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Short Communication

Ionic liquid as a promising biobased green solvent in combination with microwave irradiation for direct biodiesel production

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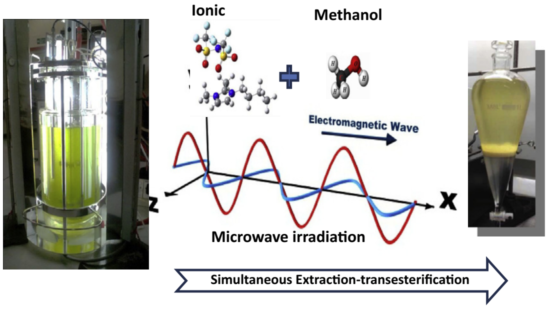
HIGHLIGHTS

- Wet microalgae was directly transesterified in ionic liquid (IL) assisted by MW.
- Methanol-IL combination increased cell fractal dimension proven by FESEM images.
- Conversion yield reached 36.79% (per dried biomass) using [EMIM][MeSO$_4$]-methanol.
- IL as co-solvent in MW enhanced cell disruption releasing more lipids.
- Subsequently the lipids are rapidly transesterified to biodiesel in IL under MW.

GRAPHICAL ABSTRACT



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ABSTRACT

The wet biomass microalgae of *Nannochloropsis* sp. was converted to biodiesel using direct transesterification (DT) by microwave technique and ionic liquid (IL) as the green solvent. Three different ionic liquids; 1-butyl-3-metyhlimidazolium chloride ([BMIM][Cl], 1-ethyl-3-methylimmidazolium methyl sulphate [EMIM][MeSO$_4$] and 1-butyl-3-methylimidazolium trifluoromethane sulfonate [BMIM][CF$_3$SO$_3$]) and organic solvents (hexane and methanol) were used as co-solvents under microwave irradiation and their performances in terms of percentage disruption, cell walls ruptured and biodiesel yields were compared at different reaction times (5, 10 and 15 min). [EMIM][MeSO$_4$] showed highest percentage cell disruption (99.73%) and biodiesel yield (36.79% per dried biomass) after 15 min of simultaneous reaction. The results demonstrated that simultaneous extraction-transesterification using ILs and microwave irradiation is a potential alternative method for biodiesel production.

1. Introduction

Environmental friendly concept has placed a renewed emphasis on careful solvent selection and search for less harmful alternatives. Increasing, solvent use during manufacture is viewed in terms of avoiding the costs associated with disposal, legal liabilities and regulatory constraints (Owen et al., 2010; McCurdy et al., 2014). Over the years, our society has become more concerned about environmental problems. This awareness has led to interest in cleaner and greener technologies which reduce or even eliminate the use of hazardous and toxic materials (Cheng et al., 2014; Parmar et al., 2011; Chen et al., 2015).

Ionic liquids (ILs) are molten salts with low melting temperatures or below the ambient temperature. ILs are comprised of relatively large asymmetric organic cations coupled with smaller

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inorganic or organic anions (Choi et al., 2014). The cation is generally composed of a nitrogen containing ring structure (e.g. imidazolium or pyrinidine) to which a broad range of functional side groups can be attached (Young et al., 2010). Ionic liquids (ILs) have been touted as "green" alternatives to traditional molecular solvents because of their fascinating properties such as non-volatile, non-flammable, recyclability, good dissolving ability, excellent microwave absorbance, thermally stable solvents, relatively undemanding and inexpensive to manufacture (Jonathan et al., 2001).

