ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Variables Affecting the In-Situ Transesterification Via Ultrasonic from Microalgae and Comparing with Other Methods of Transesterification.

Abo El-Enin SA, El-Ibiari NN, Ola El-Ardy, and Guzine El Diwani.

Chem. Eng. and Pilot Plant Dept., National Research Centre, Dokki, Egypt.

ABSTRACT

The aim of this study is to investigate the variables affecting the in-situ transesterification via ultrasonic bath for *Spirulina platensis* microalgae species. The optimum conditions were the presence of co-solvent to algae oil mass ratio 3:1 v/w, catalyst percentage 100% to the oil weight and molar ratio of alcohol to algae oil mass 30:1 which gives 70% yield at room temperature for 30 minute. *Scendismus* species was subjected to one step transesterification via ultrasonic bath under the optimum conditions of *Spirulina platensis* and recorded 76% yield of fatty acid methyl ester. *Chlorella species* obtained from Alexandria University through MED algae project was subjected to two step transesterification via ultrasonic bath under the previous optimum conditions. Comparison between different methods of transesterification (extraction followed by transesterification process, direct transesterification process and direct transesterification via ultrasonic bath) was studied. The extraction and transesterification process resulted in higher ester yield than direct transesterification process either via ultrasonic bath or without. The GC analysis of crude biodiesel from *Spirulina platensis* , *Scendismus species* and *Chlorella species* showed that they are promising algal species for biodiesel production with high oxidation stability and high predicted cetane number due to high percentage of saturated fatty acid methyl ester (SFAME).

Keywords: In-situ transesterification - ultrasonic bath - *Spirulina platensis -Scendismus species- Chlorella species*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Biodiesel has proved to be a good substitute for fossil-based fuels due to its environmental advantages, engine performance, emissions reduction, lubricity and availability from renewable resources such as refined and waste vegetable oils. The increasing popularity of biodiesel has generated great demand for its commercial production methods, which in turn calls for the development of technically and economically sound process technologies.

Using microalgae to produce biodiesel has several advantages over production from terrestrial plant crops. Microalgae are fast growing photosynthetic microorganisms that can complete an entire growing cycle in few days, and can be cultivated in fresh water, sea water, or wastewater. Microalgae can be used to sequestrate carbon dioxide and can produce lipids at up to 77 wt. % of total biomass [1]. One option for producing biodiesel from microalgae is to convert algal lipids to fatty acid alkyl esters via transesterification [2, 3]. Transesterification occurs as a series of three reversible reactions: triglycerides (lipid compounds) are sequentially converted to di-glycerides, mono-glycerides and, finally to alkyl ester and glycerol .Conventionally, the oil is extracted and refined from microalgae prior to conversion to FAME. Several studies have focused on transesterification of microalgae oil using alkaline catalysts [4, 5] or acid catalysts [6–10].Most of the current research on the application of the conventional transesterification scheme on biomass oils has focused on the use of alkaline catalysts and virgin biomass oils with a free fatty acid (FFA) content of <0.5% w/w (based on oil weight). However, in considering the production of biodiesel from microalgae, the use of the alkaline catalyzed transesterification technology would not be suitable, due to the characteristically high FFA content of microalgae lipids. This is because the use of alkaline catalysts with high FFA containing oils would result in a partial saponification reaction, leading to the soap formation [11] and difficulties in the biodiesel separation and purification downstream. The use of inorganic acids, such as sulphuric or hydrochloric acids, as reaction catalysts have therefore been considered for microalgae lipid transesterification, due to its insensitivity to the free fatty acid (FFA) content of this oil feedstock, since both biodiesel producing through transesterification and esterification reactions are facilitated via acidic catalysis.

Another alternative to the conventional process, which is considered to have potential of reducing the processing units and costs of the fuel conversion process, is the 'in situ' transesterification or reactive extraction. The in situ process facilitates the conversion of the biomass oil to FAME directly from the oil bearing biomass, thereby eliminating the solvent extraction step required to obtain the oil feedstock as in the conventional method. This biodiesel production scheme could therefore aid in the simplification of the fuel conversion process, potentially reducing the overall process cost, hence lowering the final fuel product costs [12] as well. This method may be especially advantageous for use with microalgae, since the extraction of microalgae lipids is usually accomplished via solvent extraction and not with the use of cheaper physical extraction methods (for example, expellers) as utilized for conventional oil crops. The alcoholysis of the oil in the biomass directly has been shown to result in increased biodiesel yields, compared to the conventional route [13]. Process wastes and pollution could also be reduced [12] by this method.

