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Solvent Extraction Techniques of Lipid from Algal Species in Wastewater Treatment Station

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BIODIESEL production process relies on various key steps one of which is the lipid extraction from microalgae cells using economic techniques. In this study, Algal community structure and physicochemical parameters of the wastewater treatment plant (Beni-Suef, Egypt), are determined from March 2016 to February 2017. In this study, we focused on the lipid extracted from wild mixed culture of *Chlorella sp.* which is the dominant from December 2016 to February 2017. Lipid content and fatty acid profiling of *Chlorella sp.* are investigated using three different methods (1) conventional extraction (CE), (2) ultrasonic assisted extraction (UAE) and (3) microwave assisted solvothermalextraction (MASE). MASE showed the highest lipid yield of 37.9±0.13%. SEM micrographs showed that MASE had the most distributive effect for algae cells compared to UAE and CE. Six different solvents are tested for MASE technique namely hexane: isopropanol (3:2), hexane: ethanol (1:1), chloroform: methanol (1:1), diethyl ether, ethanol and methanol. A strong linear relation between the direct electric constant and yield percent is obtained with methanol showing the highest yield. The GC-analysis of extracted lipids shows that methanol resulted in the highest percentage of palmitic acid (45.5%). The chemical composition of extracted lipids shows promise towards further conversion to biodiesel.

Keywords: Solvent extraction, Lipids recovery, Wastewater treatment plant, Saturated fatty acid, Microwave solvothermal, Ultrasound techniques, Solvent polarity, GC analysis.

Introduction

Due to intenseness of human population and consumption of fresh water, large volumes of domestic, agricultural and industrial wastewater are generated[1]. Wastewater scan cause serious problems if not properly managed [2,3]. Domestic wastewaters are rich in nutrients that enhance microalgal growth. Its use as culture medium will reduce the requirement of fresh water and nutrients. At the end of the process, a clean effluent may be verified to discharge in a watercourse. Hence, algae are considered as a more environmental-friendly way due to its ability to treat wastewater by uptake nutrients. The algal cells convert these nutrients to cellular substances such as lipid, protein and carbohydrate[4,5].

Production of biodiesel from wastewater microalgae have multi advantages: it can be produced around year and at very high rates depending on climate and solar energy[6–9]. Microalgae biomass is ownerless in lignocellulosic material and full of lipids and protein, so it is considered as an important alternative feedstock for biofuels [10]. The differences in cell size, shape and structure of algae species, and the characteristics of the wastewater media can effect

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on the lipid recovery efficiency[11,12]. Bernaerts et al. [12]demonstrated that algal biomass components can be altered by modifying the cultivation conditions, such as nutrients concentration and temperature. Patrícya et al. demonstrated that maximum total lipid of mixed algae cultures cultivated in a stabilization pond system treating sewage was 33.7±5.3%[7]. Chinnasamy et al. stated that about 63.9% of algal oil obtained from the consortium of 15 native algae grown in carpet wastewater could be converted into biodiesel[9]. Biodiesel can be recognized as a substitute for petroleum diesel or used in any proportion mixing with petroleum diesel for conventional diesel engines without modification [7,13]. Biodiesel is an eco-friendly environment fuel which has low emissions, nontoxic and biodegradable[8,14]. Nautiyal et al. (2014) reported that the algae biomass harvested from natural pond water is considered as a cheaper feedstock for biodiesel production than pure cultures of Chlorella [14].

Cell disruption techniques such as autoclave, bead-beating, sonication, microwave and osmotic shock are usually followed by solvent extraction for improving lipid yield and quality [15]. The mechanism of these techniques isn't similar but most of the techniques include cell disruption to facilitate the extraction of lipids [16]. Conventional technique consumes several hours for completing the lipid extraction and results in low lipid yield [15,16]. Microwave can overcome some of the problems attributed with other techniques and is recognized as more economic and environmental-friendly technique [17]. Microwave is characterized by shorting processing times, little solvent requirement and rapid heating affording better products [18]. The microwave effect is dynamically based on the nature of both solvent and material [19]. On the other side, disruption of microalgal cells through sonication is based on cracking the cell walls and membranes by cavitation effect [20,21]. Microwave-assisted extraction (MAE) technique is provided a higher extraction efficiency of total steroid saponins from Dioscoreazingiberensis C.H. Wright than ultrasonic-assisted extraction (UAE)[22]. MAE and UAE exhibited higher efficiencies for extraction of polysaccharide from *F. velutipe* with high yields and short extraction times compared to CE[23].

The mechanisms of organic solvents used in lipid extraction process are not fully understood. Hidalgo et al. (2016) and Halim et al. (2012) explained a solvent–microalgal biomass interaction mechanism, where the organic solvent can interact with the neutral lipids in the cytoplasm by Van der Waals forces forming an organic solvent–lipid complex. The neutral lipids are released from the cells by diffusion of organic solvent–lipid complex across the cell membrane based on a concentration gradient. In microalgae, the neutral lipid–polar lipid complexes are linked to proteins by hydrogen bonds. The non-polar organic solvents aren't suitable to disrupt these membrane–lipid–protein linkages. However, polar organic solvents can break the lipid–protein linkage by hydrogen bonds formation with the polar lipids in the complex [24,25]. Solubility of lipids depends heavily on the proportion of polar lipid to non-polar lipid in the algal cells. Hence, the choice of solvent system is important for efficient lipid extraction [26].