There has been increasing interest in microwave irradiation for lipid extraction and biodiesel production from microalgae biomass. Microwave irradiation involves rapid volumetric heating, producing higher product yields, lower quantities of side products, efficient, and does not require samples be devoid of water (Wahidin et al., 2014; Lee et al., 2010; Matthieu et al., 2008; Patil et al., 2011; Wahlen et al., 2011). Hemwimon et al. (2007) reported that rapid generation of heat and pressure within the biological system forced out compounds from the biological matrix in the microwave-assisted extraction system. It also enhanced the quality of extract with better target compound recovery (Patil et al., 2011). To the best of our knowledge, the combined use of ionic liquid as green solvent in microwave system for direct biodiesel production has not been explored yet. Thus, in this study a novel simultaneous extraction-transesterification technique in explored using a combination of ionic liquids and microwave heating; where microwave irradiation offers energy saving and rapid heating in which molecules are directly heated through the interaction between microwave energy and molecular dipole moments of the starting materials. In this part of the study DT for biodiesel production was performed using different types of solvents at three level reaction times. In order to identify the structural impact of the microwave irradiation with organic solvents (hexane and chloroform) and ILs (IL1, IL2 and IL3) at cellular scale, FESEM analyses were performed to determine whether cell walls were disrupted after DT.

2. Methods

2.1. Microalgal cultures and cultivation

Marine Heterokontophyta, *Nannochloropsis* sp. was obtained from the culture collection of Borneo Marine Research institute (BMRI), Universiti Malaysia Sabah, Malaysia. *Nannochloropsis* sp. was cultivated in a 5 L photobioreactor at 23 ± 0.5 °C, pH 8 ± 0.2 under a light intensity of 100 μmol m^{-2} s^{-1} with an 18:06 h light/dark cycle. The *Nannochloropsis* sp. cells were cultured in sterilized seawater enriched with Walne's medium (Wahidin et al., 2013).

2.2. Simultaneous extraction-transesterification reaction

The simultaneous reaction in the microwave irradiation was operated at 65 °C and 350 rpm at three different reaction times of 5, 10 and 15 min using five types of solvents; hexane, chloroform, 1-butyl-3-metyhlimidazolium chloride; [BMIM][Cl] defined as IL1, 1-ethyl-3-methylimmidazolium methyl sulphate; [EMIM][$MeSO_4$] defined as IL2 and 1-butyl-3-methylimidazolium trifluoromethane sulfonate; [BMIM][CF_3SO_3] defined as IL3. The DT method was carried out with 1 gram of wet microalgae biomass (water content, 80 wt%) charged into a three necked mini reactor. The output of microwave power was set at 700 watt during the heating process. A MAS-II microwave synthesis workstation, 1000 W (Shanghai Sineo Microwave Chemistry Technology, Co. Ltd., China) with an operational frequency of 2450 MHz was used so as to allow the extraction of lipid and the transesterification to take place simultaneously (Patil et al., 2011; Cheng and Zheng, 2013). The mixture was cooled at room temperature and centrifuged at 4000 rpm for 5 min to separate the methanol-IL and biodiesel phases. The 2 factor general factorial design was used to determine the effect of simultaneous extraction-transesterification reaction time and type of co-solvents on the cell disruption and also the biodiesel yield. An ANOVA analysis was performed to determine the *p*-values.

2.3. Disruption quantification of cell walls

The number of intact cells was counted after 5–15 min using Neubauer Haemocytometer. The control value was taken at the start of each experiment and also samples were taken after each reaction was completed. A microscope (Olympus CX31, Japan) was employed in the brightfield configuration, using between 10× and 40× objective. The microscope was equipped with the auxiliary Olympus Xcam-Alpha CCD camera (Olympus U-CMAD 3, T7 Tokyo, Japan) using DigiAcquis image software. The total cell disruption, (C_t) at time *t*, was measured in terms of the ratio of the average number of intact cells counted after a certain time (N_t) to the initial counts of intact cells (undamaged cell) (N_o) at $t = 0$. The total cell disruption, C_t was calculated using the following Eq. (1):

$$C_t\ (\%) = \left(1 - \frac{N_t}{N_o}\right) \times 100 \tag{1}$$

where the ratio N_t/N_o indicates the undamaged and intact cell probability and C_o indicates the total number of cell disrupted for the control sample (Halim et al., 2012). The initial disruption of microalgae was counted for the control sample value and the percentage of total cell disruption was calculated according to Eq. (2):