The application of ultrasonic stirring to improve transesterification yields and reduce reaction times has been reported in the literature for the conventional alkaline catalyzed transesterification of various extracted biomass oils [14, 15]. This is due to the possibility for increased mass transfer between the immiscible liquid phases. The oil-alcohol phase boundary was reported to be disrupted due to the collapse of ultrasonically induced cavitations bubbles, in turn leading to an accelerated alkyl ester formation [15]. Few reports on the use of ultrasound stirring with acid catalysts or/and using the in-situ transesterification process with microalgae as the reaction feedstock were however found in the literature.

The aim of investigating ultrasound mixing for the in-situ process was to assess the extent to which the reacting alcohol volumes could be reduced, providing useful information on how the percentage mass oil to FAME conversion efficiency, biodiesel yields and reaction times would be affected.

Furthermore, the use of co-solvents in the in-situ transesterification process was also explored. Co-solvents have been reported [16-18] to improve the solubility of alcohol and accelerate the in-situ transesterification reaction.

This work aimed to study the variables affecting the in-situ transesterification via ultrasonic bath for *Spirulina platensis* microalgae species, and to compare the biodiesel yield percentage of the transesterification process on lipid extracts with direct transesterification of dry biomass and direct transesterification via ultrasonic bath. Under the optimum conditions of *Spirulina platensis, Scendismus* species was subjected to one step transesterification via ultrasonic bath and *Chlorella species* was subjected to two step transesterification via ultrasonic bath.

EXPERIMENTAL SECTION

Materials

Local strains of microalgae *Scendismus species* [19] and *Spirulina platensis* dry biomass were obtained from the Algal Biotechnology Unit, NRC, and Microbiology Department Soils, Water and Environment Res. Inst., ARC, respectively. While *Chlorella species* was obtained from Alexandria University through MED algae project. All chemicals and solvents used were analytical grade.

Culturing of Chlorella species

Chlorella sp. was cultivated in a cylindrical Plexiglas photo bioreactor with 15L working volume provided with mechanical stirring, aeration unit (v/v). Fluorescent lamps were arranged around the photo bio reactor (5000LUX) to supply illumination, at ambient temperature (25-30˚C). The growth medium, modified Guillard medium, was inoculated with the micro algal strain followed by daily determination of OD in a batch system. After harvesting, washing and drying, biomass dry weight (2g/L).

Lipid Extraction

Lipid extraction using magnetic stirring for two algal species

A mixture (75ml) of n-hexane and iso-propanol (3: 2 v/v), based on the principle that the lipid extraction co- solvent system must be containing one solvent adequately polar to remove the polar lipids from cell membranes and tissue constituents and the second solvent is being non-polar to extract the neutral lipids (20), was added to 1g of algal powder. The mixture was agitated at 800 rpm for 5-minutes using homogenizer Model Wise Tis HG-150 and then subjected to magnetic stirring at room temperature (30-35^{o}C) for 2hrs. Cells residue were removed by filtering through GF/C paper. The filtrate was transferred into a separating funnel and sufficient water (approximately 20 ml) was added to induce biphasic layering. After settling, the solvent mixture was partitioned into two distinct phases: a top dark-green hexane layer containing most of the extracted neutral lipids and a bottom light green aqueous-iso-propanol layer containing most of the co-extracted non-lipid contaminants and polar lipid. The hexane is evaporated using a rotary evaporator to enable gravimetric quantification of the lipid extract. The crude lipid was re-dissolved in hexane (approximately 5 ml) and transferred into a sealed glass vial for storage.

