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OPTIMIZATION OF MICROALGAE OIL EXTRACTION UNDER ULTRASOUND AND MICROWAVE IRRADIATION

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Abstract

Background. Microalgae are one of the most promising biofuel sources that the world has to offer; nevertheless the conversion process is hampered by technical and economic problems that are mainly related to de-watering and extraction. The efficiency of the process can be dramatically improved by means of non-conventional techniques such as ultrasound (US) and microwaves (MW). Scaling-up feasibility is strictly linked to reactor efficiency, energy consumption, environmental impact and overall cost. In the present work, the optimization of lipid extraction from *Nannochloropsis gaditana* microalga is investigated.

Results. A series of selected solvent mixtures and procedures have been tested and compared. Conventional extraction procedures with chloroform/methanol mixtures and fast US- and MW-assisted extractions with methanol gave comparable fatty acid (FA) w/w% from dried microalgae. The highest extraction yield and lowest energy

consumption was found to occur under MW irradiation, especially at high temperatures and under pressure.

Conclusion. This study highlights the advantages of US- and MW-assisted lipid extraction from microalgae, both in term of efficiency and operational costs.

Keywords

Microalgae; Extraction; Microwaves; Ultrasound; Biofuels

Introduction

One of the main scientific tasks of the third millennium is achieving the ability to exploit renewable energy sources cost-effectively in the pursuit of minimal environmental impact.[1] Many alternatives have been proposed and, in the case of the transportation industry, biofuels seem to be the most promising. They are already in use in some countries and further expansion is expected.[2-4] Several technological, economic and social barriers have yet to be overcome in conventional biofuel production. The fact that it competes for use of arable land with food production has also started an ethical debate in emerging economies because of high water and fertiliser requirements and the issue of bio-diversity conservation.[5] For these reasons, the replacement of classical biofuel crops by microalgae is gaining ever more interest because they can produce up to 10 times more oil per cultivated area than traditional oil plants.[1, 6-11] There are other benefits to be gained from the use of aquatic as opposed to terrestrial biomass; (i) relatively fast growth allows harvesting to be carried out on a daily basis, (ii) microalgae use light more efficiently, (iii) their growth is unaffected by weather conditions, (iv) they have lower water consumption needs than oilseed crops, (v) there

is no need for the use of herbicides and pesticides in their cultivation, (vi) they can be grown in brackish water on non-arable land and use waste water as a source of nutrients (specially nitrogen), (vii) microalgae biomass production can affect the biofixation of waste CO_2 (1 kg of dry algal biomass utilises about 1.83 kg of CO_2), (viii) a larger number of species are available and their genetic manipulation in order to modify their chemical composition (e.g. lipid content) is relatively easy, (ix) besides biofuels, several valuable co-products (such as omega-3, carotenoids and poly unsaturated fatty acids "PUFA") with numerous applications (human nutrition, animal feed and aquaculture, biofertilization, as a source of PUFA and proteins) can be obtained in the process.[4, 8, 10-17] All these advantages explain why microalgae are regarded as "biotechnology's green gold".[18] Despite these advantages, several reviews have recently attempted to answer the questions about the true commercial viability of large scale production of biodiesel from microalgae, analyzing all the steps of the process from the energy balance point of view.[19, 20] Currently, the drying and extraction processes represent the most critical steps in terms of energy consumption.[6-8, 10, 11, 18, 19]

Conventional extraction techniques are usually time-consuming and may cause degradation or unwanted chemical changes in the products. Working at higher temperatures can lower treatment times but leads to processes with high energy demands. Of the novel extraction techniques that are gaining interest, US- and MW-assisted processes (UAE and MAE respectively) are playing the leading role. Microalgae extraction accomplishes two of the "Six Principles of Green Extraction"[21] *per se* (innovation by selection of varieties and use of renewable plant resources, and secondly, the production of co-products instead of waste that can include the bio- and agro-refining industries). The use of UAE and MAE covers two additional principles

(reducing energy consumption by energy recovery and the use of innovative technologies, and secondly, reducing unit operations and favouring safe, robust and controlled processes) making this an even *greener* process. Several works have been published on the efficiency of the extraction techniques, from the extraction yield point of view, and have concluded that the UAE and MAE processes are the most efficient.[4, 6-8, 22, 23]

Recent papers have proposed different lipid extraction methods from microalgae, and some show improvements with MW or US-assisted protocols.[14, 24, 25] However, no work has so far dealt with their efficiency from an energy viewpoint. The aim of the present work is to fill this gap by focusing on the yields and energy consumption of the UAE and MAE of bio-oils from the microalgae *Nannochloropsis gaditana*, using the most suitable solvent mixture.

