Bioresource Technology 184 (2015) 305–313

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Cascade approach of red macroalgae *Gracilaria gracilis* sustainable valorization by extraction of phycobiliproteins and pyrolysis of residue

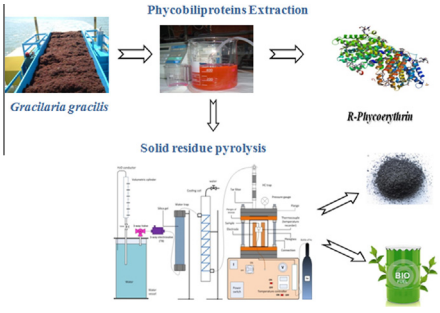
M. Francavilla [a,c], P. Manara [b], P. Kamaterou [b], M. Monteleone [a], A. Zabaniotou [a,b,*]

[a] STAR*AgroEnergy Research Group, University of Foggia, Foggia, Italy
[b] Biomass Group, Dept of Chemical Engineering, Aristotle University of Thessaloniki, Greece
[c] National Research Council, Institute of Marine Science, Lesina, Italy

HIGHLIGHTS

- Cascade biorefinery of *Gracilaria g.*, seaweed harvested in Lesina Lagoon performed.
- R-phycoerythrin, allophycocyanin, and phycocyanin were primary extracted.
- Extraction's residue pyrolyzed for bio-oil and biochar production, at 400–600 °C.
- High bio-oil yielded (~65 wt%) but not suitable for fuel without de-nitrogenation.
- Pyrolytic char (26.5–33 wt%) including P, K, Ca, Fe and Mg, suggested as biochar.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 27 October 2014
Accepted 29 October 2014
Available online 5 November 2014

Keywords:
Macroalgae
Red seaweed
Gracilaria gracilis
Phycobiliproteins
Pyrolysis

ABSTRACT

Phycobiliproteins extraction (primary refining) from *Gracilaria gracilis* seaweed, harvested in Lesina Lagoon (Italy) and further valorization of the residual algal via pyrolysis (secondary refining), were investigated with a cascade biorefinery approach. R-phycoerythrin (7 mg/g d.w.), allophycocyanin (3.5 mg/g d.w.) and phycocyanin (2 mg/g d.w.) were the main phycobiliproteins extracted. Pyrolysis of *G. gracilis* residue followed, aiming to investigate the production of bio-oil and biochar within a pyrolysis temperature range of 400–600 °C. Results showed that the bio-oil yield is high (~65 wt%) at pyrolysis temperature ~500 °C, but its high content in nitrogenous compounds prevents its use as a biofuel, unless some further de-nitrogenation takes place. Biochar yield ranged between 33 wt% (400 °C) and 26.5 wt% (600 °C). Interestingly, inorganic nutrients including P, K, Ca, Fe and Mg were detected in biochar, suggesting its potential use as recovering system of natural mineral resources from the oceanic reservoir.

1. Introduction

The upcoming depletion of fossil fuels necessitates their replacement by biofuels derived from various types of biomass, terrestrial either marine. Due to the fact that macroalgae are rich in valuable compounds, they constitute an interesting feedstock for the production of bio-fuels and more specifically bio-oils (Sahoo et al., 2012). Macroalgae, also known as seaweed, have been regarded as a promising feedstock for biorefinery due to their high photosynthetic efficiency, fast growth rate, and considerable sea-farming scale for mass cultivation. As a potential 3rd generation biofuel feedstock, algae have a number of desirable features including also high biomass conversion rate, ease of handling and reduced potentially-zero net CO_2 emissions (Wargacki et al.,

* Corresponding author at: Biomass Group, Dept of Chemical Engineering, Aristotle University of Thessaloniki, Greece. Tel.: +30 6945990604.
E-mail addresses: azampani@gmail.com, azampani@auth.gr (A. Zabaniotou).

http://dx.doi.org/10.1016/j.biortech.2014.10.147
0960-8524/

2012; Kerton et al., 2013). Compared with land biomass, macroalgae have higher contents of nutrient such as nitrogen and phosphorus, as well as various trace metals since macroalgae uptake and accumulate these elements over whole body from seawater (Bird et al., 2011). In general seaweeds are important components in marine ecosystems providing essentially unique ecological functions (Anderson, 1997).

