S., Rhodes, I., Lammers, P., Nirmalakhandan, N., 2011. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. Bioresour. Technol. 102, 118–122.
Qian, J., Wang, F., Liu, S., Yun, Z., 2008. In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. Bioresour. Technol. 99, 9009–9012.
Refaat, A.A., 2010. Different techniques for the production of biodiesel from waste vegetable oil. Int. J. Environ. Sci. Technol. 7, 183–213.
Sanchez, A., Maceiras, R., Cancel, A., Rodríguez, M., 2012. Influence of *n*-hexane on *in situ* transesterification of marine macroalgae. Energies 5, 243–257.
Shiu, P.J., Gunawan, S., Hsieh, W.H., Kasim, N.S., Ju, Y.H., 2010. Biodiesel production from rice bran by a two-step in situ process. Bioresour. Technol. 101, 984–989.
Velasquez-Orta, S.B., Lee, J.G.M., Harvey, A., 2012. Alkaline in situ transesterification of *Chlorella vulgaris*. Fuel 94, 544–550.
Xu, R., Mi, Y., 2011. Simplifying the process of microalgal biodiesel production through in situ transesterification technology. J. Am. Oil Chem. Soc. 88, 91–99.
Zanin, F.B., Macedo, A., Vinicios, M., Archilha, L.R., Wendler, E.P., Dos Santos, A.A., 2013. A one-pot glycerol-based additive-blended ethyl biodiesel production: a green process. Bioresour. Technol. 143, 126–130.