Lipid extraction using ultra-sonic bath

The same steps described above were carried out but under ultra-sonic bath (Model WUC-D10H, 60 Hz, 230 volts, 665 W, 3 AMPS) instead of magnetic stirrer for 30 minutes at room temperature under reflux condenser to avoid the solvent evaporation.

Analysis of crude lipid

The fatty acid profile of the extracted oil sample of two species were determined by converting the fatty acids in the oil to fatty acid methyl esters (FAMEs).The FAME composition was determined using a Gas-Chromatography (GC) with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm.) . Helium was used as carrier gas at a flow rate of 1 ml/min. The column was held at 150 ºC for 1 min and ramped to 240 ºC at rate 30 ºC/min, and it was then held at 240 ºC for 30 min. Standards were used to give rise to well-individualized peaks that allow the identification of the fatty acid composition.

Biodiesel production

Conventional Transesterification:

After lipid extraction for microalgae *Scendismus species*, *Spirulina platensis* and Chlorella species, the transesterification reaction is carried out using H_2SO_4 acid (98% concentration) as a catalyst (100% of the lipid mass), and methanol as solvent (methanol/lipid 30:1 ml/g) .When the reaction mixture (oil and methanol) reached 60 ^{o}C, sulphuric acid was added to start the reaction time (4-hours). After complete reaction, the excess alcohol is removed by evaporation using a rotary evaporator. The reaction mixture was transferred to a separating funnel after adding hexane to facilitate settling. Then mixture was partitioned into two distinct phases: a top hexane layer containing fatty acid methyl ester FAME and a bottom layer containing the glycerol. Then hexane phase was washed with distilled water and collected in a pre-weighed flask and left to dry at 60 ^{o}C till constant weight (21-22). The percentage yield of biodiesel was calculated using equation (1):

$$\text{Yield of biodiesel } \% = \frac{\text{Grams of biodiesel produced}}{\text{Grams of the oil used}} * 100 \quad \text{---------- (1)}$$

Conventional In-situ Transesterification

This method was carried out for both strains (*Scendismus species* and *Spirulina platensis*), where 10 grams of dried algae are charged into a conical flask (500ml) under reflux in which the acid catalyst 100% based on oil content by weight, methanol to algae oil biomass v/w ratio (30:1) and hexane as co- solvent to algae mass v/w ratio (3:1) were added to allow the extraction of lipid and its transesterification to take place simultaneously at 60^{o}C, for 240 minutes under constant stirring.

In- situ process via ultra- sonic waves for *Spirulina platensis*

10 grams of dried algae is charged into a conical flask (500ml) under reflux in which the acid catalyst [10,30,60,100,120 and 150 %] based on oil content, methanol at various methanol to algae oil biomass v/w ratio[5:1,10:1,15:1,20:1,30:1 and 35:1] and hexane as co-solvent to algae oil mass v/w ratio[0:1, 1:1, 2:1, 3:1, 4:1and 5:1] were added to allow the extraction of lipid and transesterification to take place simultaneously. The reaction was performed at room temperature, for 30 minutes in an ultrasonic bath.

In situ process via ultra- sonic waves for *Scendismus species*

The same process was carried out at the optimum conditions obtained in case of *Spirulina platensis*.

RESULTS AND DISCUSSION

Lipid content

In case of *Scendismus species* and *Spirulina platensis*, ultra –sonication technique led to a higher yield of extracted lipid than the conventional method. The lipid content of *Scendismus species* recorded 6% via ultra-sonic bath and 3.5% by conventional method. The ultra-sonic technique of *Spirulina platensis* produced 9% lipid content, while the conventional method resulted 6.5%, as shown in Figure (1).

It is clear that using ultrasonic bath increases the oil percent recovery due to the high frequency of ultrasonic bath employed and also reduces the time of extraction from 2hr to 0.5 hr. These results agree with previous studies [23].

The analysis of fatty acid composition in lipid content of *Scendismus species* and *Spirulina platensis* are given in Table 1 which shows that the most abundant saturated and unsaturated fatty acids are palmitic and oleic acid respectively which are reasonable for fuel properties (biodiesel) as reported before [24].