Marine biomass (e.g. seaweed) has attracted considerable attention as a potential biofuel, food and biomaterials feedstock (Lordan et al., 2011; Alvarado-Morales et al., 2013). Currently, the macroalgae industry is primarily focused on food products for human consumption, which account for 83–90% of the global value of seaweed. Algal hydrocolloids extracted from macroalgae, such as alginate, agar and carrageenan, account for most of the remaining value (Wargacki et al., 2012). The vast majority of the macroalgae for these products is produced by aquaculture, which included some 3.1 million dry metric tons of annual global production in 2006 as compared to only 22,000 dry metric tons by harvesting of wild algae stocks (Kerton et al., 2013). *Laminaria*, *Undaria*, *Porphyra*, *Euchema*, and *Gracilaria* represent 76% of the total tonnage of macroalgae production by aquaculture (Wei et al., 2013).

Red macroalgae (*Gracilariales*, *Rhodophyta*) are important for industrial and biotechnological uses and they are considered economically valuable resources due to their ability to produce high yields of commercially valuable biomass (Capo et al., 1999). In fact, besides other compounds, they contain phycocolloids, the main source of agar. Phycocolloids are gelatinous non-toxic colloidal carbohydrates present in the cell wall and intercellular spaces of the algae, having wide use in the preparation of food, ice creams, jellies, soups, bacteriological samples and cosmetics (Kerton et al., 2013).

Recently, the red seaweed *Gracilaria gracilis* has been reported as an interesting source of a plethora of compounds with intriguing applications including phycobiliproteins which have a great economic potential in medical diagnostic and natural colorants for food and cosmetic, proteins for food and feed (Francavilla et al., 2013a), agar polymer as mesoporous material (Francavilla et al., 2013b) and sacrificial biotemplate for nanoparticles production (Francavilla et al., 2014).

The valorization of extracted macroalgal residues via thermochemical conversion technologies, and more specifically via pyrolysis appears to be notably interesting. During pyrolysis a significant amount of bio-oil is produced. It could be used as biofuel if its quality is appropriate. In addition, the pyrolytic gas can be used as a combustible fuel, while the char has various applications including fertilizer, activated carbon material (Bird et al., 2011) and carbon sequestration (Bird et al., 2011). Algal biochar has lower carbon content, surface area and cation exchange capacity compared to the lignocellulose biochar, but it has higher content of nitrogen, ash and inorganic elements (P, K, Ca and Mg) and also higher pH (Zhao et al., 2011).

Although many studies over pyrolytic oils derived from brown or even green macroalgae have already come to light, few studies have been performed on the investigation of red macroalgae as a feedstock for bio-oil production via pyrolysis process. Furthermore, in the light of our knowledge, pyrolysis of red algal residues after phycobiliproteins extraction has not been investigated so far.

Given the fact that fast pyrolysis gives the highest bio-oil yields and bearing in mind the role of bio-oil as a bio-fuel, the evaluation of the properties of pyrolytic oil from *G. gracilis* residue **(R)**, is the main aim of the present study, while the objective is the valorisation of *G. gracilis* (originated from Lesina Lagoon, Italy) by means of extraction of valuable products, followed by pyrolysis of macroalgal residue in a cascade biorefinery integrated approach. In addition to pyrolysis of macroalgal residue **(R)**, pyrolysis of *G. gracilis* **(MA)** was performed for comparison, as well.

2. Methods

2.1. Raw materials

The red seaweed *G. gracilis* was collected in the Lesina Lagoon (Southern Adriatic Sea, Italy), where a stable coverage was found. The macroalgal biomass was initially dried in the harvesting facilities before being supplied to the laboratory for the experiments.

The samples were ground to suitable particle size and sieved to powder of 1 mm diameter. The elemental analysis (in triplicate) of the samples was achieved with the method LECO-ASDM-D 5291. This method was accomplished with an elemental analyzer CHNS-LECO 680. Samples of dried seaweed **(MA)** and residue **(R)** (0.5 g) were wet digested in HNO_3 in a closed vessel microwave digester (CEM-Mars6). The solution was analyzed for metals by inductively coupled plasma spectrometry-optical emission spectroscopy (ICP-OES Agilent 720). The raw macroalgal biomass **(MA)** and the residue obtained as by-product after the phycobiliproteins extraction **(R)** were characterized.

2.2. Phycobiliproteins extraction

The algal sample (0.5 g d.w.) was ground manually with pestle and mortar. The mixture was suspended in 10 mL of 1 M acetic acid–sodium acetate buffer (pH 5.5) with 0.01% of sodium azide for 30 min in the dark. After the incubation with buffer, samples were ground for 5 min using a Potter homogeniser (Marconi, model MA099). The mixture was transferred in a centrifuge glass tube centrifuged at 5 °C, 15,000 rpm for 20 min. Supernatant was collected and pellet was extracted again with buffer as described for three times. Supernatants were combined and the final volume of the extract was about 40.0 mL. Phycobiliproteins (identified as R-phycoerythrin R-PE, phycocyanin PC and allophycocyanin APC) were quantified by spectrophotometry according, to Kursar et al. (1983). The wet solid residue was dried in oven (105 °C) overnight.