$$C_o\ (\%) = \left(\frac{C_o}{C_o + N_o}\right) \times 100 \tag{2}$$

2.4. Surface structure of microalgae biomass

The microalgae biomass surface structure before and after the lipid extraction was observed and characterized by field emission scanning electron microscopy (JSM-6071F, Jeol, USA Inc, USA). FESEM sample preparation includes chemical fixation using modified method with 2.5% glutaraldehyde in sodium phosphate buffer for 4 h and the samples were post-fixed in 1% of osmium tetroxide (OsO_4) solution at room temperature. The samples were then rinsed three times and washed with fresh buffer. Samples were dehydrated in ethanol series (three times) and critically point dried. The samples were glued to a stub, sputter-coated with gold and examined under FESEM at 10 kV (Wahidin et al., 2014).

2.5. Fatty acid methyl esters (FAMEs) composition analysis

The compositions of FAMEs contained in the biodiesel were analyzed using a gas chromatography (GC-7820A, Agilent, USA) system equipped with a flame ionization detector (FID) and with a HP-88 Agilent column (length: 60 m, ID: 0.25 mm, Film: 0.20 mm). Nitrogen gas (99.9% purity) was employed as the carrier gas with a column flow rate of 1 ml/min. The injection volume and temperature were 1 μL and 220 °C respectively with a split ratio of 1:50. The initial oven temperature was 80 °C held for 3 min and then increased to 220 °C at the rate of 5 °C/min, and held at 220 °C for 5 min.

3. Results and discussion

3.1. Microscopic images of microalgae cell wall using different solvents after DT

The microscopic images of *Nannochloropsis* sp. cells before the direct transesterification are completely intact and mostly spherical in shape were taken (not shown). The presence of insoluble non-hydrolyzable biopolymers in the outer cell walls of *Nannochloropsis* sp. are unusually resistant to drastic non-oxidative chemical treatments (Allard and Templier, 2000). These biopolymers, termed algaenans (Allard and Templier, 2000; Gerlin et al., 1997) have a highly aliphatic structure that serves as a protective coat surrounding the cells (Mendes-Pinto et al., 2001). Microalgae fragments collected after simultaneous extraction-transesterification at three different reaction time (5, 10 and 15 min) were observed by FESEM in order to identify the structural impact of the microwave irradiation with organic solvents (hexane and chloroform) and ionic liquids (IL1, IL2 and IL3).

Nannochloropsis sp. cell structure appears to be deformed exhibited by the shrinkage observed when the microalgae were extracted with methanol-hexane co-solvent assisted by microwave heating at 5 min. The images also illustrate a minimum level of damage to the cell walls structure which is associated with noticeable leakage of cell content and it is assumed that disruption occurs as a simple puncture when the reaction time were prolonged from 10 to 15 min. This is due to the non-polarity of hexane which does not allow combination of methanol-hexane heat up under microwave effect. When the reaction time was increased from 10 to 15 min, that microalgae cell wall is still present but it is drastically damaged and ruptured. Methanol-chloroform is most frequently used as organic solvent mixture for lipid extraction and also for co-solvent mixture for transesterification process from microalgae biomass. Extraction lipid using chloroform is fast and quantitative (highly disrupted cell walls) compared with hexane when assisted with microwave radiation.

The microscopic image of microalgae cell walls were taken (not shown); when the reaction used IL1 ([BMIM][Cl]) as co-solvent system. After 5 min of reaction, it can be noted that the microalgal cell walls become exhausted and badly damaged and the degree of cell walls damage was higher than those placed in chloroform and hexane. Ultimately, the more prolonged the reaction time (10–15 min) the higher the extent of disruption of the cell walls under microwave irradiation, the ionic liquid receives large amounts of thermal energy thus further disrupts the microalgal cells. When the cells received this energy, local internal superheating occurred lead to instantaneous temperature rise within the matrices and rapid pressure increase thus rupturing the cell walls or the membrane structure of microalgae.