Experimental

Raw Materials

The microalgae selected for the extraction study was *Nannochloropsis gaditana* supplied by Exeleria, S.L. - Spain (fatty acid percentage in cell dry weight near 13%, CleanAlgae). The algal biomass was dried by the supplier.

Equipment

UAE was performed using probe systems developed in our laboratories in collaboration with Danacamerini (Torino, Italy). The working power setting was 100 W. Two high-power devices were used: an immersion horn (19.5 kHz), and a cavitating tube, which is a cup horn-like system consisting of a thin hollow titanium cylinder fixed to a booster

(21.5 kHz).[22] The extraction temperature was kept between 50 and 60°C by means of a thermostated cooling system (Fig. 1).

MAE was carried out in a professional multimode oven (2.45 GHz, Microsynth-Milestone, BG Italy), in a closed PTFE (Teflon®) vessel. The extraction temperature was kept constant at either 60 or 90°C and monitored by an optical fibre thermometer. The MW device modulated the power used with the aim of keeping the operating temperature constant. The power varied in the range of 25-30 W for the extractions carried out at 60°C and in the range of 30-35 W for the extraction performed at 90°C.

FIGURE 1

Lipid extraction

A weighed amount of dried microalgae (5 g) was suspended in the solvent (50 mL, ratio of 1:10 g/mL, and separately 250 mL, ratio of 1:50 g/mL). The different techniques were applied in a time range of 5 - 60 minutes and at temperatures from room temperature (rt) up to 90°C.

A number of solvents were tested; a $H_2O/CHCl_3$/MeOH 1:1:2 mixture (Bligh and Dyer),[26] a $CHCl_3$/MeOH 2:1 mixture (Folch)[27] hexane, acetone and MeOH. Once the extraction was completed, the mixture was filtered by means of a sintered glass Buchner funnel and the solvent was evaporated. In the case of the $H_2O/CHCl_3$/MeOH 1:1:2 mixture, H_2O and $CHCl_3$ (1:1) were added to form a biphasic system after filtration. In the case of the $CHCl_3$/MeOH 2:1 mixture, H_2O was added to form a biphasic system after filtration giving a final ratio of $CHCl_3$/MeOH/H_2O 8:4:3. The organic phase

containing the lipid fraction was separated and evaporated under vacuum. When necessary, the aqueous layer was extracted with $CHCl_3$ (1-2 x 20-50 mL).

Fatty acid (FA) characterization

Several derivatization methods were tested and the most efficient protocol for the transesterification of the triglycerides and other ester derivatives (i.e. carotenoids FA esters) and the esterification of any free FA present in our vegetal matrix was selected.

Method A was proposed by Ríos *et al*. in 2013.[25] A weighed amount of extract (ca. 30 mg) and an internal standard (FA C23, ca. 0.4 mg) were suspended in a MeOH/HCl/$CHCl_3$ (4.5 mL) mixture and heated at 80°C under magnetic stirring for 4 h. After cooling, H_2O (1.5 mL) was added and the sample was well mixed. Finally, a 4:1 Hex/H_2O mixture (3 x 4 mL) was added to the mixture to facilitate the extraction of the lipidic fraction. The organic layers were collected, dried on anhydrous Na_2SO_4 and filtered before GC analysis.

In method B,[28] a weighed amount of extract (ca. 30 mg) and an internal standard (FA C23, ca. 0.4 mg) were suspended in a MeOH/H_2SO_4 mixture (5 mL) and heated at 80°C under magnetic stirring for 4 h. After cooling, H_2O (10 mL) was added and the sample was well mixed. Finally, hexane (2 x 3 mL) was added to the mixture to facilitate the extraction of the lipidic fraction. The organic layers were collected, dried on anhydrous Na_2SO_4 and filtered before GC analysis.