2.3. Thermogravimetric analysis

Thermogravimetric analysis was applied to analyze the mass loss of the algae biomass and extraction residue versus heating. A TGA analyzer unit (LECO-TGA701) was used in nitrogen atmosphere (10 L min^{-1}). Approximately 250 mg of sample was heated in a porcelain crucible from 25 up to 800 °C at a rate of 10 °C min^{-1}. Analyses were performed in duplicate. Differential thermogravimetric (DTG, in unit of wt%/°C) curves were also obtained through differential calculations of the thermogravimetric data (TG, in units of wt%).

2.4. Fast pyrolysis

The macroalgae (*G. gracilis*) and the residue obtained from the phycobiliproteins extraction process were used as feedstock in fast pyrolysis. Pyrolysis experiments were performed in a laboratory scale, wire mesh type reactor (Fig. 1). The experimental apparatus comprised of two electrodes, an electrical circuit, a water cooling coil, a moisture trap, two filters for liquid hydrocarbons a helium providing section, temperature controller and a gas sampling collection system. The produced pyrolysis gas was analyzed offline in a gas chromatographer (Model 6890N, Agilent Technologies) fitted with two columns, HP-PlotQ and HP-Molsive type. The samples (0.5 g) were placed in an envelope of stainless steel 100 mesh. A thermocouple inside the sample provided the temperature evolution and regulated eventually the heating rate. The experiments were carried out at three temperatures (400, 500 and 600 °C), with a heating rate of approximately 50 °C/s at atmospheric pressure