Increasing the reaction time of simultaneous extraction-transesterification to 10 min resulted in a greater damage to the cell walls thus releasing more lipids. The combination of microwave irradiation and mixture of methanol and ionic liquid as solvents are more effective in destroying the cells walls during a short period of time. Prolonged reaction time also has the tendency to disrupt the permeability of the cell walls, and the lipids released were further converted to biodiesel resulting in higher yield of biodiesel. The cell walls are mostly completely broken; leaving no intact cells and transformation from intact cells to the broken cells is clearly visible. These microscopic images indicated that the methanol-IL2 mixture allowed penetration of microwave irradiation which could significantly destroy the cell walls very rapidly in 5 min releasing high amounts of lipids which subsequently reacted with solvent to form biodiesel thus promotes simultaneous extraction-transesterification.

3.2. The percentage of cell disruption

Fig. 1a shows the average percentage cell disruption as a function of reaction time for all the co-solvents system used in the direct transesterification. The disruption level of microalgae cell walls after simultaneous extraction-transesterification process was higher when microwave reaction time was increased from 5 to 15 min. As depicted in Fig. 1a, the rate of intact cell count reduction appeared to continuously decrease with increase in the reaction time for all the co-solvents used. The error bars show the standard deviation of the mean for total cell disrupted. IL2 co-solvents system was found to be the most effective co-solvent in disrupting *Nannochloropsis* sp. cell. It was seen that at 5 min reaction time the cell disruption achieved was 93.88%. A maximum of 99.73% cell disruption was achieved after 15 min of reaction, which was slightly better than 10 min with an increase of only 5.85%. The efficiency of the IL's in disrupting cell walls can be arranged in the following order IL2 > IL3 > IL1.

3.3. Biodiesel yields

Fig. 1b shows the effect of DT or simultaneous extraction-transesterification reaction time on biodiesel yield for each solvent used. The prolonged reaction time assisted the co-solvent system and methanol to disrupt the cell walls, releasing more lipids as well as accelerate the transesterification reaction. Maximum biodiesel

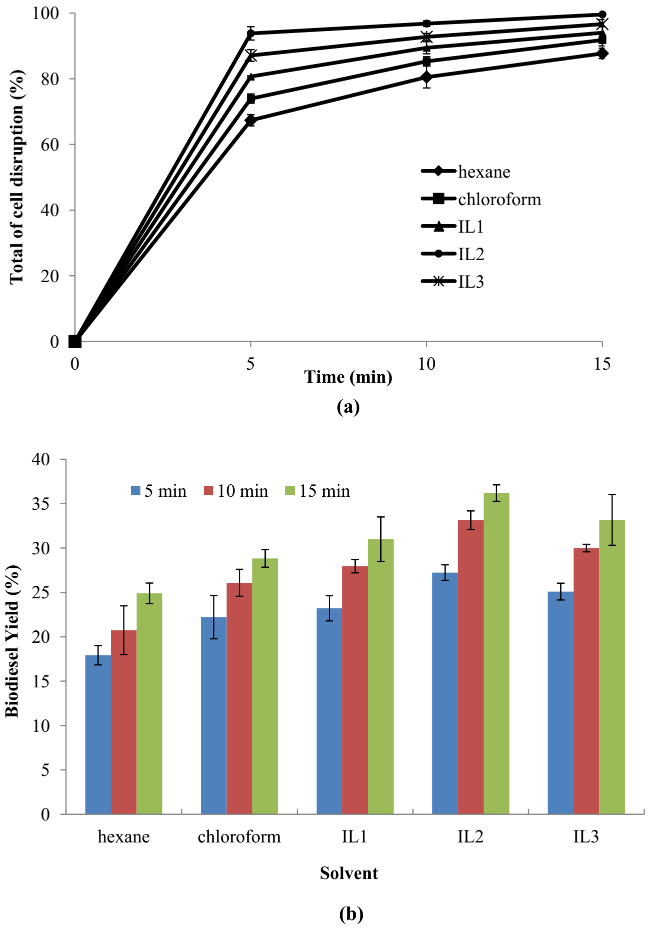

Fig. 1. (a) Percentage of cell wall disruption at different reaction times; (b) Biodiesel yield at different reaction time for the different type of solvents.

