Research Article

Lipid extraction from oleaginous microorganism with electrochemical method†

Lipid extraction with electrochemical method

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ABSTRACT

In this study, electro-treatment was used to disrupt the microbe cells for facilitating the lipid extraction from oleaginous yeast. Two types of the electrochemical assisted lipid extraction were performed. One was that electrochemical method was used as pre-treatment to break cells, and thereafter solvents (hexane, methanol, and mixture of chloroform: methanol) were used to dissolve the lipid, defined as electrochemical pre-treatment followed extraction. The extraction efficiency reached 43% and 92% in the electrochemical involved treatment with hexane and chloroform: methanol, respectively. In addition, simultaneous electrochemical treatment and lipid extraction was also conducted. In the process, the biomass dispersed in the solvents was subjected to the electrochemical treatment for extraction, defined as simultaneous electrochemical treatment and lipid extraction. The highest lipid extraction efficiency was 92.17% w/w dry biomass obtained at 48 h extraction time with chloroform: methanol:water as solvents. Electrochemical treatment showed great potential in lipid extraction as it somewhat reduced the toxic chloroform utilization. Increase of current of electro-treatment has led to the increase of saturation degree of the biodiesel converted from lipid extracted with electro-treatment. It was found that the current of electro-treatment should be kept below 0.4 A in order to avoid the impact on biodiesel property.

Practical applications: The study provided a way of lipid extraction from oleaginous microorganism with electrochemical method. It has great potential to be utilized in lipid extraction from microorganism.

Keywords: lipid extraction; wastewater sludge; electrochemical; biodiesel; oleaginous microorganism

1 Introduction

Increasing attention has been paid to biodiesel production from microbial oil due to the predication of fossil fuel depletion and the increase of price of traditional feedstocks (vegetable oils and animal fat) of biodiesel production. Biodiesel can be produced from vegetable oils, animal fats, and microbial oil by a simple chemical reaction in which oil reacts with methanol in the presence of catalyst (acid or base) to form biodiesel and glycerol [10,12]. Microbial oil is accumulated by oleaginous microorganisms inside their bodies as energy source. To obtain microbial oil from oleaginous microorganisms, extraction has to be performed.

Organic solvent (mixture of chloroform and methanol with 2:1 v/v and/or 1:1 v/v, respectively) extraction is the most utilized technology for microbial oil extraction from oleaginous microorganism biomass due to its high efficiency [4,7,11]. However, there is increasing concern on the operation safety and environmental pollution due to the inflammable and toxic properties of chloroform. Supercritical fluid (mostly CO_2) lipid extraction has also been widely studied as it is a rapid, clean and cost acceptable process [13,16,17]. However, it is generally employed in small scale attributed to (1) the design of extraction condition is difficult due to the complexity of phase equilibrium between solvent/solute; (2) co-solvent is required in order to achieve high extraction efficiency; (3) the requirement of high pressure leads to high operation safety concern.

Many methods including ultrasonication, microwave, pressure, enzyme, and homogenization have been conducted to achieve microbial oil separation from cells in water [2,3,5,9,19]. These methods were aimed to obtain oil by cell disruption as oil could not dissolved in water and would float to the top of water solution. However, the methods still had to depend on organic solvent. Lipid droplets inside of the cells were not hydrophobic but hydrophilic as there was a monolayer of phospholipid with hydrophilic head towards outside, which led to a hydrophilic appearance in the lipid drop in overall. Electrochemical process is another alternative to achieve cell disruption. It was reported that microalgae cells were ruptured after electrochemical treatment and enhanced microbial oil extraction [6]. However, the study was performed on dried biomass suspended in chloroform and methanol. In fact, biomass drying is an energy and cost intensive process and thus required to be eliminated.

Therefore, the aim of this study is to investigate electrochemical treatment effect on lipid extraction from wet biomass. The parameters including current, power, electro materials, electrolytes, treatment time, and solvent types have been studied to investigate their effect on lipid extraction.

2 Materials and Methods

2.1 Biomass production

2.1.1 Wastewater sludge

In this study, the used wastewater sludge was municipal secondary wastewater sludge obtained from a local wastewater treatment plant, Québec, Canada. The sludge was allowed to settle at 4 ºC for 24 h after collection. Thereafter, centrifugation at 5000 rpm for 15 min was performed to further concentrate the sludge to obtain a high suspended solids (SS) concentration (around 100 g/L).

2.1.2 Oleaginous microorganism

The employed microorganism in the study was *Trichosporon oleaginosus* (ATCC 20509).

2.1.3 Production of pre-culture

The pre-culture medium was prepared from 30 g/L of sludge (SS concentration) and 30 g/L of yeast extract-peptone-dextrose. The medium was first sterilized at 121°C for 15 min and then inoculated with one loop full of *T. oleaginosus* preserved in malt extract agar plates. Thereafter, the medium was incubated at 28 °C and 170 rpm for 24 h prior to being used as inoculum.