In method C, the extract was treated according to the protocol first proposed by Lepage and Roy[29] and later modified by Xu *et al.*.[30] A weighed amount of extract (ca. 30 mg) and an internal standard (FA C23, ca. 0.4 mg) were suspended in a 0.5 N NaOH solution in MeOH (3 mL) and heated at 75°C under magnetic stirring for 10 min. After

cooling, a 1 N solution of acetyl chloride in MeOH (1 mL) was added to the mixture and kept at 75°C under magnetic stirring for 10 min. Finally, H_2O (3 mL) and hexane (2 x 2 mL) were added to the mixture. The heterogeneous sample was vigorously shaken. After phase separation, the upper layers (hexane) were collected, dried on anhydrous Na_2SO_4 and filtered before GC analysis.

After some analyses on two different extracts, method A was chosen for all samples, seeing as it gave the best FA recovery results (for details, see Supporting Information).

The GC-MS qualitative analyses were performed in an Agilent Technologies 6850 Network GC System with a 5973 Network Mass Selective Detector and 7683B Automatic Sampler, using a capillary column (HP-5MS 5% Phenyl Methyl Siloxane, length 30 m; i.d. 0.25 mm; film thickness 0.25 μm).

GC-MS quantitative analyses were performed in an Agilent Technologies 7820A Network GC System equipped with a FID detector, using a capillary column (Mega WAX, length 30 m; i.d. 0.25 mm; film thickness 0.25 μm) on the basis of the internal standard amount.

FAME identification was performed by checking the correspondence with C8-C24 saturated and unsaturated external standards (Sigma-Aldrich), which were prepared in solution with GC grade cyclohexane and with Wiley275 and NIST05 GC libraries (only for GC-MS analyses). Additional experimental information is provided in the supplementary material.

Energy calculation

Energy consumption determination was different for each technique and depended on the equipment used. In the case of US devices, a working power was set which,

multiplied by the extraction time, gives the total energy consumption. In the case of the MW device, as mentioned previously, the power provided by the device is modulated with the aim of maintaining the operating temperature. Thus, it is not possible to multiply the power by extraction time, since power is not constant. However, the device software has the facility of integrating the power vs. time curve in order to obtain the energy consumed.

Results and discussion

Solvent selection

The first step in the research procedure was to select the best solvent or solvent mixture and plant/solvent ratio. The experiments were performed at room temperature for 1 h under magnetic stirring (conventional extraction). Table 1 shows the yields achieved in each experiment. The results are expressed as follows:

$$Extraction\ yield\ (\%) = \frac{mass\ of\ extract}{mass\ of\ dried\ microalgae} \cdot 100$$

$$FA/Ex\ (\%) = \frac{mass\ of\ extracted\ FA}{mass\ of\ extract} \cdot 100$$

$$FA/DM\ (\%) = \frac{mass\ of\ extracted\ FA}{mass\ of\ dried\ microalgae} \cdot 100$$

TABLE 1

$CHCl_3$/MeOH mixtures, unlike hexane, enable both polar and non-polar lipids to be extracted. In the literature, two different methods are proposed for lipid extraction; namely the Bligh and Dyer (BD) and Folch (FO) methods. These protocols were tested at different plant/solvent ratios in order to identify the best conditions for a reference extraction (Table 1). The BD protocol with a 1:10 plant/solvent ratio gave the lowest extraction yield (8.9%), showing, however, high selectivity in lipid extraction (81.24%) with a FA/DM w/w av. % of 6.74. This percentage was increased to 12.18% with a 1:50 plant/solvent ratio. The FO procedure generally gave a higher extraction yield and also to a higher FA/DM w/w av. %. Using a 1:50 ratio, the extraction yield was 28.1%, with 15.40% free FA in dried microalgae. The weight of these extract can be considered a gravimetric measurement of the lipid content in the vegetal matrix.[31]

Other solvents were tested in lipid extraction from microalgae using the lowest plant/solvent ratio (1:10) in order to find an alternative to $CHCl_3$/MeOH mixtures. The extractions carried out in hexane and acetone were not satisfactory and gave only 0.73% and 1.11% yields, respectively. As reported in Table 1, the best solvent was MeOH which gave a 33.00% extraction yield and a comparable value of FA/DM w/w % to FO protocol (1:10), 9.71 *vs* 10.56%.