ISSN: 0975-8585

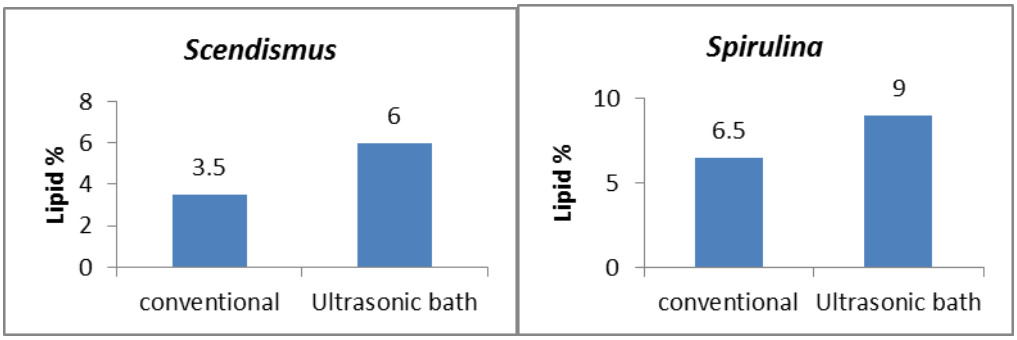

Figure 1: Effect of lipid extraction technique on the lipid percentage for both *Spirulina platensis* and *Scendismus species*

Table 1: Fatty Acid profile of *Spirulina platensis* and *Scendismus species* lipid

Fatty Acids	% Mass Fraction of *Spirulina platensis*	% Mass Fraction of *Scendismus species*
Lauric Acid C_{12}	1.8	5.5
Myristic Acid C_{14}	2.6	10.1
Palmitic Acid C16	61.1	60.6
Stearic Acid C_{18}	7.5	5.4
Arachidic Acid C_{20}	4.2	4.5
Palmitolic Acid $C_{16\,(1)}$	2.0	4.5
Oleic Acid $C_{\,18(1)}$	15.2	7.1
Linoleic $C_{18\,(2)}$	5.3	2 .1
Total Fatty Acid (TFAME)	99.7	99.8
Unsaturated (USFAME)	22.5	13.7
Saturated (SFAME)	77.2	86.1

The lipid percentage of *Chlorella species* was 7% of the dry weight using ultrasonic bath.

Transesterification

Conventional process

According to equation (1), transesterification of *Spirulina platensis* oil recorded 90.5% as yield percentage of fatty acid methyl ester (FAME). While, the yield percentage of FAME for *Scendismus species* and Chlorella species oils were 85%for both.

Analysis of fatty acid methyl ester for Chlorella species recorded the presence of saturated, monounsaturated and polyunsaturated fractions. Fatty acids are mostly saturated more than 60% are palmitic acid (C16:0) and stearic acid (18:0), which are considered as the most common fatty acids contained in biodiesel. They give good cetane number and oxidative stability to biodiesel.

Conventional In situ process

The yield percentage of FAME through in-situ transesterification of *Spirulina platensis* and *Scendismus species* by conventional in situ method was 67% and 75% respectively.

In situ via ultrasound Waves for *Spirulina platensis* strain

Effect of co-solvent

As shown in figure (2) the %yield was positively influenced with the addition of the co-solvent volume. As the hexane ratio to algae oil mass increases, the yield percentage increases till it reached 79%yield at ratio 4:1then it decreased to 56% at 5:1 .This is agree with [25] who explained that beyond the optimum level, the co-solvent acts as a barrier between the reactant methanol and oil molecules in the algal biomass. This was a

hindrance for the transesterification reaction to complete. Hence the optimum amount of co-solvent is important for the in-situ transesterification reaction to achieve the maximum percentage yield.