2.1.4 Fermentation

A 15 L fermentor with working volume of 10 L was utilized for *T. oleaginosus* fermentation in this study. The fermentation was performed in similar way as described in our previous study [18]. The 8.5 L sludge with SS of 32 g/L was transferred to the fermentor after adjusting its pH to 12 with 4M NaOH, and then subjected to 121°C for 20 min. After the temperature of the medium was dropped to 28 °C, the medium pH was re-

adjusted to 6.5 with 2M H_2SO_4. Pre-sterilized crude glycerol (0.5 L) was then added to the fermentor followed by inoculating 1 L seed culture (pre-culture). The fermentation lasted for 48 h. Thereafter, the broth was harvested and stored for further utilization.

2.2 Determination of suspended solids concentration and lipid content

Before lipid extraction, the determination on SS concentration was performed. Ten mL (0.01 L) of fermentation broth was centrifuged at 8000 rpm for 15 min. The solid (pellets) collected from the centrifuge was washed with distilled water till a clear supernatant was obtained The solid part was then transferred to a pre-weighed aluminum cup, and subjected to 105 °C till weight constant. The SS concentration (=(W_{c+ss}-W_c)/0.01, g/L) was calculated based on the weight difference of the aluminum cup before (W_c, g) and after (W_{c+ss}, g) containing solids.

In this study, wastewater sludge was used as cultivation medium. There are inert materials (residual sludge) in the sludge, which would remain in the broth after fermentation as they were not degraded. The microbial biomass cannot be separated from the residual sludge; therefore, all the solids collected namely SS were used in the lipid extraction. The lipid extraction was performed as following: the 30 mL of fermentation broth was used to determine the total lipid content of the biomass with bead milling extraction method. The broth was subjected to centrifugation (at 8000 rpm for 15 min). The resulting solid was then washed two times with distilled water. Thereafter, 30 mL of solvent mixture of chloroform and methanol (2:1 v/v) was added to the solid and transferred to a 50 mL tube containing 6 mL of Zirconia beads (1 mm diameter). The

beads added in the tube were used to provide milling action to the cells. The 50 mL tube was then subjected to a continuous shaking in a wrist action shaker (Burrell Model 75) for 12 h. The sample was allowed to stand for phase separation. The bottom layer (lipid dissolved in chloroform) was collected after 12 h shaking. Second extraction was then conducted by addition of 20 mL of a mixture of chloroform and methanol (1:1 v/v) to the tube containing the residual biomass and beads. Shaking was performed for 2 h and the bottom layer was collected, and then united with the bottom layer obtained in the first extraction. The obtained solution was then filtrated. The filtrate was added 5 mL of water and centrifuged at 6000 rpm for 15 min. The bottom layer (in the centrifuge tube) was then transferred with pipet to a pre-weighed glass tube and subjected to nitrogen gas for solvent evaporation till the weight was constant. The lipid content was calculated as shown in Eq. 1:

$$\text{Lipid content} = (W_{t+L}-W_t)/(SS\times V)\times 100\% \quad (1)$$

Where W_{t+L} is the weight of the glass tube after evaporation (g); W_t is the weight of the empty glass tube (g); SS is the suspended solids concentration (g/L); V is the volume (30 mL) of the broth taken for lipid extraction. An average value of triplicate samples was reported in this study.

2.3 Electrochemical lipid extraction

The extraction was conducted in the setup including a power supply, peristaltic pump, and an electrochemical cell (working volume 1 L). The electrochemical cell was fitted with an anode (Ti/IrO_2) and a cathode (stainless steel) 1 cm apart. The surface areas of

the electrodes were 110 cm^2 with width 10 cm and height 11 cm. The distance between the bottom edge of the electrodes and the bottom of cell was about 2 cm. The anode and cathode were connected with positive and negative outlets of the power supply, respectively.

The electrochemical treatment lipid extraction included the electrochemical pre-treatment followed extraction and the simultaneous electrochemical treatment and lipid extraction.

In electrochemical pre-treatment followed extraction, 1 L of fermentation broth was used and the experiments were conducted at 25 °C. During the treatment, the current was adjusted to 0.3, 0.4, 0.5, or 0.6 A; recycle flow rate was set at 300, 400, or 500 mL/min; the electrolyte used was either Na_2SO_4 or NaCl with concentrations of 0.01, 0.02, 0.04, or 0.08 mol/L by directing added the chemicals to the broth, the treatment time was kept for 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min, or 120 min. After treatment, sample was taken and used to determine the lipid in the biomass.