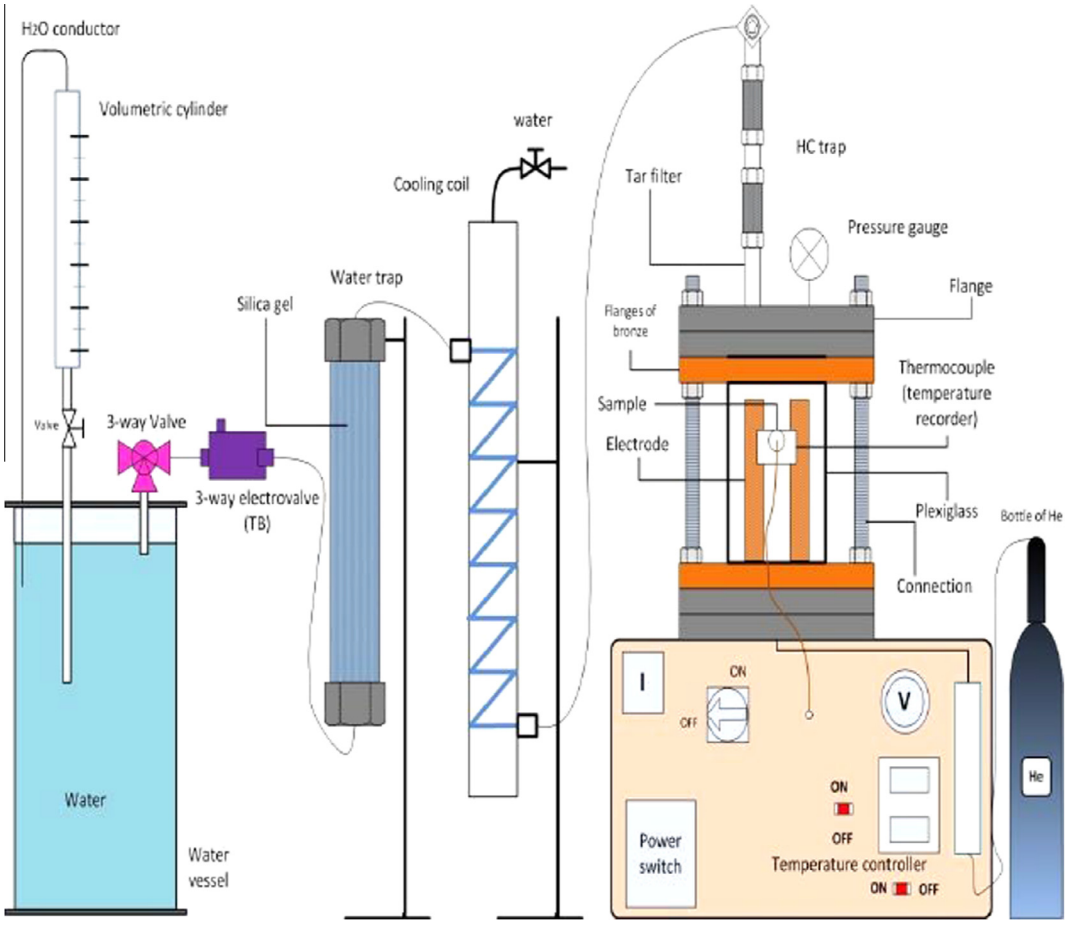


Fig. 1. Experimental set up for fast pyrolysis experiments.

and inert atmosphere. Pyrolysis products included char, liquid and non-condensable gas. The products collection was carried out, as follows:

Char was collected after the termination of the experiment and measured gravimetrically. The liquid product included tar and liquid hydrocarbons. Tar was defined as the material condensed within the reactor vessel, on the walls, flanges and at the paper filter placed at the outlet of the reactor, at ambient temperature. The condensed tar at the walls of the apparatus was removed after the termination of the experiment by washing with an acetone (C_3H_6O) soaked filter paper and measured gravimetrically. Hydrocarbons in the vapor phase at room temperature were collected by a lipophilic trap (80:100 mesh Porapaq Q chromatographic packing), placed at the exit of the reactor and then measured gravimetrically.

It is evident that since the capacity of the reactor was very small and the collection of very small amounts of tar and char was difficult, any loss in collection had an impact on mass balance.

2.5. Characterization of bio-oil via GC–MS and FT-IR

The produced pyrolytic oil was analyzed using a Gas Chromatograph-Mass Spectrometer System (Agilent 7890A/5970C), equipped with an HP-5MS 30 m × 0.25 mm, ID × 0.25 μm Column. The Electron Energy was 70 eV, the emission 300V and the Helium flow rate 0.7 cm^3/min. Internal libraries were used in order to identify the compounds.

The water content of the produced bio-oil was measured with Karl-Fischer titration (ASTM E203-08) method, while the elemental composition determined by an elemental CHN LECO-800 analyzer.

FT-IR analysis of bio-oil was performed using a Perkin–Elmer Spectrum 400 Series spectrometer, working in diffuse reflectance mode (DRIFT). Measurements were carried out at 4 cm^{-1} resolution in the range of 4000–450 cm^{-1}, with 64 scans. Spectra were baseline corrected, normalized and transformed to absorbance spectra.

3. Results and discussion

3.1. Feedstock characteristics

Proximate, ultimate and elemental analysis, of the raw *G. gracilis* **(MA)**, as well as, of the solid residue **(R)**, by-product after the extraction of phycobiliproteins, are presented in Table 1.

Compared to other species of red macroalgae, both *G. gracilis* **(MA)** and its residue **(R)** have high hydrogen, nitrogen and oxygen content. The high nitrogen content renders this kind of macroalgae a promising potential precursor for the production of potential high value products (Ross et al., 2009). Compared to other macroalgae, *G. gracilis* has low ash content (Table 1).

Three main phycobiliproteins were detected and measured in *G. gracilis*. The R-phycoerythrin (R-PE) was found to be the most abundant (7 mg g^{-1} d.w.) compared to allophycocyanin (APC) and phycocyanin (PC) (3.5 mg g^{-1} d.w. and 2 mg g^{-1} d.w., respectively). These data are in accordance with the data on phycobiliproteins reported by Francavilla et al. (2013b) in a previous study. Phycobiliproteins have a great economic potential (Niu et al., 2006; Bermejo et al., 2007) and they are already widely used as

Table 1
Proximate and ultimate analysis of *Gracilaria* **(MA)**, its residue **(R)** after extraction of phycobiliproteins and biochar produced by fast pyrolysis (500 °C) of **(MA)** and **(R)** - comparison with various species of red macroalgae.