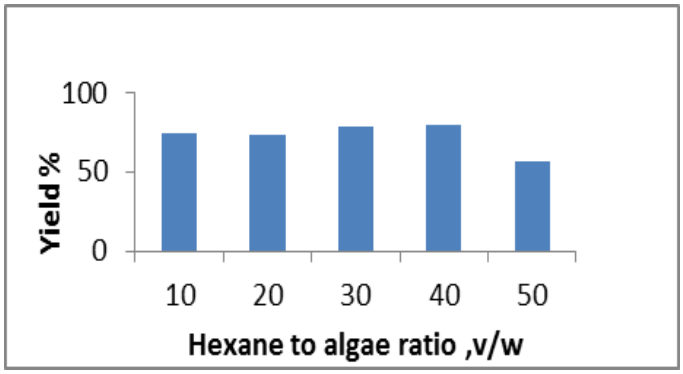

Figure 2: The effect of co-solvent ratio on the yield % of FAME

Effect of catalyst percentage

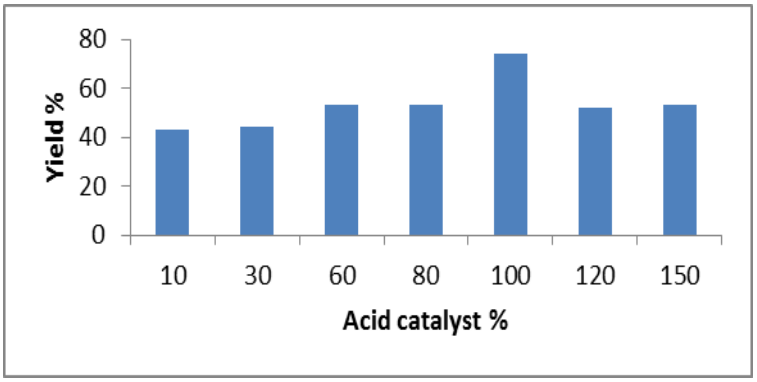

Figure 3: The effect of Acid catalyst % on the yield % of FAME

The effect of H_2SO_4 percentage was studied, with various percentages ranging from 10-150% to the oil content. The other parameters, such as methanol to biomass ratio 30:1, co-solvent ratio 3:1 at room temperature were maintained constant for 30 min in an ultrasonic bath. As shown in figure (3) the yield of methyl ester increased with an increase of catalyst percentage from 10 to 100%, then it decreased when the catalyst percentage increases to 120 and 150%. This may be due to the excess amount of catalyst causing the formation of water and soap that interferes the transesterification reaction. This result agrees with Prommuaket al [26] who found that biodiesel yield of *Chlorella vulgaris* increases with increasing amount of the catalyst from 2 to 6 % then it decreased when excess amount of catalyst 8% was used. Suganya et al [25] reported that 10% of H_2SO_4 concentration was found to be an optimum level for in situ transesterification of *E. Compressa;* also Shuit et al [27] reported that 15% wt H_2SO_4 concentration of seed biomass was found to be optimum for the in situ transesterification to achieve the best yield of 99.8% from Jatropha curcus L. seeds.

Effect of alcohol to biomass ratio

As shown in figure (4), the alcohol to biomass oil ratio has no significant difference on the biodiesel yield for the range employed from 5:1 to 25:1 and recorded a significant increasing at ratio 30:1and returns to sharp decreasing at higher ratio. Suganya et al [25] reported that the methyl ether yield was increased when increasing the methanol ratio from 4:1 to 5.5:1. For further increase of methanol ratio up to 6:1, there was no significant difference on the biodiesel yield. Moreover, for the excess of methanol to biomass ratio above 6:1, the methyl ester yield was observed to be decreased and also slowed down the separation process [27].

ISSN: 0975-8585

From the above results, the optimum conditions for direct transesterification via ultrasonic bath to produce biodiesel were the presence of co-solvent at ratio 3:1 v/w, catalyst percentage 100% to the oil weight and molar ratio of alcohol to algae oil weight 30:1.