The solvent utilized in the extraction included hexane, methanol, and methanol/chloroform (1:1 v/v). In the extraction, 30 mL sample of electrochemical treated was centrifuged at 8000 rpm for 15 min, and then the solids were washed two times with distilled water after collecting the supernatant. Adding 30 mL of solvent (hexane, methanol, or methanol/chloroform) to the washed solid, the mixture was then kept for shaking for 12 h followed by centrifugation (6000 rpm for 15 min). The solvent part was then collected and added 20 mL of solvent to the solid. The mixture was then subjected to shaking for another 2 h, and then centrifuged (6000 rpm for 15 min). The solvent was transferred to the tube which containing the solvent obtained from the first

extraction. The solvent was then centrifuged at 6000 rpm for 15 min. For hexane as solvent, the top layer was collected and subjected for evaporation with nitrogen gas. In the extraction with methanol as solvent, the supernatant was collected and subjected for evaporation with nitrogen gas. In the extraction with methanol/chloroform as solvent, the bottom layer liquid was collected and subjected for evaporation with nitrogen gas. In order to investigate if there was lipid release from the cells after electrochemical pre-treatment, the supernatant was undergone the same procedure as the solid. However, no lipid was detected in the supernatant. Thus it was considered that there was no lipid in the supernatant, and only the lipid extracted from solid was considered. The lipid extracted with different solvents was calculated by dividing the biomass with the lipid obtained after evaporated the solvent [$=(W_{et+L}-W_{et})/SS$].

The simultaneous electrochemical treatment and lipid extraction was that the wet biomass (water content around 85%) obtained after distilled water washing was mixed with methanol, or chloroform: methanol and then subjected to the electrochemical treatment. As hexane, methanol and chloroform are organic solvent and have no electroconductivity. In order to generate the current in the system, certain portion of water had to been added. However, hexane is insoluble in water, thus it had not been studied in the combined extraction system. For the simultaneous electrochemical treatment and lipid extraction, methanol and the mixture of chloroform: methanol were used as solvent. In every 1 volume of methanol or in every 1 volume of chloroform and 1 volume of methanol, 0.9 volume of tap water was added [15]. Biomass concentration in solvent was the same as that of the broth at the end of the fermentation (25.90 g/L). Similar as in the electrochemical pre-treatment process, the current was adjusted to 0.3,

0.4, 0.5, or 0.6 A; recycle flow rate was set at 300, 400, or 500 mL/min; the electrolyte used was either Na_2SO_4 or NaCl with concentrations of 0.01, 0.02, 0.04, or 0.08 mol/L by directly addition of the chemicals to the solvent. The samples were taken at 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 18 h, and 24 h. After the extraction, the sample was centrifuged at 6000 rpm for 15 min. The supernatant (solvent) was collected and subjected to evaporation with nitrogen gas. Similarly as the method used to calculate lipid extracted in the process of electrochemical pre-treatment followed extraction, lipid extraction efficiency was obtained by dividing the biomass with the lipid obtained after evaporated the solvent.

The lipid extraction efficiency with different solvents was calculated as shown in Eq. 2. The sample after electrochemical treatment without adding solvent was used as control, called plain extraction.

Lipid extraction efficiency=lipid amount extracted with different solvents/total lipid amount in the SS ×100% (2)

3 Results and discussion

3.1 Lipid extraction with bead milling

The total lipid content of SS was 41.49%+1.46% w/w (0.4149 g lipid was obtained from 1 g of biomass in dry basis), which was determined by utilization of chloroform: methanol 2:1 (v/v) as solvent under bead milling extraction. Based on this value, lipid extraction efficiency with other solvents was calculated with Eq. 2.When utilization of hexane, methanol, and chloroform: methanol (1:1 v/v) as solvents to extraction lipid from

untreated (without electrochemical treatment) biomass, the lipid extraction efficiency was 92.17%±3.13% (0.3824 g lipid was extracted from 1 g biomass in dry basis) for hexane, 49.68%±2.14% (0.2061 g lipid was extracted from 1 g biomass in dry basis) for methanol, and 99.68%±3.78% (0.4136 g lipid was extracted from 1 g biomass in dry basis) for chloroform: methanol (1:1 v/v). When hexane was used under bead milling process to extract lipid, around 92.17% lipid could be extracted (only 26.33% was extracted without beads). Bead milling could somewhat disrupt the cell walls, cell membrane as well as the monolayer phospholipid covering lipid droplets, and let the hexane (non polar) get contacting with lipid droplet (non polar) and hence extract the lipid out. The disruption through bead milling in 14 h was limited. When bead milling time can be increased, hexane as solvent could also extract all the lipids (100% extraction efficiency) from SS. When methanol was used as solvent to extract lipid from SS under bead milling process, just around half of the lipid (efficiency is 49.68%±2.14%) was extracted, and was only 15.93% lipid was extracted without the presence of beads. It was due to that methanol was polar solvent and could partially dissolve the lipid droplets (non polar). Chloroform and methanol with volume to volume ratio of 1:1 was as efficient as those of 2:1, which extracted all the lipids from SS when bead milling was performed (only 67.45% lipid was extracted without the presence of beads). Similar as mentioned, bead milling helped the cell wall, cell membrane, and monolayer phospholipid disruption. Chloroform (non polar) thus could dissolve and extract lipid droplets. The mixture of chloroform: methanol (regardless of the volume ratio) displayed great advantage in lipid extraction. But the usage of chloroform has great concern due to its reverse environmental effect. In fact, the mixture of hexane and methanol could also extract all the lipids from SS under bead

milling as hexane and chloroform have the similar function (get close to lipid droplets and dissolve them) in the extraction. In addition, providing pre-treatment on biomass before extraction could also be a way to help recovery all the lipid from SS with hexane or methanol.