	Ultimate Analysis (wt% dry)					Proximate Analysis (wt%)		HHV MJ/kg	References
	C	H	N	S	O	Moisture	Ash		
G. gracilis **(MA)**	31.53	5.13	4.07	1.61	37.68[a]	9.13	19.98	13.53	Present study
G. gracilis **(R)**	31.67	5.17	3.98	1.58	36.72[a]	1.32	20.88	13.58	Present study
Biochar **(MA)**	36.83	0.23	2.32	2.64	10.57[a]	4.08	47.41	11.4	Present study
Biochar **(R)**	41.18	2.17	2.32	1.32	22.31[a]	5.42	30.7	14.5	Present study
P. yezoensis	–	–	–	–	–	9.2	31.3	10.6	Li et al. (2011)
P. telfairiae	–	–	–	–	–	11.7	33.2	12.3	Li et al. (2011)
C. pilulifera	–	–	–	–	–	10.5	38.6	9.7	Li et al. (2011)
D. ramentacea[b]	30.9	4.7	3.4	–	26.2	10.7	34.8	14.5	Kebelmann et al. (2013)
O. dentata[b]	35.3	5.2	3.1	–	22.2	9.4	34.2	15.2	Kebelmann et al. (2013)
P. rubens[b]	32.0	5.4	2.9	–	29.7	8.9	30.0	14.3	Kebelmann et al. (2013)
P. arctica[b]	34.4	5.0	1.8	–	31.3	11.3	27.6	14.9	Kebelmann et al. (2013)
G. skottsbergii[b]	23.7	3.9	1.9	–	30.3	10.1	40.2	13.8	Kebelmann et al. (2013)
H. crustigena[b]	28.8	4.2	4.0	–	18.9	9.8	44.2	14.5	Kebelmann et al. (2013)
M. manginii[b]	22.3	4.9	3.4	–	30.5	8.7	38.9	13.1	Kebelmann et al. (2013)
K. antarctica[b]	31.5	4.9	3.5	–	15.4	7.5	44.7	14.5	Kebelmann et al. (2013)
P. cartilagineum[b]	30.1	4.4	3.6	–	25.6	10.6	36.2	14.5	Kebelmann et al. (2013)

[a] Calculated by difference, O (%) = 100-C-H-N-S-Ash.
[b] Ash free basis.

Table 2
Elemental analysis of *Gracilaria* **(MA)**, its residue **(R)** after extraction of phycobiliproteins and biochar produced by fast pyrolysis (500 °C) of **(MA)** and **(R)** - comparison with various species of red macroalgae.

Elemental analysis (ppm)	**(MA)** Present study	**(R)** Present study	Biochar **(MA)** Present Study	Biochar **(R)** Present Study	*Laminaria digitata* Ross et al. (2008)	*Fucus vesiculosus* Ross et al. (2008)	*Macrocyctis pyrifera* Ross et al. (2008)
Al	548.24	427.30	1702.44	874.66	186	1275	1830
Cd	n.d.	n.d.	n.d.	n.d.	1.1	2.4	6.5
Ba	15.68	5.97	61.54	19.98			
Ca	11,913.00	3978.94	47,024.56	15,603.29	10,600	10,650	31,950
Co	1.25	0.61	5.27	1.12	–	–	–
Cr	0.59	9.16	634.42	66.98	2.3	7.2	17.2
Cu	3.29	2.14	70.89	14.37	17.5	13.7	15.2
Fe	210.49	258.80	5129.09	1221.80	1980	2420	3500
K	55,802.00	16,027.30	141,201.75	14,945.71	36,600	37,450	26,250
Mg	2233.70	393.41	6659.40	684.22	9325	7710	10,600
Mn	316.32	178.09	1023.69	404.54	29.3	66.2	24.9
Na	5468.62	73,980.10	11,546.04	106,048.40	43,300	29,350	54,300
Ni	2.60	4.58	319.65	39.99	–	–	–
P	2347.9	1267.69	6951.30	1458.83	8750	24,970	12,650
Pb	1.17	0.83	5.67	2.45	7.0	6.0	1.8
Si	1015.89	818.22	1909.02	1776.63	1215	3060	5875
Sr	251.81	73.18	1024.01	300.94	524	480	1320
V	8.66	5.41	25.54	9.69	–	–	–
Zn	21.09	14.33	104.80	38.13	205	282	70

fluorescent probes in medical diagnostic and natural colorants for food and cosmetic.