Table 1
Composition of FAMEs (% of total FAMEs) from DT using different types of co-solvent at 15 min of reaction time.

Fatty acid methyl esters composition	Hexane	Chloroform	IL1	IL2	IL3
C14:0	3.54	3.12	4.66	5.33	5.09
C16:0	12.62	8.19	12.16	19.01	19.03
C16:1	26.51	21.04	31.25	37.64	34.28
C18:0	5.92	6.16	8.92	3.26	5.02
C18:1n9c	22.11	23.25	16.88	13.28	13.25
C18:2n6c	23.52	26.63	24.15	21.48	23.34
C18:3	2.12	6.55	0	0	0
C20:0	3.66	5.06	0	0	0

yield was obtained at 15 min when IL2 was used as co-solvent (36.79%) and when the reaction time was increased from 5 to 10 and 15 min, the biodiesel yield obtained increased from 29.22% to 33.13% and 36.79%, respectively. The maximum biodiesel yields under hexane and chloroform co-solvent system were only 24.89% and 28.82% respectively at 15 min of reaction time as shown in Fig. 1b. The conversion yield reached a saturated value after a specific time, even if the reaction time was increased, indicating that the reaction has almost approached equilibrium.

The biodiesel yield was higher when ionic liquid was used as the co-solvent system compared to organic co-solvents (hexane and chloroform) because the higher rate of cell disruption achieved allowed the lipids to be transesterified immediately to biodiesel. In terms of methanol-ionic liquid content, the limiting step in the direct transesterification process appeared to be the extraction of the lipid from the microalgae biomass. The mixture of solvents required a certain amount of the polar covalent molecule. The methanol-ionic liquid was able to extract the lipids rapidly from the biomass under microwave irradiation. In terms of efficiency the combination of co-solvent system are as follows; IL2 > IL3 > IL1 > chloroform > hexane. The ANOVA analysis was performed and both factors; simultaneous extraction-transesterification reaction time and type of co solvent were found to be significant with p values less than 0.0001.

3.4. FAMEs composition

The composition of FAMEs obtained from the DT using the combination of microwave irradiation and the various solvents are summarized in Table 1. The content of the FAMEs in the final product was calculated quantitatively by comparing the peak area of FAMEs to the peak of the external standard. The major composition of FAMEs; palmitic (C16:0, 10–35%), palmitoleic acid (C16:1, 19.61–37.77%) oleic acid (C18:1n9c, 11–23%) and linoleic acid (C18:2n6c, 20.31–26.63%) were found in all samples produced. From the GC peak, it was also observed that the biodiesel also consisted of methyl ester of myristic acid (C14:0) and stearic acid (C18:0) in minor quantities in all samples. Table 1 shows a lower percentage of methyl esters having a carbon chain of arachidic acid (C20:0) was obtained when using hexane and chloroform as the co-solvents. An increase in reaction time has a positive effect on the amount of FAMEs that can be produced from the wet microalgae biomass. However when all ILs were used as the co-solvent, a complete absence of arachidic acid methyl ester (C20:0) and linolenic acid methyl ester (C18:3) were observed. The long chain arachidic acid methyl ester (C20:0) tend to break down to shorter chain of FAMEs under the influence of IL's and microwave.

4. Conclusion

The presence of methanol-ILs in DT disrupted the rigid cell walls efficiently, rapidly releasing the lipids which were subsequently transesterified into FAMEs under microwave-irradiation. The microscopic images depicted the structure of microalgae cell walls was totally ruptured after exposure to irradiation wave and ILs as a solvent. The small amount of IL to the solvent mixture can induce dramatic changes in the heating characteristics of the solvent under microwave-irradiation thus improving the overall dielectric properties of the reaction medium. Methanol-ILs interacted very efficiently with microwave through ionic conduction mechanism and was quickly heated without any considerable pressure increase.

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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.2016.01.084.

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