Based on my literature, few studies are interested with lipid extraction from waste-grown mixed-algae cultures. The aim of this study is to evaluate the effect of different extraction techniques and solvent systems on the lipid yield and fatty acid composition of wild mixed culture of *chlorella sp*. harvested from the plant.

Materials and Methods

Study area and sampling protocol

Wastewater entering the domestic wastewater treatment plant (Beni-Suef, Egypt) is divided into two identical flow lines starting with an anaerobic pond followed by the facultative and four maturation ponds. Only one of the two parallel flow lines of the plant is sampled. Seven sites (influent (S1), outlet anaerobic pond (S2), outlet facultative pond (S3), outlet maturation pond1(S4), outlet maturation pond 2(S5), outlet maturation pond 3(S6) and outlet maturation pond 4 (S7)) in the plant are sampled from March 2016 to February 2017. Samples are collected once or twice every month.

Wastewater analysis

Samples are preserved in glass bottles during transportation from the plant to laboratory. Analysis of total phosphorus (TP), ammonia (NH_3-N), nitrate (NO_3-N), nitrite (NO_2-N), chemical oxygen demand (COD) and biological oxygen demand (BOD) are measured following standard methods described in APHA[27].

Algae identification, harvesting, and drying

Algae species are identified following monographs on algae[28]. The cells are harvested by gravitational settling for 24 h. The wet algal biomass is dried for 12 h in a drying oven at 60ºC. The dried biomass is milled in a mortar with pestle and stored at -25 ºC for analysis.

Lipid extraction

Three different methods are used for lipid extraction (1) conventional extraction (CE), (2) ultrasonic assisted extraction (UAE) and (3) microwave assisted solvothermal extraction (MASE).For each method a 1:6 W/V suspension of the dried wild mixed culture of *Chlorella sp*. in methanol is prepared. During CE, the suspension is stirred at 30-35 ºC for 2 h at 300 rpm under reflux. For UAE, the suspension is subjected to transient ultrasonic waves (9 seconds on/ 3 seconds off) by ultrasonic probe device (Newtown, CT 06470, 40 kHz & 750 W) for 30 min. MASE is conducted by placing the suspension in Teflon lined closed ceramic tubes placed in microwave-assisted hydrothermal oven (Anton Paar GmbH, A-8054 Graz/ Austria-Europe). The reaction temperature is set to 100 ºC for 30 minutes using 400 W input microwave power.

Applying microwave technique,an aliquot weight of the dry biomass is mixed with each solvent of the following solvent systems [hexane: isopropanol (3:2), hexane: ethanol (1:1), chloroform: methanol (1:1), diethyl ether, ethanol and methanol]at a constant ratio (1: 6 W/V)to determine which solvent system perform better in the extraction process.

After the extraction process, the produced suspension was filtered to separate cell residues. The total lipid percentage (after solvent evaporation) is calculated by the following equation:

$$\%\ oil\ content = \frac{weight\ of\ oil\ obtained\ after\ extraction}{weight\ of\ dry\ sample} \times 100$$

Scanning electron microscopy (SEM)

The surface morphologies of both the raw and the ruptured cells by the three different extraction techniques were observed by FESEM (High Resolution Field Emission SEM Quanta 250) at 16000x magnification mode.

GC-analysis

The extracts are analyzed by gas chromatography (GC) with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm). Helium is used as carrier gas at a flow rate of 1 ml/min. The column is held at 150 ºC for 1 min and ramped to 240 ºC at a rate of 30 ºC/min, then held at 240 ºC for 30 min. Fatty acids are identified by comparing the retention time of each peak to that of reference standards.

Statistical analysis

Data on the Physico-chemical variables are represented as means ± the standard deviations of the means. The extraction experiments are conducted in triplicates. Results are analyzed using one way ANOVA and Tukey's post hoc analysis through the SPSS program 22. The level of significant difference was at $P < 0.05$.

Result and Discussion

Wastewater characteristics and Algal community composition

Developing enhanced extraction processes for algae harvested from wastewater streams can be regarded as a promising approach towards wastewater treatment and biodiesel production. Algae samples are collected from a wastewater plant in Beni-Suef governorate, Egypt. A full study during the period from March 2016 to June 2016 is conducted to monitor the dominant algae species and wastewater composition. The characteristics of wastewater are summarized in Tables 1, 2 and 3.

From March 2016 to June 2016, the algal community structure in the facultative and maturation ponds included eighteen algal species belonging to three algal groups, namely; *Chlorophyta*(green alga), *Cynanophyta*(blue-green algae) and *Bacillariophyta*(diatoms). 12 species belong to *Chlorophyta,* 2 species to *Cyanophyta*and 4 species to *Bacillariophyta*where *Euglena sanguinea* is dominated the five ponds, as shown in Table 4.