The ultimate analysis of the residue **(R)** was almost similar to original biomass **(MA)**. The extraction process seemed not to affect the algal biomass composition in terms of C, H, N, S and O concentration (Table 1), and it is in accordance with the composition reported by Kebelmann et al. (2013) for macroalgae belonging to *Rhodophyta* division (red algae). However, significant differences in elemental composition between the two samples **(MA)** and **(R)** were found; according to literature reported data, the elemental composition of seaweeds presents a high concentration of alkali metals (Ross et al., 2009; Trinh et al., 2013). The most abundant element was K (55802.00 ppm), followed by Ca (11,913.00 ppm), Na (5468.62 ppm) and Mg (2233.70 ppm) (Table 2). The treatment of **(MA)** with sodium acetate/acetic acid buffer (pH 5.5) for phycobiliproteins extraction affected the concentration of Na which considerably increased (from 5468.62 ppm to 73,980.10 ppm). On the other hand, K, Ca and Mg decreased at concentration of 16,027.30 ppm, 3978.94 ppm and 393.41 ppm respectively (Table 2). Comparing with other species of macroalgae (Table 3), the red seaweed *G. gracilis* appears to be especially rich in valuable compounds. Specifically, the red seaweed *G. gracilis* has higher content of crude protein and crude fat and lower content of carbohydrates, in comparison with other species of macroalgae (Table 3).

3.2. Thermal degradation mechanism

The TG and DTG curves that were obtained from the pyrolysis of *G. gracilis* **(MA)** and the residue **(R)**, at a heating rate of 10 °C min^{-1}, are presented in Fig. 2a and b.

The experimental data clearly indicated that different shaped TG and DTG curves were obtained for the two analyzed samples. Three stages of thermal decomposition, including dehydration (stage I), devolatilization (stage II) and decomposition of carbonaceous solids (stage III) were evident, as shown in Fig. 2a. For **(MA)** and **(R)** a minor weight loss of ca. 11.1% and 3.1% respectively was observed during stage I, at temperatures < 146 °C. This corresponds to dehydration and evaporation of highly volatile compounds. The two samples exhibited the main weight loss during stage II, related to the main devolatilization reactions during the pyrolysis process.

Table 3
Chemical composition of *Gracilaria gracilis* **(MA)** and comparison with various species of macroalgae.

Macroalgae	*Sargassum* sp. Borines et al. (2013)	*Sargassum pallidum* Li et al. (2010a)	*Laminaria japonica* Li et al. (2010a)	*Enteromorpha prolifera* Li et al. (2010b)	*Gracilaria gracilis* Present study
Compounds					
Crude protein (wt%)	10.25	10.59 ± 1.82	8.95 ± 1.51	14.3 ± 0.34	31–45
Crude fat (wt%)	0.75 ± 0.02	1.56 ± 1.14	1.03 ± 0.99	1.1 ± 0.04	1.12–1.98
Crude fiber (wt%)	9.84 ± 0.07	13.66 ± 0.89	9.56 ± 1.21	15.4 ± 0.12	
Carbohydrates (wt%)	41.81			45.1 ± 0.57	24.8-34.1

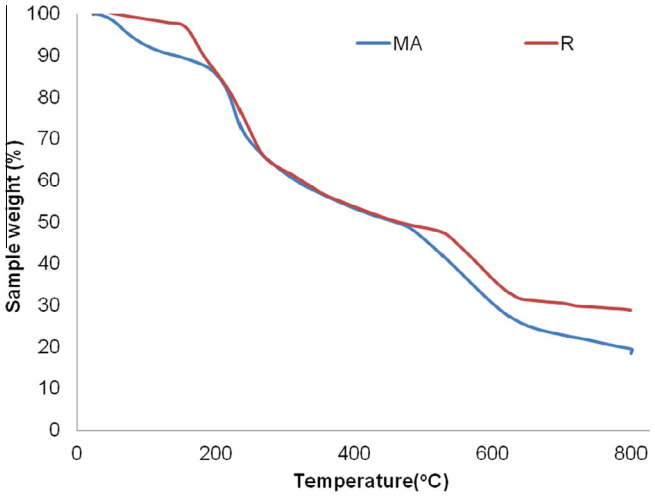


Fig. 2a. TG pyrolysis profiles of *Gracilaria gracilis* biomass before **(MA)** and after **(R)** phycobiliproteins extraction at a heating rate of 10 °C min^{-1} (nitrogen atmosphere).