We selected MAE and UAE protocols with MeOH (1:10) to maximize extraction yields and a FA/DM w/w% ratio to reduce solvent and energy consumption while also avoiding the use of toxic chlorinated solvents.

Extraction yields

With conventional extraction methods, yields increased with temperature; around 33% at rt (1 h), 38.3% at 60°C (45 min) (Tables 1 and 2, respectively). All extractions were,

therefore, carried out at 60°C (MeOH boiling point 65°C), with the exception of MAE which was also performed at 90°C and under pressure (MW u.p.).

Table 2 summarizes the work of optimising extraction time and results are outlined in Graph 1.

TABLE 2

Conventional extraction protocols only gave a 36.2% yield and a 12.27% FA/DM % in 30 min at 60°C. When the time was extended to 45 min, yield grew up to 38.2% and FA/DM % to 13.59%.

GRAPH 1

UAE carried out in the cavitating tube provided the same extraction yield in 20 min as the conventional technique did in 45 min (around 38.1%), whereas the US horn was not able to equal this value (36.2% yield) in the same time (see Graph 1). However, from the FA/DM % value, it can be seen that both the US extraction in the cavitating tube and with the US horn gave a higher FA yield in 20 min than conventional extraction did in 45 min (see Table 2 and Graphic 2), 14.76% and 14.11%, respectively.

Both techniques were superior to conventional extraction. However, the cavitating tube protocol was preferred as it afforded better process control. The results obtained using MAE were extremely interesting. When the extraction temperature was set at 60°C, we obtained a high extraction yield in 20 min (39.6%) and a FA/DM % that was slightly higher than conventional extraction could give in 45 min and the US horn in 20 min. Extractions carried out in 10 min gave significantly lower yields, but, conversely, the best FA selectivity (41.53%). However, when the extraction temperature was increased

to 90°C under pressure, the best results were achieved in only 10 min (see Table 2 and Graphic 2).

The comparison of yields and efficiency in UAE and MAE is strictly related to the type of reactor employed and the mode of use (temperature, pressure).

GRAPHIC 2

Table 3 reports extract characterization data that was obtained under the best conditions, compared to conventional BD and FO extractions. The FA composition of the extracts obtained under US irradiation (cav. tube, 20 min, 50-60°C, 1:10 plant/MeOH ratio) and MW u.p. (10 min, 90°C, 1:10 plant/MeOH ratio) show no significant difference to the conventional FO protocol characterization results (60 min, rt, 1:50 plant/solvent- $CHCl_3$/MeOH 2:1 mixture- ratio).

TABLE 3

Energy consumption

Table 4 shows the energy consumption data expressed as energy consumed per gram of total extract ($W \cdot h/g_{Ex}$), per gram of FA extracted ($W \cdot h/g_{FA}$) and per gram of dried, treated microalgae ($W \cdot h/g_{DM}$).

TABLE 4

Generally, the energy consumption in MAE is lower than in UAE, however fast sonication treatments (5 min) at high power density may be competitive, this is also related to the reactor efficiency. The lowest energy consumption was obtained when the

extraction was carried out at 60°C in 10 min, but the extraction yields were slightly higher at 90°C in 10 min. In the light of these findings, it is clear that the selection of the best operating conditions needs to be addressed using a wider approach that includes the whole production process, from microalgae cultivation to final product.

To show how far this technology has progressed and to underline the need for further development, the energy consumption of these techniques may be compared with the theoretical maximum energy that can be obtained from microalgae. In a report entitled National Algal Biofuels Technology Roadmap,[9] the U.S. Department of Energy established that a maximum amount of energy of approximately 5 Wh/g can be obtained. If this is the case, only MAE can currently be used for the extraction of bio-oils from microalgae to produce biofuels.