A total of 15 species are identified during the period from July 2016 to November 2016. A total of 10 species of *Chlorophyta*, 3 species of *Cynanophyta* and 2 species of *Bacillariophyta* are recorded where *Spirulina maxima* was dominant in the five ponds, as shown in Table 5.

From December 2016 to February 2017, there was a shift in dominance from *Spirulina maxima* to *Chlorella sp.*12 genera of algae are identified in the facultative and four maturation ponds and divided into three classes. Chlorophyta showed the highest number of taxa with eight, followed by Cynanophyta with three genera and Bacillariophyta with one (Table 6).

TABLE 1. Mean ± standard deviation of water quality variables in the wastewater treatment plant measured during the 4 months (March2016-June 2016).

Site	COD mg/L	BOD mg/L	$N\text{-}NH_3$ mg/L	$N\text{-}NO_2$ mg/L	$N\text{-}NO_3$ mg/L	TP mg/L	PH
S1	692.5±73.8	381±48.8	36.3±11.2	0.8±0.8	2.9±2.5	5.6±3.9	7.1±0.1
S2	365±154.2	228.5±127.5	43.8±11.3	0.3±0.2	3.6±3.1	4.9±3.5	7±0.1
S3	292.5±119.8	179.5±93.9	30±8.2	0.5±0.4	3.3±2.6	4.2±4.2	7.7±0.5
S4	262.5±68.5	141.7±66.5	28.1±13.3	0.8±0.4	2.9±2.3	4.2±3.9	8±0.3
S5	207.5±55.8	113.4±47.4	25±11.4	1±1.1	2.9±2.4	4.2±2.4	8.1±0.3
S6	170.5±21.5	83.9±28.1	24.1±15.2	1.1±1.4	3.3±2.8	3.5±2.6	8.1±0.1
S7	119.3±46.5	49.8±12.7	14.1±11.2	0.9±0.7	5.7±3.9	4.1±4.2	8.1±0.1

TABLE 2. Mean ± standard deviation of water quality variables in the wastewater treatment plant measured during the 5 months (July2016-November 2016).

Site	COD mg/L	BOD mg/L	$N\text{-}NH_3$ mg/L	$N\text{-}NO_2$ mg/L	$N\text{-}NO_3$ mg/L	TP mg/L	PH
S1	338.7 ±290.2	259.5±129	18.3±9.5	0.1±0.1	1.8±2.2	3.4±2.5	7.2±0.1
S2	142.7 ±115.6	162.7±72.9	31.2±9	0.1±0.1	2±2.2	5.3±2.5	7.4±0.2
S3	104.4 ±80.6	136.2 ±60.3	28±5.5	0.9±0.8	2±2.5	2.3±1.3	8.1±0.2
S4	119.2±103.3	153±83.4	16.6±6.4	11.5±5.1	1.9±2	5.5±1	8.2±0.1
S5	119.5±106.7	150±78.4	7.1±4.5	9.3±4	4.5±4.1	5.7±1.7	8.5±0.4
S6	93.4 ±72.3	171.2±130.2	2.7±1	8±3.1	3.5±5.1	4.5±2.1	8.6±0.2
S7	114.5 ±98.5	129.5±96.1	3.3±1.9	5.2±1.9	3.6±5	3.5±3.1	8.7±0.2

TABLE 3. Mean ± standard deviation of water quality variables in the wastewater treatment plant measured during the 3 months (December2016-February 2017)

Site	COD mg/L	BOD mg/L	$N\text{-}NH_3$ mg/L	$N\text{-}NO_2$ mg/L	N-NO3 mg/L	TP mg/L	PH
S1	830.5±261.9	456.2±75.4	57.5±37.6	2±2.3	3.7±3.1	4.8±5.6	6.9±0.2
S2	539.5±86.6	294±45.5	61.2±15.8	2.1±2.8	4.5±4.5	6.7±9	7±0.2
S3	402.2±87.8	234.2±18.8	38.7±9.4	2.4±3.1	7.9±9.1	4.2±4.6	7.8±0.3
S4	306.2±59	186.7±18	32.8±18.1	2.3±3.3	3.9±4	3.8±4.1	8±0.1
S5	246±46.9	148±32.5	29.3±9.6	2±2.6	3.3±2.8	4±4.3	8±0.1
S6	171.7±33.2	100±14.62	34.7±14.6	1.86±2.12	3.3±3.2	4.2±4	8.2±0.3
S7	115.2±20.9	49.5±17.6	39.4±13.4	1.8±2.3	3±2.5	3.3±2.9	8±0.47

TABLE 4. Algae species composition identified during the 4 months (March2016-June 2016).