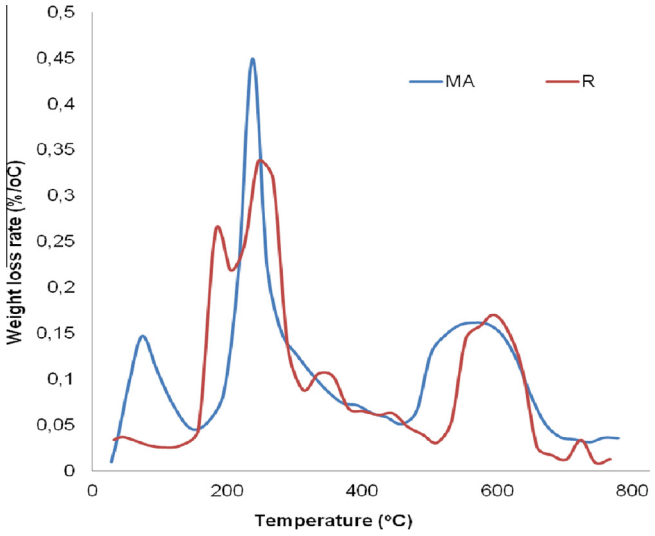


Fig. 2b. DTG curves of *Gracilaria gracilis* residue after phycobiliprotein extraction (R-MA) and raw *Gracilaria gracilis* at a heating rate of 10 °C/min (nitrogen atmosphere).

The thermochemical characteristics observed including decomposition temperatures and released volatiles are presented in Table 4.

Devolatilization occurred at two separate regions: the first region covered a wide temperature range between ca. 150 and 500 °C; the second region followed and extended to a high temperature (671 and 658 °C respectively). Initial pyrolysis temperature (T_i) obtained for **(MA)** was 172 °C while a lower T_i (156 °C) was found for the residue **(R)**. The final temperature (T_f) of the main

Table 4
TGA of *Gracilaria gracilis* biomass before **(MA)** and after **(R)** phycobiliproteins extraction (TGA at a heating rate of 10 °C min^{-1} in N_2 atmosphere).

Sample	Temperature (II)			Volatiles (wt%) (II)
	T_i	T_m	T_f	
MA	172	237	671	74
R	156	245	658	67

T_i: initial temperature; T_m: maximum temperature; T_f: final temperature.

pyrolysis process was found at 671 and 658 °C for **(MA)** and **(R)** respectively. A release of 74 wt% volatiles was observed for **(MA)** while a lower value was observed for **(R)** (67 wt%).

The maximum degradation temperature (T_m) observed in the second stage were 237 and 245 °C for **(MA)** and **(R)**, respectively. Moreover, they showed a large pick in DTG at 586 and 594 °C. A similar large pick was found by Kan et al. (2014) for the marine microalgae *Ulva ohnoi*. Interestingly, the extracted residue **(R)** showed other two different picks at 182 and 334 °C which did not appear in DTG of **(MA)**. Within decomposition stage, at temperature above 650 °C, a release of 4% and 2.5 wt% volatiles was observed for **(MA)** and **(R)** respectively. The char residue at 800 °C was higher for **(R)** (29.7%) than **(MA)** (19.5%), (Fig. 2a). This fact is attributed to O/C ratio in the materials (Table 1). Usually, high ratio of O/C in biomass promotes gasification reactions during the thermochemical process rather than pyrolytic/carbonization reactions and thus decreasing char yield. In this study, pyrolysis of **(MA)** which has higher O/C ratio than **(R)**, gave decreased char yield.

The different thermal degradation behavior that the samples **(MA)** and **(R)** presented (Fig. 2a and b) are attributed to the differences in the inherent structural and chemical characteristics of the samples. The devolatilization includes the stepwise decomposition of the different bio-polymers fraction. The major polysaccharides of *Gracilaria* biomass are sulfated galactans such as agar. Agar is a biopolymer built on a disaccharide-repeating unit of 3-linked β-D-galactose (G) and 4 linked 3,6-anhydro-α-L-galactose (AG) residues, with possible occurrence of sulfate, methoxyl, and/or pyruvate substituents at various positions in the polysaccharide chain (Rees, 1970). Treatment with acetate buffer (pH 5.5) used for phycobiliprotein extraction reduced the temperature required for onset of pyrolysis of macroalgal residue **(R)**. This is likely to be a consequence of hydrolysis and selective extraction of certain biopolymers from macroalgal biomass agreeing with the results reported by Ross et al. (2009) who investigated the effect of different pretreatment of brown seaweed on the pyrolysis behavior.

The difference in the amount of alkali earth metals in **(MA)** and **(R)** could also affect the degradation behavior of the two materials. According to previous studies (Kan et al., 2014), metals react with CO_2 during algal pyrolysis (decarboxylation process) generating different carbonates which are decarbossilated between 550 and 750 °C. Metals, in particular calcium, sodium, potassium and magnesium are also largely present in bound with macroalgal carbohydrates polymers (Ross et al., 2011; Pathak et al., 2010). The change

in cation composition can significantly influence the pyrolysis behavior. In fact, Ross et al. (2011) reported that the change from Ca^{2+} to Na^{+} in macroalgal alginate (biopolymer extracted from brown seaweeds) caused a decrease of devolatilization temperature, differentiated the range of volatile components and it also provoked a significant swelling of char produced. In our study we found similar results that can be partially explained by the higher amount of Na and the lower amount of Ca, K and Mg in the residue **(R)** than in macroalgal biomass **(MA)**.