3.2 Electrochemical pre-treatment followed extraction

3.2.1 Current effect on the extraction

Electroporation has been used mainly to introduce molecule into cells [1,8]. To achieve the transportation across the cell membrane, in fact, electroporation creates transient holes in cell membranes. It indicates that electro-treatment through providing electric field on the cells could disrupt cell membrane. It was reported that some organelles and cytosolic material had been detected to be released from cells after electroporation [14]. It suggested that electroporation was capable of breaking cells to release intercellular products. Thus, in order to disrupt cells and enhance the recovery of intercellular lipid, direct current (0.3, 0.4, 0.5, and 0.6 A) electric field was applied to the microbe cells in this study. Electroporation was used as pre-treatment to disrupt the cell; thereafter, the treated cells were subjected to solvent (hexane, methanol, chloroform:methanol).

The current used in the treatment included 0.3, 0.4, 05, and 0.6 A. During the treatment (0.5 A and 0.6 A), small bubbles were observed which would be due to that the water was decomposed to H_2 and O_2. In addition, temperature of the solution was slightly increased from 0.7 to 1 °C. The lipid extraction efficiency with different solvent after

treated for different time was shown in Table 1. For hexane as solvent, the extraction efficiency was around 40% to 43% under current 0.3 to 0.6 A, respectively, which was 31.42±1.58% in the control (solo hexane no pre-treatment) extraction. For methanol as solvent, the efficiency was around 22% to 27% under current 0.3 to 0.6 A, respectively, which was 17.88±0.45% in the control (solo methanol no pre-treatment) extraction. For chloroform: methanol as solvent, the efficiency was round 84% to 88% under current 0.3 to 0.6 A, respectively, which was 79.26±2.47% in the control (solo chloroform:methanol no pre-treatment) extraction.

The results showed that variation of current (0.3, 0.4, 0.5 and 0.6 A) has no significant impact on the extraction efficiency. It suggested that electro-treatment with current 0.3, 0.4, 0.5, and 0.6 A on cells didn't provide significantly contribution on cell disruption to release the intercellular lipid. Largely increase in the current might be a way to improve the lipid extraction efficiency, but it would also increase O_2 production as it was observed that bubbles generated during electro-treatment (0.5 A and 0.6 A). The produced O_2 can oxidize unsaturated fatty acids to saturated ones and hence increase the saturation degree of the lipid. It would lead to high viscosity in the biodiesel produced from this lipid. In fact, it has been observed that when the current was greater than 0.5 A, oxidation of lipid occurred (Table 2) in this study,, which should be prevented.

In addition, it was found that with electric pre-treatment, extraction was enhanced compared to the control (hexane, methanol, and chloroform:methanol plain solvent extraction) as the highest efficiency of extraction was around 42.83±1.05%, 26.81±0.64% , and 87.28±2.55% for electro treated followed by hexane, methanol, and chloroform:methanol and 31.42±1.58%, 17.88±0.45%, and 79.26±2.47% for solo hexane,

methanol, and chloroform:methanol without pre-treatment, respectively. It indicates that electro treatment could lead to the cell disruption and then improve the extraction.

3.2.2 Recycle flow rate effect on the extraction

The recycle flow rate used during electrochemical treatment included 300, 400, and 500 mL/min. The lipid recovered with different solvent after being treated for different time was shown in Table 2.The increase in the recycle flow rate from 300 to 500 mL/min in fact didn't impact on the lipid extraction efficiency as it was 41 to 46% for hexane, 23 to 27% for methanol, and 85 to 91% for chloroform: methanol, respectively. The recycle flow is to provide a well mixing in the solution. The variation on recycle flow rate changed the residence time of the biomass in the cell. As shown, increase in treatment time from 0 to 120 min didn't give any effect on the lipid extraction efficiency. It suggests that it would not be a good method to enhance lipid extraction efficiency by increase the recycle flow rate (treatment time on cells).

3.2.3 Electrolyte effect on the extraction

Electrolyte added to the lipid sample included NaCl and Na_2SO_4 with concentration of 0.01, 0.02, 0.04, and 0.08 mol/L. In fact, the anion or cation concentration was 0.02, 0.04, 0.08, and 0.16 mol/L when Na_2SO_4 was used as electrolyte. The lipid extraction efficiency from electrochemical pre-treatment biomass was around 42-46%, 22-26%, and 86-89% for hexane, methanol, and chloroform:methanol, respectively. Increasing of electrolyte concentration and changing the electrolyte didn't improve the extraction efficiency. The variation of electrolyte concentration caused slight change in voltage, but

the current was manually kept the same (0.4 A) during the experiments. As discussed in the section of Current effect on the extraction, current 0.4 A couldn't enhance the extraction efficiency. When NaCl was used as electrolyte, ClO· would be generated. The radical has strong oxidation property. It could help with the cell disruption. But the experiment results told that the addition of NaCl (ClO· formation) didn't effect on the extraction. Increase of current to increase the formation of ClO· could be a way to help with the cell disruption and thus enhance lipid extraction efficiency. However, at the same time, it would also lead to the oxidation of lipid and increase the saturation degree of the final product biodiesel.