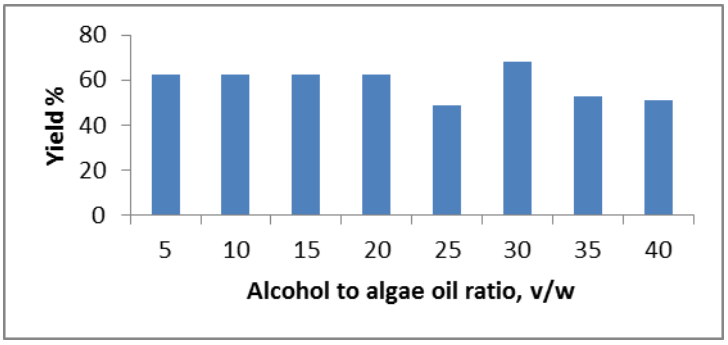

Figure 4: The effect of alcohol to biomass oil ratio the yield % of FAME

The biodiesel yield at the optimum conditions was 70% and shows a dark green color. The analysis of GC showed that the purity percentage of biodiesel under the optimum conditions was 87% having a high proportion of saturated (78.5%) and mono unsaturated (18.7%) fatty acid methyl esters. The major fatty acids methyl ester were palmitic acid (C_{16-0}, 65.6%), stearic acid (C_{18-0}, 7.5%), oleic acid (C_{18-1}, 17.2%).The high percentage of palmitic acid methyl ester which is not liable to degradation, considering its high stability [28], [29]. The predicted cetane number (CN) of biodiesel obtained under the optimum conditions according to equation, [$CN = \sum X_{ME}\ (wt.\ \%)\ CN_{ME}$] (30) was 68.The higher the cetane number the better is its ignition properties (31, 30).

In situ transesterification via ultrasound Waves for *Scendismus species*

The experiment was done at the optimum conditions of *Spirulina platensis* which were 100% of acid catalyst, 30:1 (v/w) ratio of alcohol to biomass oil, 3:1 co-solvent at room temperature and 30 min. in ultra sound bath. The yield percentage of fatty acid methyl ester was 76%. The GC analysis recorded that the produced biodiesel having purity of 98% with high stability due to the high percentage of palmitic acid methyl ester (C_{16-0}, 55.0%) [28, 29].The high percentage of total saturated fatty acids [C_{12-0} - C_{20-0} , 84.4%] recorded high value of cetane number (67%).These results agreed with the previous studies(30,31),and shows that the ignition properties of such algal biodiesel is satisfactory.

Comparison between different methods:

The application of extraction followed by transesterification process to both stains *Spirulina platensis* and *Scendismus species* was compared with in-situ process and in-situ via ultra-sonic process and the biodiesel yield was expressed as its weight relative to the dry biomass. According to figure (5) the extraction at room temperature using n-hexane and iso-propanol in an ultra-sonic bath followed by transesterification at 60 $^{\circ}$C (a two stage method) resulted in high biodiesel yield (90.5%,85% respectively)than direct transesterification of the algae biomass (67%,75%) in case of in-situ and (70%,76%) in case of in-situ via ultrasonic respectively. These results agree with [21] who mentioned that the extraction at room temperature using methanol followed by transesterification at 60^{o}C or 100^{o}C (a two stage method) resulted in high biodiesel yield than direct transesterification of the algae biomass.

Prommuak [26] studied the performance of a single step biodiesel production with that of the conventional two step method using *C. vulgaris* as a model system. They concluded that the yield of FAMEs obtained from the conventional method appeared to be significantly higher. These results are inconsistent with Harrington [13] who discussed that the application of in situ acid catalyzed process offered conversion rate improvements when compared with the conventional process. Also Johnson and Wen [32] revealed that the greater yield was obtained by applying the single step method with *Schizochytrium limacinum* .The reason for these differences in results may be due to the different bio-characteristics of the microalgae strain. In this study the in-situ or single step process can probably be modified by using ultrasonic probe instead of ultrasonic bath and increase the time and temperature.