Algal Taxa	Sites				
	S3	S4	S5	S6	S7
Green Algae					
Ankistrodesmusacicularis	++	++	++	+	+
Actinastrumhantzschii	-	++	++	+	-
Chlamydomonusvariabills	+	-	-	-	-
Dictyosphaehrenbergianumerium	-	-	+	-	-
Euglena sanguinea	++++	+++	++++	+++	+++
Euglena acus	-	+	++	++	-
Golenkinaradiata	-	-	-	-	+
Micractinumpusillum	+	+	+	-	-
Pediastrumtetrus	++	-	-	-	-
Phacuslongicauda	+	++	+	+	++
Phacustorta	-	++	++	++	++
Trachelomonasvolvocina	++	++	++	++	+
Blue-green Algae					
Gomphosphaerialacustris	++	-	+	+	++
Oscillatorialimnetica	++	++	+	+	+
Diatoms					
Cyclotellacomta	-	+	+	-	+
Gomphonemaolivaceum	-	-	-	-	++
Nitzschialinearis	-	+	-	-	+
Syndera ulna	-	-	++	-	-

Predominant; +++ = Dominant; ++ = Many + = Appreciable; - = absent = ++++

TABLE 5. ALgae species composition identified during the 5 months (July2016-November 2016).

Algal Taxa	Sites				
	S3	S4	S5	S6	S7
Green Algae					
Ankistrodesmusacicularis	++	++	++	++	++
Actinastrumhantzschii	++	+	++	+	+
Chlamydomonusvariabills	+	-	++	-	+
Euglena sanguinea	++	++	++	++	++
Golenkinaradiata	+	-	-	-	-
Micractinumpusillum	+	-	-	-	+
Phacuslongicauda	++	++	+	++	++
Phacustorta	++	++	++	++	++
Scenedesmusquadricuda	+	-	+	+	++
Tetraedronmuticum	-	+	+	-	-
Blue-green Algae					
Oscillatorialimnetica	+	-	++	++	++
Spirulina maxima	++++	++++	++++	++++	++++
Spirulinaplatensis	-	+	-	++	+
Diatoms					
Cyclotellacomta	-	+	+	-	+
Nitzschialinearis	++	+	++	+	++

Predominant; +++ = Dominant; ++ = Many + = Appreciable; - = absent = ++++

TABLE 6. Algae species composition identified during the 3 months (December2016-February 2017).

Algal Taxa	Sites				
	S3	S4	S5	S6	S7
Green Algae					
Ankistrodesmusacicularis	++	-	+	++	+
Actinastrumhantzschii	++	-	+	-	-
Euglena sanguinea	++	++	++	++	++
Phacuslongicauda	+	+	+	+	++
Phacustorta	-	-	-	-	+
Trachelomonasvolvocina	+	-	+	+	++
Chlorella sp.	+++	+++	+++	+++	+++
Pediastrumtetrus	+	+	+	++	++
Blue-green Algae					
Oscillatorialimnetica	+	++	-	+	+
Spirulina maxima	-	-	+	-	-
Spirulinaplatensis	-	+	-	-	-
Diatoms					
Nitzschialinearis	-	-	-	+	-

Predominant; +++ = Dominant; ++ = Many + = Appreciable; - = absent = ++++

Comparison of lipid extraction techniques

Figure 1 showed the lipid content of *Chlorella sp.* recovered by three different extraction methods associated with methanol. Statistical analyses showed that there were significant differences between the techniques tested. MASE technique presented the highest lipid yield, with percentages of 37.87±0.14 %. When a material containing water or other polar compounds exposed to electromagnetic microwave radiation, polar molecules oscillated causing inter- and intra-molecular friction. Coupling of This friction coupled with the movement and collisions of charged ions result in rapid heating (within seconds) of the material. Later, intracellular heating results in pressurized effects rupturing the cell membranes and releasing desired contents from within[17]. This could be supported by the findings of Koberg et al. (2011) reported that microwave radiation is more effective in the destruction of the algal cells and accelerates the transesterification reaction [8]. Patrícya et al. (2014) stated that a lipid content of microalgae collected from a wastewater treatment plant (33.7%) using microwave is higher than using ultrasonication (13.3%)[7]. Prabakaran et al (2011) demonstrated that lipid released from Tolypothrix sp. through microwave method was higher than bead beating, osmotic shock and sonication methods [13].

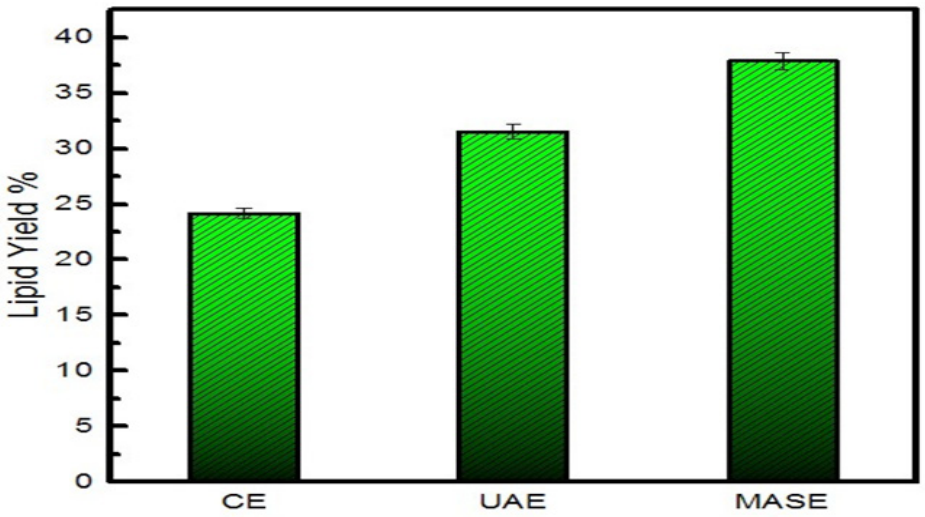

Fig. 1. Chlorella lipid extracted through different extraction methods.