3.3 The simultaneous electrochemical treatment combined and lipid extraction

In the simultaneous electrochemical treatment and lipid extraction, the biomass in the solvent was subjected to different current, recycle flow rate, and electrolytes for different period. The study firstly determined the extraction time effect on lipid extraction with different solvent by keeping electric current at 0.4 A and recycle flow rate at 400 mL/min with NaCl concentration of 0.01 g/L. It was observed that the extraction efficiency of combined extraction of electrochemical treatment with methanol: water (11.52±0.74%) was rather lower than that of the control (only methanol extraction without bead milling or electrochemical treatment) (17.88±0.45%), and lower than that of the pre-treated with electrochemical followed by methanol extraction (26.81±0.64%) (Fig. 1). It would be due to the water presence in the combined extraction system, and hence weakened the methanol function in the extraction.

For the extraction with of chloroform: methanol: water (1:1:0.9 v/v), the lipid extraction efficiency increased as the extraction time going on. The maximum extraction efficiency were 88.76±2.57% in the extraction with chloroform: methanol: water occurred at 24 h. It indicates that extraction time has significant impact on the extraction efficiency. As mentioned above, electroporation generated with application of electro current is capable of creating transient holes in cell surface. With the increase of extraction time, cell was gradually disrupted and solvent could excess to the lipid droplets, and more and more lipid was extracted which led to the increase of the extraction efficiency.

It can be seen that the lipid extraction efficiency had the trend to continually elevate if the extraction time was prolonged (Fig. 1). In order to investigate if the extraction efficiency could be further increased, similar extraction was performed and the lipid extraction efficiency at extraction time of 36 h and 48 h were determined. In the extraction with methanol: water, the extraction efficiency was almost the same with time increased to 36 and 48 h compared to that at 24 h.

In the electro treatment with the mixture of chloroform: methanol: water, lipid extraction efficiency achieved 90.65±4.51% at 36 h and 92.17±1.64% at 48 h, which were comparable with that used to determined the total lipid amount in biomass (2:1 v/v chloroform: methanol with bead milling). It indicates that electrochemical treatment combining with chloroform: methanol: water could be used to extract lipid from biomass. However, long reaction time was required to obtain high lipid extraction efficiency (over 90%). It required 36 h in combined extraction of electrochemical treatment with

chloroform: methanol: water, but only 12 h in that of bead milling with chloroform: methanol to obtain the similar extraction efficiency.

The study revealed that the in the simultaneous electrochemical treatment and lipid extraction system the one with chloroform: methanol: water was comparable with the bead milling with chloroform: methanol extraction which was normally used to determine the total lipid in cells; however, the extraction time was rather long. In order to improve the extraction and reduce the time, variation on current and recycle flow rate, and electrolytes in the system have been conducted. The extraction time was set at 14 h which was the same as used in the bead milling with chloroform: methanol extraction, but the results were not exciting. It was observed that the extraction efficiency was not highly enhanced by varying the current and recycle flow rate as well as the addition of electrolytes.

In the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water, the extraction efficiencies in all the cases lain between 73 and 78%. The maximum lipid extraction efficiency with extraction time 14 h was 77.66±2.83% occurred at current 0.5 A, recycle flow rate 500 mL/min, and NaCl concentration of 0.04 g/L. However, it was known that current 0.5 A caused the change of biodiesel profile (Table 2), thus it was not suggested to apply the current 0.5 A in the extraction.

It was obvious that the extraction efficiency of the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water was still too low to compare with the bead milling extraction using the same extraction time. However, in the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water (adding 10 mL chloroform for 1 mg dry biomass), the toxic chloroform amount

used was largely reduced compare to the bead milling extraction withchloroform: methanol (adding 18 mL chloroform for 1 mg dry biomass). It indicates that electrochemical treatment is more environmentally friendly compared to the generally used chloroform: methanol extraction. The main obstacle is the low extraction efficiency. In fact, increase of extraction time could improve lipid extraction but the increase of the efficiency was only about 2% for every 12 h extraction time increase (88.76±2.57% at 24 h; 90.65±4.51% at 36 h; 92.17±1.64% at 48 h). It is not worth to increase 12 h extraction time just for winning the 2% extraction efficiency increment. To utilize the combined extraction of electrochemical with chloroform: methanol: water, other low energy input technology such as mild heating could have to be applied simultaneously to improve mass transfer and hence enhance lipid extraction.