Conclusions

This work confirms the advantages of UAE and MAE in the production of bio-oils from microalgae. The best solvent for the classic extraction process was a $CHCl_3$/MeOH mixture and MeOH for UAE and MAE. All of these optimized processes gave comparable FA/DM w/w%. Extraction under MW and US required a lower amount of solvent, avoided chlorinated waste and proceeded in a shorter extraction time. These techniques also enable one-pot sequential extraction/transesterification for biodiesel production.[32] All these advantages, together with the lower energy consumption in MW reactors, may further reduce the environmental impact of the extraction process. Recent industrial advances in MW-assisted biodiesel production in MW-flow reactors[33] make it easy to expect a fully automated continuous flow- microalgae MAE in the near future.

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Table 1. Extraction yield comparison of different solvents and plant/solvent ratio, at rt for 1 h (derivatization method A)

Sample	Plant/solvent ratio	Extraction Yield (%)	FA/Ex[a] (% av.)	FA/DM[b] (% av.)
Bligh Dyer	1:10	8.9	81.24	6.74
Bligh Dyer	1:50	15.5	78.47	12.18
Folch	1:10	12.3	85.90	10.56
Folch	1:50	28.1	54.76	15.40
Hexane	1:10	0.73	-	-
Acetone	1:10	1.1	-	-
MeOH	1:10	33.0	32.00	9.71

[a] FA/Ex (% av.) = FA w/w average percentage in the extract, [b] FA/DM (% av.) = FA w/w average percentage in dried microalgae.

Table 2. Yields obtained using different extraction techniques.

Technique	Temp. (°C)	Time (min)	Extraction yield (%)	FA/Ex[a] (%)	FA/DM[b] (%)
Conventional	60	15	31.3	33.04	10.33±0.29
Conventional	60	30	36.2	33.90	12.27±0.35
Conventional	60	45	38.3	35.50	13.59±0.39
US horn	50-60	5	31.4	38.28	12.00±0.34
US horn	50-60	10	33.0	37.97	12.52±0.36
US horn	50-60	15	35.8	36.09	12.92±0.37
US horn	50-60	20	36.2	38.91	14.11±0.40
US cav. tube	50-60	5	31.5	35.66	11.21±0.32
US cav. tube	50-60	10	32.6	37.89	12.34±0.36
US cav. tube	50-60	15	36.9	36.04	13.29±0.38
US cav. tube	50-60	20	38.1	38.72	14.76±0.42
MW	60	10	29.7	41.53	12.33±0.36
MW	60	20	39.6	36.24	14.36±0.41
MW (u.p.)	90	10	40.0	37.06	14.82±0.43

[a] FA/Ex (% av.) = FA w/w average percentage in the extract, [b] FA/DM (% av.) = FA w/w average percentage in dried microalgae.

Table 3. FA w/w percentage in dried microalgae: comparison of the best result achieved with each technique.

FA	**BD 1:50** **1 h, rt**	**FO 1:50** **1 h, rt**	**Conv. 1:10** **45 min, 60°C**	**US horn 1:10** **20 min, 50-60°C**	**US cav. tube 1:10** **20 min, 50-60°C**	**MW 1:10** **20 min, 60°C**	**MW (u.p.) 1:10** **10 min, 90°C**
C14	0.416	0.560	0.673	0.575	0.615	0.583	0.595
C16	3.104	3.680	3.628	3.567	3.691	3.598	3.651
C16:1 (n9)	1.934	2.440	2.226	2.274	2.342	2.351	2.327
C16:2 (n6)	0.711	0.926	0.863	0.872	0.943	0.884	0.935
C16:3 (n3)	0.908	1.172	1.038	1.142	1.174	1.136	1.175
C18:1 (n9)	0.517	0.637	0.526	0.613	0.638	0.627	0.622
C18:2 (n6)	1.730	2.309	1.833	2.049	2.196	2.093	2.258
C18:3 (n3)	1.438	1.947	1.530	1.712	1.773	1.742	1.801
C20:4 (n6)	0.259	0.335	0.252	0.265	0.286	0.286	0.278
C20:5 (n3)	1.047	1.392	1.020	1.045	1.098	1.055	1.176

Table 4. Energy consumption of MAE and UAE.