The lipid yield under conventional extraction (24.17±0.34%) was significantly lower than that through microwave. This difference is due to faster heating up the reaction mixture in microwave than that in conventional reflux [8].

The lipid yield by UAE (31.53±0.87%) was significantly higher than conventional reflux. The efficiency of the ultra-sonic depends on the extreme high pressure and temperature generated by bursting the cavitation bubbles. When bursting of cavitation bubbles is repeated, the cell walls of microalgae are damaged and more oil release [29, 30].

Compared to MASE, the UAE technique showed less lipid yield. It is believed that this can be caused by the internal pressure built inside the reaction vessel in case of the MASE as compared to conducting the extraction process in an open vessel as in case of UAE. CE method relies on just conventional heating through conduction and convection with simple mechanical stirring which showed the least lipid yield. CE suffers from poor heat transfer inside the reaction vessel compared to the even heating caused by the microwave radiation. Moreover, the simple mechanical stirring has the least effect on shearing algae cells as compared to ultrasonic waves that locally enhance cell rupture.

Effect of different technique on cell disruption of chlorella sp. through SEM

The SEM observations of the algae cells before and after extraction showed in Fig. 2. The raw cells of *chlorella sp.* before extraction (Fig. 2a) appeared without destructive. Figure 2b showed the limited change in the cells morphology after the conventional technique. SEM analysis of algal cells subjected to ultrasonic (Fig. 2c) cleared cell wall intermediate damage allowing the solvent to access the interior of disruptive algal cells and releasing the lipid to the medium. The microwave technique achieved a wide destruction of cells, as shown in Fig. 2d. MAE can cause severe breaks due to the absorption of algae cells to of microwave energy. This accumulated energy leads to increase the temperature instantly and the possible pressure being exceeded. Results in present study are in compliance with studies

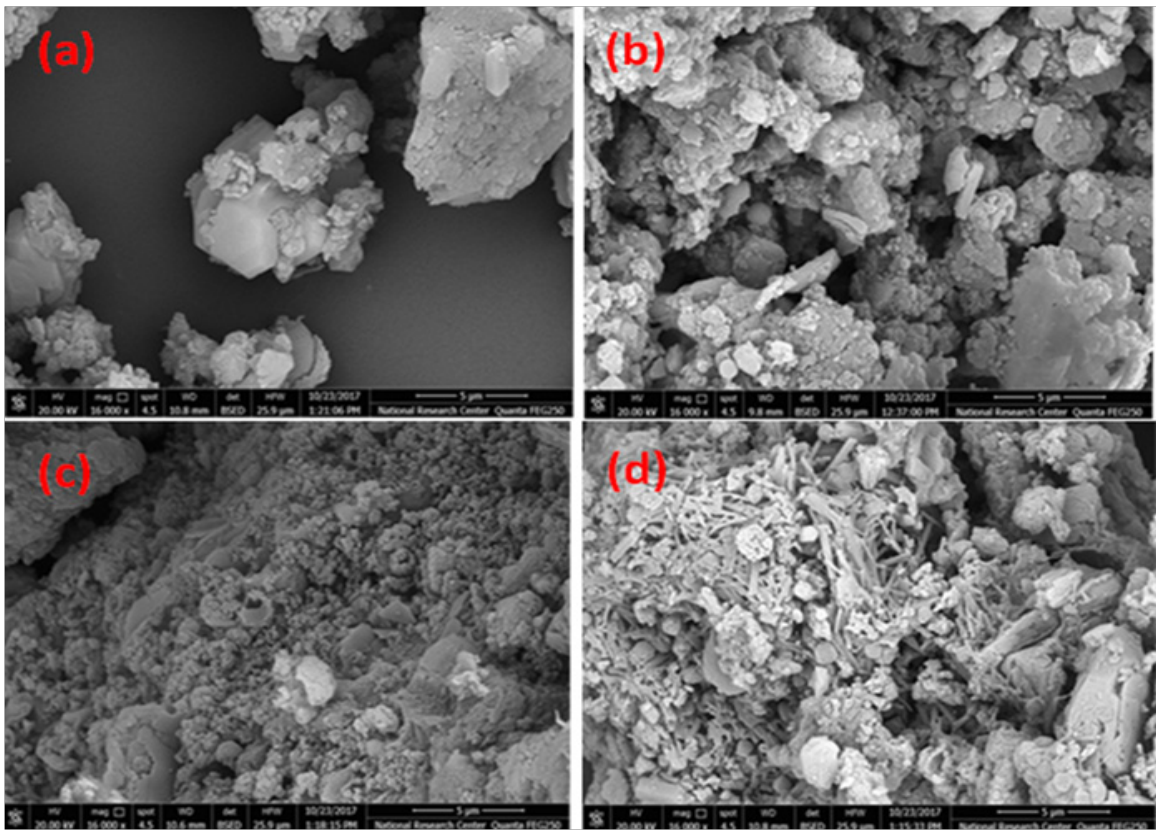

Fig. 2. SEM micrographs of (a) raw cells and ruptured cells by (b) CE, (c) UAE and (d) MASE.

by [18,30]

Impact of solvent polarity on algal lipid recovered from chlorella sp.