3.4 Electro-treatment effect on biodiesel composition

The composition of biodiesel converted from the lipid extracted from biomass with different solvent is presented in Table 3. When the mixture of chloroform and methanol was used as solvent either with bead milling alone or pre-treated with electro-treatment followed solvent extraction, the extracted lipid composition was similar, but it has slight change in the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water (Table 3). The sum of C (14, 16,17,18, 20):0 was higher in the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water than that of bead milling alone or pre-treated with electro-treatment followed extraction. It would be due to the higher oxygen solubility in chloroform than in

water, and more oxidation occurred in the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water than that in the electrochemical pre-treatment followed chloroform: methanol extraction. In the bead milling chloroform: methanol extraction, the extraction took place in a closed system, but the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water occurred in an open system, hence more oxygen would have presented in the extraction and caused the high oxidation extent in the simultaneous electrochemical treatment and lipid extraction with chloroform: methanol: water than that in bead milling chloroform: methanol extraction.

The composition of the lipid extracted with hexane was different from that extracted with mixture of chloroform and methanol. Hexane seemed more efficient to extract C14 and C18, but not C20 (Table 3). There was significant different on the composition of lipid extract from methanol and the mixture of chloroform and methanol. The major fatty acids of lipid in *T. oleaginosus* were C16 (41.55% w/w total fatty acids including 24.69% C16:0 and 16.86% C16:1) and C18 (40.01% w/w total fatty acids including 15.23% C18:0, 22.46% C18:1, 2.31% C18:2, and 1.01% C18:3). Other fatty acids (C14, C15, C17, and C20) took up small content of the lipid of *T. oleaginosus*. Methanol as solvent has extracted only around 22-27% lipid out of total lipid (100%), and they were mainly C16 (C16:0 and C16:1) and C18 (C18:0 and C18:1) (Table 3) as they were more abundant in the lipid of *T. oleaginosus*. Other fatty acids could be also extracted but the quantity was too small to be detected.

As stated above, the extraction efficiency wasn't impacted by the electro pre-treatment (current, recycle flow rate, and electrolytes). However, it was found that the fatty acid composition was greatly affected by the treatment. The extraction with the mixture of chloroform and methanol in the solo bead milling and electrochemical pre-treatment followed extraction provided similar extraction efficiency, thus they were used to compare the effect of electro pre-treatment on fatty acid composition of the extracted lipid. There was clear trend that the saturation degree (saturated fatty acid) increased as current increased (Table 2). It would be due to the fact that the high current caused more oxygen radical formation and thus induced the oxidation of unsaturated fatty acid to saturated ones. Low recycle flow rate led to high saturation degree (57.70% at 300 mL/min, 54.74% at 400 mL/min, 54.21% at 500 mL/min) (Table 2). It suggested that well mixing (high recycle flow rate) reduced the chance of oxidation of the unsaturated fatty acid. In the case of NaCl as electrolyte, the increase of its concentration increased the saturation degree. It would be caused by the formation of ClO^- (Eq. 3, 4, and 5), and thus the unsaturated fatty acids were oxidized to saturated ones. High saturation degree means that the biodiesel has high viscosity which causes engine problems at low temperature.

$$2Cl^- - 2e^- \rightarrow Cl_2 \quad (3)$$

$$2H_2O + 2e^- \rightarrow 2OH^- + H_2 \quad (4)$$

$$2OH^- + Cl_2 \rightarrow ClO^- + Cl^- + H_2 \quad (5)$$

When Na_2SO_4 was used as electrolyte, the saturation degree didn't affected. It indicates that it is safe to use Na_2SO_4 as electrolyte when considering preventing the change of the composition of fatty acids during electro-treatment.

4 Conclusion

The study showed that the c simultaneous electrochemical treatment and lipid extraction with solvent could extract more than 92% total lipid from wet biomass when using the mixture of chloroform: methanol: water as solvent. Though it was still lower than that of bead milling chloroform: methanol: water, but due to the addition of water in the combined extraction of electrochemical treatment with chloroform: methanol: water, the required amount of chloroform could be extensively reduced. However, the required extraction time was long in the simultaneous electrochemical treatment and lipid extraction compared with bead milling solvent extraction. Study on other technologies such as heating and mild pressure to add into the combined extraction of electrochemical treatment with chloroform: methanol: water should be conducted in order to improve the extraction efficiency.

Current showed great impact on the saturation degree of the fatty acid extracted from biomass. The selection on current in electrochemical involved lipid extraction should be given great attention as high saturation led to high viscosity of the final product (biodiesel). The study showed that electrochemical treatment combined with chloroform: methanol: water could efficiently extract lipid from biomass, but it also affect on the composition of the final product biodiesel. If the aim of a study is to extract products from microbes and preserve their nature, the utilization of electrochemical treatment should be well evaluated.

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6 Conflict of interest

The authors have declared no conflict of interest.

7 References

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Table 1

Lipid recovery efficiency after different current electro-chemical treatment (Recycle flow rate=400 mL/min).