Technique	**Temperature (°C)**	**Time (min)**	**Consume**		
			W·h/g $_{Ex}$	**W·h/g $_{FA}$**	**W·h/g $_{DM}$**
US horn	50-60	5	5.3	13.9	1.7
US horn	50-60	10	10.1	26.6	3.3
US horn	50-60	15	14.0	38.7	5.0
US horn	50-60	20	18.4	47.2	6.7
US cav. tube	50-60	5	5.3	14.9	1.7
US cav. tube	50-60	10	10.2	27.0	3.3
US cav. tube	50-60	15	13.6	37.6	5.0
US cav. tube	50-60	20	17.5	45.2	6.7
MW	60	10	2.9	6.9	0.9
MW	60	20	4.3	11.8	1.7
MW (u.p.)	90	10	4.1	10.9	1.6

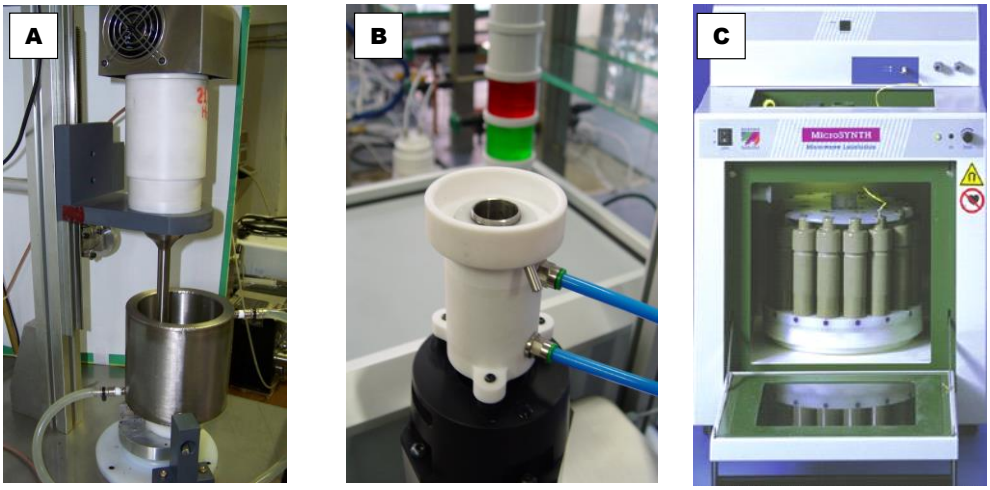