ISSN: 0975-8585

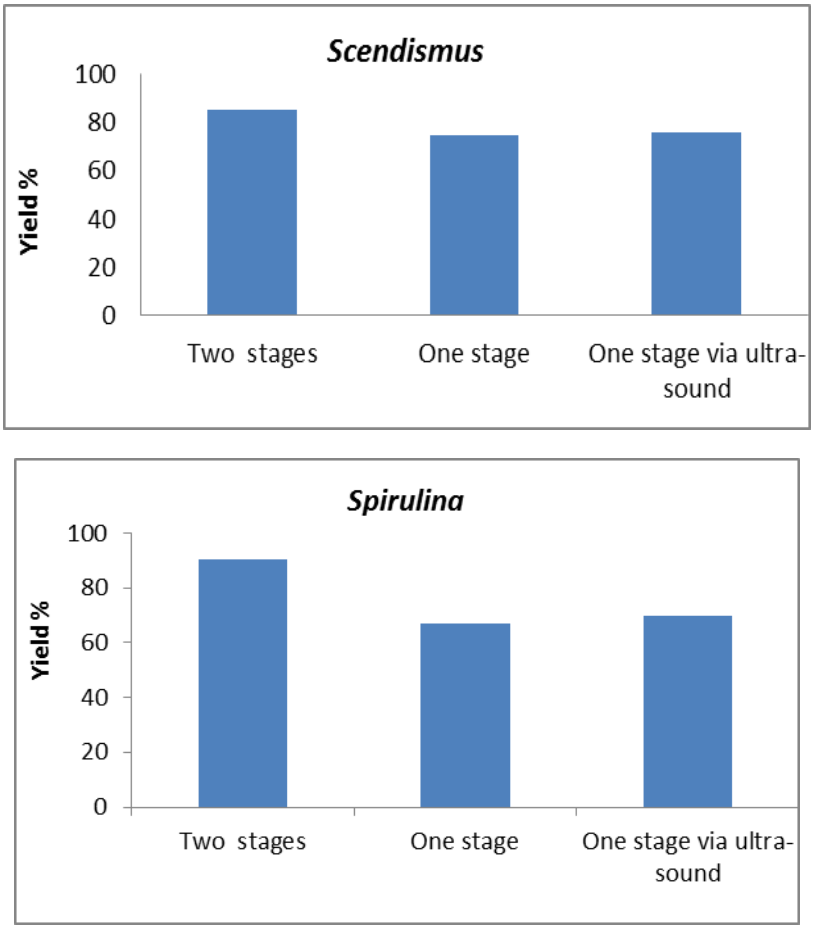

Figure 5: Comparison between different methods of transesterification

CONCLUSION

Variables affecting the in-situ transesterification via ultrasonic bath for *Spirulina platensis* microalgae species were studied. The optimum conditions were the presence of co-solvent to algae mass ratio 3:1 v/w, catalyst percentage 100% to the oil weight and molar ratio of alcohol to algae weight 30:1, which gives 70%yield at room temperature for 30 minute. *Scendismus obliques* species was subjected to one step transesterification via ultrasonic bath under the optimum conditions of *Spirulina platensis* and recorded 76% yield of fatty acid methyl ester. Direct transesterification via ultrasonic bath was compared with direct transesterification from dry biomass (one step) and transesterification process on lipid extracts (two steps). In case of two step transesterification extraction two methods (magnetic stirrer and ultrasonic bath) were applied for lipids extraction from the dry biomass using n-hexane: iso-propanol (3:2v/v). The method using ultrasonic bath showed the highest lipid extraction. The extraction and transesterification process resulted in higher ester yield (90.5 %, 85%, 85% for *Spirulina platensis, Scendismus species* and Chlorella species respectively) than direct transesterification process either via ultrasonic bath (70% for *Spirulina platensis* and76 %for *Scendismus species*) or without (67% for *Spirulina platensis* and75 %for *Scendismus species).*So extraction followed by transesterification process (two steps) may be specially advantage for microalgae biomass in comparison to direct transesterification (one step) either via ultrasonic or without. This result may be attributed to the different bio-characteristics of the microalgae strain.

The production of biodiesel from microalgae is still in the research and development stages, with laboratory scale transesterification trials (in situ via ultrasonic bath, in this study. Further experimental work is recommended to permit the larger scale and industrial scale design and economic evaluation.

ISSN: 0975-8585

ACKNOWLEDGMENT

This project was supported financially by the Science and Technology Development Fund (STDF), Egypt. GRANT NO: 4991 and supported by the project "Production of Biofuels from Microalgae in Selected Mediterranean Countries" Ref.No 1-b/2.2/099 from the Program (ENPI) European Neighborhood and Partnership Instrument- Mediterranean Sea Basin Joint Operational Program.