Time (min)	Lipid recovery efficiency (% w/w)											
	Current=0.3 A			Current=0.4 A			Current=0.5 A			Current=0.6 A		
	He	Me	Chlo/me	He	Me	Chlo/me	He	Me	Chlo/me	He	Me	Chlo/me
15	40.16 ±1.55	23.18 ±1.22	86.26 ±2.51	41.26 ±1.35	25.68 ±1.55	84.28 ±1.35	42.22 ±1.56	24.68 ±1.02	85.66 ±3.05	41.95 ±2.20	25.66 ±1.30	86.42 ±1.66
30	40.89 ±1.32	22.94 ±1.67	87.49 ±1.38	42.23 ±1.58	26.32 ±1.64	86.42 ±1.76	41.89 ±1.46	25.88 ±1.70	86.48 ±2.14	42.28 ±1.77	26.43 ±1.51	87.54 ±1.29
45	40.91 ±1.06	23.54 ±2.11	86.52 ±2.22	42.51 ±1.73	25.16 ±1.00	87.15 ±2.73	41.61 ±1.30	26.76 ±1.28	85.61 ±1.82	42.96 ±2.18	26.84 ±2.09	86.23 ±1.81
60	41.22 ±1.77	24.65 ±1.57	85.69 ±3.37	41.94 ±2.05	26.29 ±1.07	86.55 ±1.69	40.21 ±2.51	26.40 ±0.95	86.34 ±2.90	41.67 ±2.02	25.68 ±1.94	87.06 ±2.14
75	41.25 ±1.64	26.14 ±1.32	86.46 ±1.66	42.23 ±1.74	22.19 ±2.01	85.46 ±2.01	41.89 ±1.64	25.88 ±1.26	87.58 ±1.91	42.44 ±1.36	26.41 ±1.71	87.23 ±3.47
90	40.64 ±1.38	25.79 ±1.90	87.25 ±3.04	40.25 ±1.92	25.82 ±1.63	87.13 ±2.47	41.73 ±1.25	26.17 ±1.64	86.94 ±1.35	42.86 ±1.09	26.23 ±1.55	86.15 ±2.76
105	41.68 ±1.94	26.17 ±2.18	86.58 ±2.64	41.76 ±2.03	26.01 ±1.62	87.69 ±1.36	42.86 ±2.07	26.57 ±1.39	85.26 ±1.38	41.83 ±1.75	26.81 ±1.86	85.58 ±2.01
120	42.08 ±2.16	25.99 ±1.73	87.12 ±1.67	41.87 ±1.33	25.82 ±1.72	86.28 ±1.33	41.92 ±1.54	25.82 ±1.64	87.11 ±3.16	42.11 ±1.69	25.77 ±1.34	86.67 ±1.69

He: hexane; Me: methanol; chlo/me: chloroform/methanol.

w/w: lipid extracted/lipid total

Table 2

The composition of biodiesel converted from the lipid.

Fatty acids	Relative amount of total fatty acid (% w/w)														
Solvent	Ch: Me BM	Ch:Me (after electro-treatment)													
Current (A)		0.3	0.4	0.5	0.6	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
RFR (mL/min)		400	400	400	400	300	500	400	400	400	400	400	400	400	400
Eletro.		0	0	0	0	0	0	NaCl	NaCl	NaCl	NaCl	Na_2SO_4	Na_2SO_4	Na_2SO_4	Na_2SO_4
Eletro. Conc. (mol/L)								0.01	0.02	0.04	0.08	0.01	0.02	0.04	0.08
C14:0	2.33	2.94	2.85	3.16	4.58	2.66	2.17	2.7	2.81	2.75	4.16	2.41	2.96	2.94	2.62
C14:1	3.51	3.26	3.44	2.22	1.33	3.63	3.84	3.45	3.54	3.62	1.84	3.85	3.71	3.58	3.61
C15:0	3.22	3.41	3.53	4.01	4.33	3.64	3.81	3.66	3.71	3.66	4.17	3.88	3.17	3.62	3.77
C15:1	1.74	1.49	1.59	0.97	1.31	1.04	1.24	1.42	1.28	1.62	0.91	1.27	1.82	1.33	1.84
C16:0	24.69	24.23	25.31	27.38	30.06	25.94	24.77	24.94	24.33	25.25	25.73	24.88	25.44	25.59	24.37
C16:1	16.86	16.63	15.88	13.05	9.16	14.27	16.82	16.13	16.21	15.14	13.76	16.28	15.17	14.83	16.91
C17:0	3.77	3.91	3.82	4.24	3.51	4.29	4.12	3.86	4.01	3.47	3.73	3.55	3.52	3.77	3.94
C18:0	15.23	17.64	17.86	20.25	24.15	18.91	18.15	17.11	17.72	18.22	21.62	14.93	17.22	17.38	14.63
C18:1	22.46	21.79	20.62	19.43	17.64	19.26	20.34	20.16	20.09	19.07	18.36	23.55	21.52	21.84	22.37
C18:2	2.31	2.04	1.78	1.13	0.84	1.62	1.93	1.96	2.31	2.21	1.42	2.04	2.15	2.23	2.41
C18:3	1.01	1.17	1.05	1.21	0.53	1.11	1	1.17	1.18	0.58	0.72	1.29	0.73	0.82	1.01
C20:0	0.79	0.92	1.17	1.17	1.22	1.26	1.02	1.92	1.08	2.06	1.83	0.73	0.82	0.64	0.88