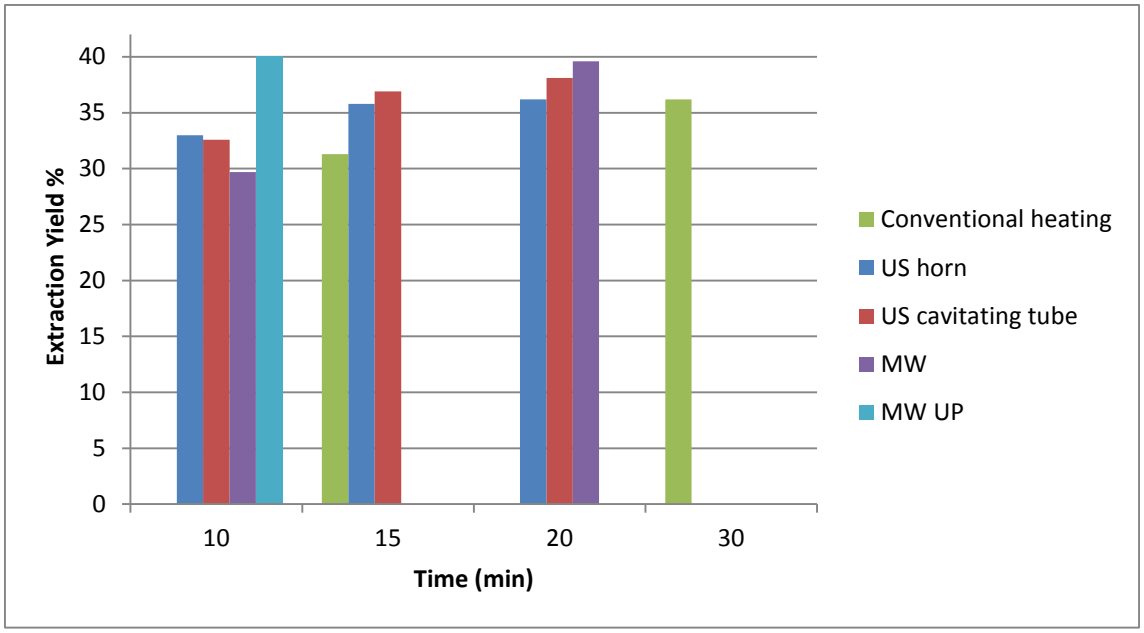
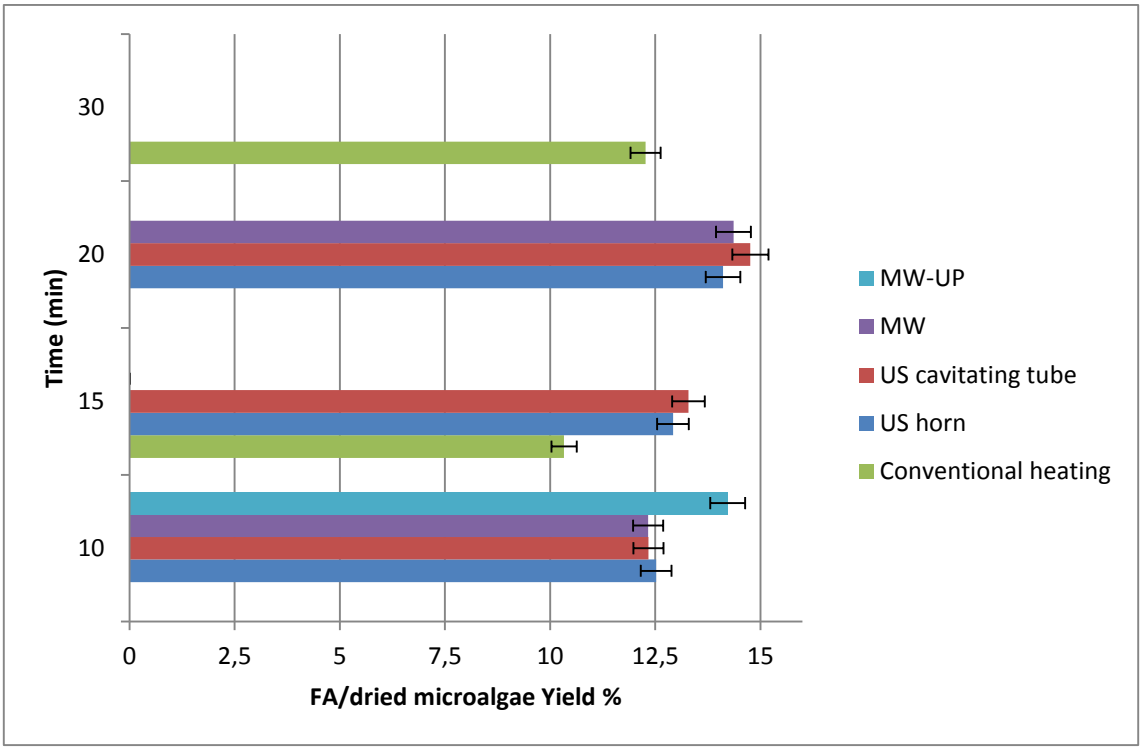


Figure 1. A) US horn B) US cavitation tube C) closed MW vessels.



Graph 1. Extraction yield (oil %) of *Nannochloropsis gaditana* using MeOH (1:10 ratio) and different techniques at different times.



Graph 2.

Free FA % (w/w) in dried microalgae from GC-MS analyses of methanolic extracts (1:10 ratio) subjected to derivatization. A comparison of different techniques and times.