REFERENCES

[1] Chisti Y. Bio-techno Adv 2007; 25:294–306.
[2] Lam MK, Lee KT, and Mohamed AR. Bio-techno-Adv 2010; 28:500–18.
[3] Vyas AP, Verma JL, Subrahmanyam N. Fuel 2010; 89:1–9.
[4] Vijayaraghavan K, Hemanathan K. Energy Fuels 2009; 23:5448–53.
[5] Hossain ABMS, Salleh A, Boyce AN, Chowdhury P, Naqiuddin M. Am J Bio-chem Bio-techno 2008; 4:250-4
[6] Miao X, Wu Q. Bioresour Technol 2006; 97:841–6.
[7] Nagle N, Lemke P. Appl Biochem Biotechnol 1990; 24–25:355–61.
[8] Xu H, Miao X, Wu Q. J Biotechnol 2006;126:499–507
[9] Li X, Xu H, Wu Q. Biotechnol Bioeng 2007; 98:764–71.
[10] Johnson MB, Wen Z. Energy Fuels 2009; 23:5179–83.
[11] Al-Zuhair S. Biofuels Bio-prod Bio-refining 2007; 1:57–66.
[12] Haas MJ, Scott KM, Foglia TA, Marmer WN. J Am Oil Chem Soc 2007; 84:963–70.
[13] Harrington KJ, D'Arcy-Evans C. J Am Oil Chem Soc 1985; 62:1009
[14] Armeta ER, Vinatoru M, Burja AM, Kralovec JA, Barrow CJ. J Am Oil Chem Soc 2007;84:1045-52.
[15] Stavarache C, Vinatoru M, Nishimura R, Maeda Y. Ultrasonics Sonochem2005; 12:367-72.
[16] Zeng JL, et al. Ind Eng Chem Re 48: 850 – 856.
[17] Mao V, SK Konar, and DGB Boocock. J American Oil Chem Soc 2004; 81:803-808
[18] Boocock DGB, SK Komar, V Mao, and H Sidi. Biomass Bioenergy 1998;11:43-50.
[19] El-Sayed AB. New Egyptian J Microbiol 2004;8:376-385
[20] WW Christie. Lipid Anlysis, 2nd edn, Pergamon Press, New York, 1982
[21] Marcelo G Montes, D'Oca, Carolina V Vieˆgas, Juliana S Lemo˜es, Eriksen K Miyasaki, Joaquı´n A Moro´n-Villarreyes, Ednei G Primel, Paulo C Abreu. Biomass and Bioenergy 2011;35:1533- 1538.
[22] Piyushi Nautiyal, KA Subramanian, MG Dastidar. Fuel Proc Technol 2014;120:79–88
[23] NN Ibiari, SA Abo El-Enin, NK Attia and G El-Diwani. J American Sci 2010; 6(12)
[24] Ola El-Ardy, Sanaa A. Abo El-Enin, Nermin A. El Semary and Guzine El Diwani. Afinidad LXIX, 558, Abril - Junio 2012
[25] Tamilarasan Suganya, Ramachandran Kasirajan, Sahadevan Renganathan. Bioresour Technol 2014;156:283-290
[26] Chattip Prommuak, Prasert Pavasant, Armando T Quitain, Motonobu Goto, and Artiwan Shotipruk. Eng J 2012;16(5).
[27] Shuit SH, Lee KT, Kamaruddin AH, Yusup S. Fuel 2010;89:527–530.
[28] Prafulla D Patil, Harvind Reddy, Tapaswy Muppaneni, Tanner Schaub, Omar Holguin Peter Cooke, Peter Lammers, Nagamany Nirmalakhandan,, Yin Li, Xiuyang Lu, Shuguang Deng. Bioresour Technol 2013;139:308–315.
[29] He H, Tao W, Zhu S. Fuel 2007;86:442–447.
[30] S Hawash, SA Abo El- Enin & G El Diwani. International Journal of Agriculture Innovations and Research 2014;2(6):2319-1473.
[31] Meher LC, Vidya Sagar D, Naik SN. Renew Sust Energy Rev 2006;10:248–268.
[32] MB Johonson and Wen. Energy Fuels 2009;23:5179-5183.