C20:1	0.6	0.41	0.73	0.62	0.36	0.64	0.48	0.89	0.71	0.89	0.46	0.53	0.47	0.62	0.47
Satur. Deg. (%)	50.78	53.14	54.74	60.92	68.52	57.70	54.21	54.53	54.21	56.23	62.04	50.79	51.31	51.88	50.80

Ch:Me BM= chloroform methanol extraction under bead milling; He=hexane; RFR=recycle flow rate;Eletro= electrolyte; ;Eletro conc.= electrolyte concentration; Satur. Deg.=Saturation degree; the extraction time was 15 min. Cn:0 presents saturated fatty acids, Cn:1 presents mono-unsaturated fatty acid, and Cn:2 Cn:3 present poly-unsaturated fatty acids. The saturation degree was calculated: $\sum$Cn:0/$\sum$(Cn:0, Cn:1, Cn:2)×100%.

Table 3

The composition of biodiesel converted from the lipid.

Items	Commercial biodiesel derived from *Jatropha curcas*	Relative amount of total fatty acid (% w/w)					
		In the initial SS		In the SS after cultivation			
Solvent		Ch:Me	Ch:Me	Hexane	Methanol	Ch:Me	Ch:Me
Method		BM	BM	Electrochemical pre-treatment	Electrochemical pre-treatment	Electrochemical pre-treatment	Combined electrochemical
C14:0	0	3.48	2.33	3.78	2.71	2.85	3.91
C14:1	0	0.51	3.51	5.19	0	3.44	2.07
C15:0	0	2.34	3.22	3.82	0	3.53	5.16
C15:1	0	0.72	1.74	3.82	0	1.59	0.89
C16:0	15.11	15.74	24.69	20.62	37.59	25.31	30.18
C16:1	0.92	11.43	16.86	18.22	2.1	15.88	11.95
C17:0	0	2.29	3.77	4.45	0	3.82	2.44
C18:0	7.10	18.19	15.23	19.63	19.66	17.86	21.06
C18:1	44.35	16.29	22.46	17.45	34.57	20.62	18.31
C18:2	31.31	12.91	2.31	1.17	1.02	1.78	1.34
C18:3	1.21	5.64	1.01	1.25	0	1.05	0.65
C20:0	0.18	0.93	0.79	0	1.66	1.17	0.94
C20:1	0	1.04	0.6	0	0	0.73	0.33

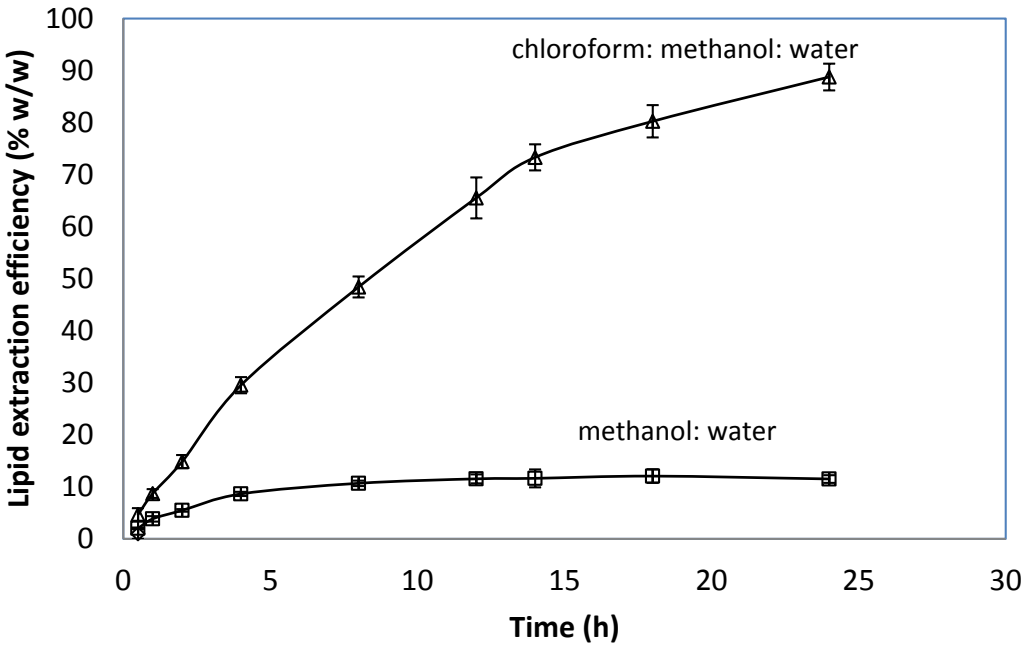

Figure 1.

Efficiency of combined extraction of electrochemical and solvent at current of 0.4 A, recycle flow rate of 400 mL/min.