Besides the extraction technique tested, the strength of solvent systems affects lipid extraction efficiency. Based on results in this study, MASE was the most effective technique for lipid extraction. Therefore, the performance of five different solvents is compared to methanol using MASE. The chemical composition and dielectric constants of all the used solvents are listed in Table 7. The dielectric constant of mixed solvents was calculated according to the relation:

$E_m = (v_1 * E_1) + (v_2 * E_2)$ [31].

Where E_m, E_1 and E_2 are the dielectric constants of the binary solvent, solvent 1 and solvent 2 respectively. v_1 and v_2 are the volumetric fractions of solvent 1 and solvent 2 respectively.

The dielectric constants for hexane, chloroform and isopropanol were equal to 1.89[32], 4.8[32] and 20.1 [33]respectively.

As shown in Fig. 3, different solvents showed different lipid yield percentages. As shown, using methanol still provided the highest yield even compared to binary solvent mixtures. In order to gain more insights into the role of solvents into the extraction process, the dielectric constant for each solvent is plotted against the yield percent as shown in Fig. 3 (right). Interestingly a strong linear relation (adjusted $r^2 = 0.91$) is obtained irrespective of the chemical nature of the single or binary solvents. These results suggest that the solvent polarity (represented by the dielectric constant) has a crucial role in the MASE technique. The more polar organic solvent used in extraction process, the higher lipid yield obtained due to the extraction of polar lipids mainly composed of phospholipids and glycolipids[34]. In the cytoplasm, some neutral lipids linked with polar lipids forming a complex. This complex is strongly bonded to proteins (in the cell membrane) via hydrogen bonds. The van der Waals forces which are formed between non-polar organic solvent and neutral lipids in the complex, aren't enough to break these lipid–protein associations. On the other side, polar organic solvent (such as methanol or isopropanol) can break the membrane-lipid–protein associations due to the formation of hydrogen bonds with the polar lipids in the complex[35]. Other variables are also tested for correlation including boiling point, vapour pressure at 100 °C and other physical properties of the solvents but none gave a strong correlation like the dielectric constant. In our study, the lipid yield recovered by the solvent system chloroform: methanol (1:1, v/v) (20.9±0.58%) was significantly higher than hexane: ethanol (1:1, v/v) (6.5 ±0.42%). A similar result was obtained from Kumar et al. (2013) when accelerated solvent extraction (ASE) was used for lipid extraction [36]. Chloroform: methanol solvent system gave significantly better lipid recovery efficiency in comparison with solvent system hexane: isopropanol (3:2, v/v) (3.1±0.21). This could be supported by the findings of Mulbry et al. (2009) who reported that isopropanol/hexane yielded significantly lower values for oil and FA content comparing to values from chloroform/methanol using ASE [37]. The extraction of lipids from *Chlorella sp.* using a non-polar organic solvent (diethyl ether) showed a lowest yield.

Fatty acid composition in lipid extracts

The nature of fatty acid composition in the *chlorella sp*. is determined by GC analysis (Fig. 4). According to Fig. 4, the major composition of *chlorella sp*. lipid produced using the illustrated techniques, consists of palmitic (C16:0) acid. The presence of a high percentage of saturated fatty acids (SFA) in the lipid guarantees the good oxidation resistance of the bio-diesel[38]. UAE is recorded highest percentage of palmitic acid (54.5%) and stearic acid (9.5%). MASE and UAE show a low percentage of fatty acids having

TABLE 7. Chemical composition and dielectric constants of the solvents used for MASE extraction

Solvent	A	B	C	D	E	F
Chemical composition	hexane: isopropanol	hexane: ethanol	chloroform: methanol	diethyl ether	ethanol	methanol
Volumetric ratio	3:2	1:1	1:1	-	-	-
Dielectric constant	9.17	13.45	19.05	4.17 [34]	25.02 [35]	33.3 [35]

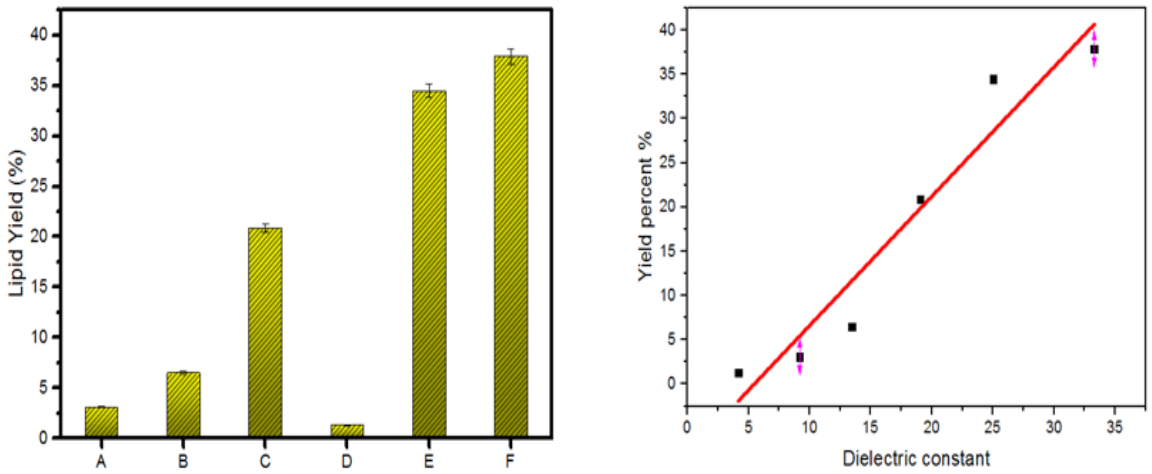

Fig. 3. (left) Lipid yield percent and (right) relation between yield percentage and dielectric constant for the different solvents used during MASE extraction technique

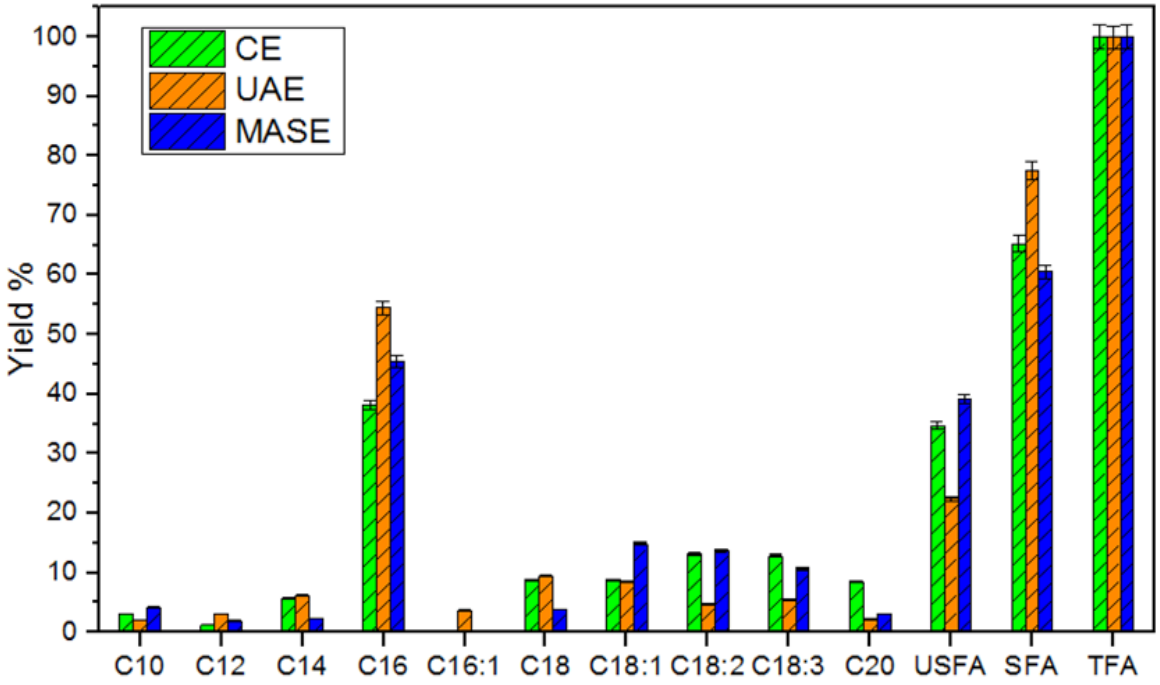

Fig. 4. Fatty acid composition of *chlorella sp.*lipid obtained using different methods

a carbon chain of >18 carbons. This is known to impart a low viscosity for the bio-diesel[8].

Figure 5 lists the composition and relative amount of each fatty acid of the lipid obtained by six solvent systems through using MASE. In a previous study, palmitic, stearic, oleic and linolenic acid are recognized as the most common fatty acids contained in biodiesel[39]. According to fatty acid profile result, methanol can extract highest concentration of palmitic acid (45.5%). The properties of a biodiesel fuel, including ignition quality, combustion heat, cold filter plugging point, oxidative stability, viscosity and lubricity are predicted by the structure of fatty acid composition of lipid. The high oleic acid content can enhance the oxidative stability for longer storage [40]. Ethanol is showed the highest oleic acid content (35.7%). The highest amount of

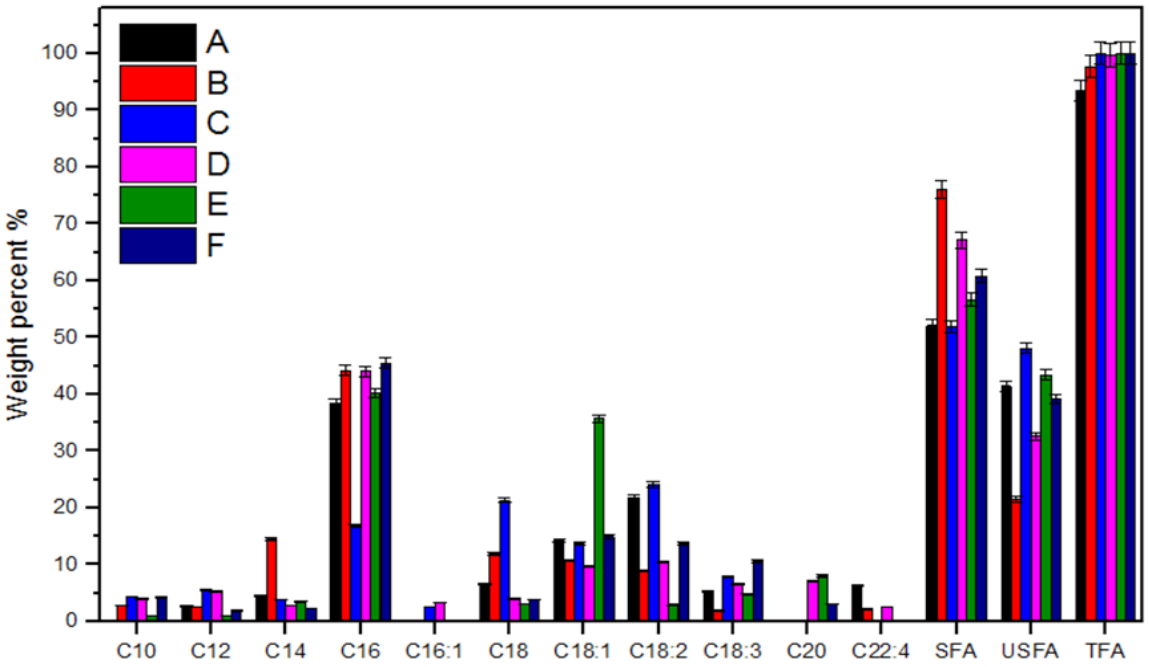

Fig. 5. Fatty acid profile of MASE *chlorella sp.* lipid extracted by different solvent systems

SFA is detected when using hexane: ethanol (1:1) (76.1%) followed by diethyl ether (1:1) (67.1%).

Conclusion

In the current study, the MASE technique showed the highest lipid yield recovered from wild mixed culture of *chlorella sp.* (37.9±0.13%). SEM micrographs showed that MASE had the most distributive effect for algae cells compared to UAE and CE. Six different solvents are tested for MASE extraction. Results indicate that a strong linear relation between the direct electric constant and yield percent. The composition of extracted lipids is promising for further conversion to biodiesel.

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تقنيات استخلاص الدهون من الطحالب في محطة معالجة المياه الصرف الصحي

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تعتمد عملية إنتاج الديزل الحيوي على خطوات أساسية مختلفة، منها استخراج الدهون من خلايا الطحالب. في هذه الدراسة، تم رصد الطحالب التي تنمو في محطة معالجة مياه الصرف الصحي (بني سويف، مصر) وكذلك الخواص الفيزوكيميائية لمياه الصرف من مارس 2016 إلى فبراير 2017. في هذه الدراسة، تم التركيز على الدهون المستخرجة من .Chlorella sp، حيث انه هو الطحلب السائد في عينات الطحالب التي تم تجميعها في الفترة من ديسمبر 2016 إلى فبراير 2017. تم تقييم محتوى الدهون و الأحماض الدهنية لـ .Chlorella sp باستخدام ثلاث طرق مختلفة (1) الطريقة التقليدية لاستخلاص الدهون(CE) و(2) الاستخلاص بواسطة الموجات فوق الصوتية (UAE) و (3) الاستخلاص بواسطة الميكروويف (MASE). أظهر MASE أعلى إنتاج للدهون وهو 37.9 ± 0.13٪. أظهرت صور SEM أن الميكروويف كان الأكثر تأثير علي خلايا الطحالب مقارنة مع UAE و CE. تم اختبار ستة مذيبات مختلفة باستخدام MASE وهي الهكسان: الأيزوبروبانول (3: 2) ، الهكسان: الإيثانول (1: 1)، الكلوروفورم: الميثانول (1: 1)، إيثيل الإيثر، الإيثانول والميثانول لدراسة العلاقة بين قطبية المذيبات وكمية الدهون المستخلصة. تم اثبات وجود علاقة خطية بين قطبية المذيبات وكمية الدهون المستخلصة، حيث ان الميثانول اظهر أعلى نسة استخلاص. من خلال تحليل GC للدهون المستخرجة أن تم استخلاص أعلى نسبة من حمض النخيلي (45.5 ٪) بواسطة الميثانول. التركيب الكيميائي للدهون المستخرجة يعطي وعودًا نحوالتحويل إلى الديزل الحيوي بجود عالية.