3.3. Fast pyrolysis product yields and mass balance

The yields of char, bio-oil and total volatiles produced during fast pyrolysis of macroalgal residue **(R)**, at three representative temperatures, are shown in Fig. 3. Total volatiles released from fast pyrolysis of **(R)** yielded ~71 wt% at 500 °C. The obtained maximum oil yields were ~68 wt% and ~65 wt% for **(MA)** and **(R)** at 500 °C, respectively.

Pyrolysis oil usually includes an organic phase and water (Karl–Fischer method). As Table 5 shows, raw **(MA)** pyrolysis resulted to higher bio-oil yield (68 wt%) than **(R)**, (65 wt%). However, the produced oil contains a lower organic phase (~27.1 wt%) compared to that of **(R)** (~34.5 wt%), probably due to the lower moisture content in the initial material (1.32 wt% in **(R)** and 9.13 wt% in **(MA)**, (Table 1).

The bio-oil yields (organic phase) that were achieved in this research work are comparable to the results presented in other literature reported studies on seaweed fast pyrolysis (Table 5). In particular, *G. gracilis* residue **(R)** presents high potential in bio-oil production via pyrolysis resulting in one of the highest bio-oil yields in comparison with the reported results.

One of the parameters affecting the yields of bio-oil derived from biomass pyrolysis is the feedstock's physicochemical behavior. Although macroalgae is rich in valuable compounds, such as carbohydrates, proteins and lipids, they contain significant amounts of ash and nitrogen, which have negative effect on the pyrolytic yields and the quality of bio-oil as a fuel. The high ash content of macroalgae is attributed to the mineral content of algae which in general decreases pyrolytic oil quality and gives high char yields. On the other hand, the alkali metals are considered to catalyze pyrolysis process (Ross et al., 2008, 2009).

3.4. Bio-oil characteristics

The composition of bio-oils derived from pyrolysis process were analyzed by GC–MS. The compounds detected in bio-oil were

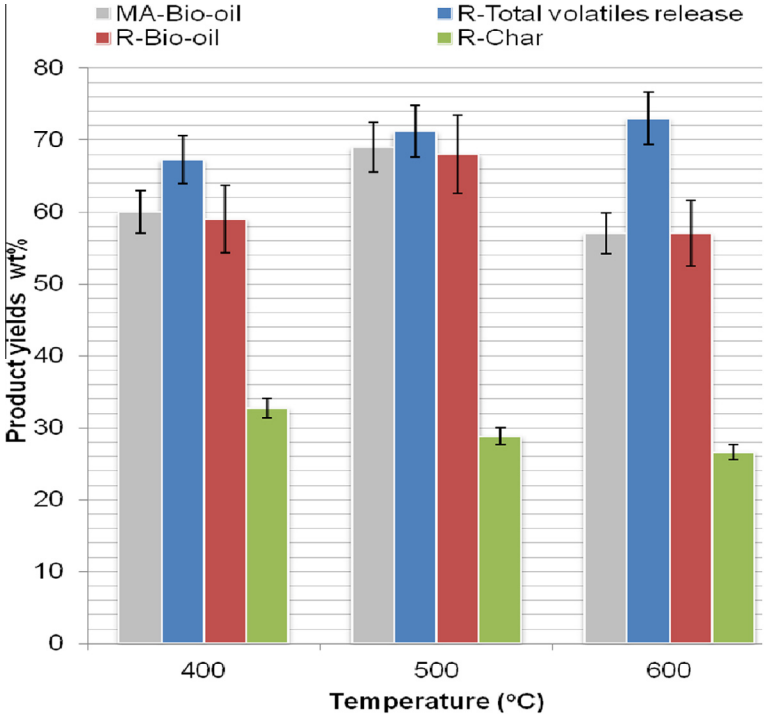


Fig. 3. Total volatiles release of macroalgae and the residue, as well as liquid and char yield results of the residue via fast pyrolysis at 450, 550 and 650 °C.

Table 5
Bio-oil yields from fast pyrolysis of *Gracilaria gracilis* **(MA)** and from residue **(R)** after phycobiliprotein extraction-comparison with literature data.

Type of reactor	Macroalgae	Temp. (°C)	Bio-oil yield (wt%)	References
Captive sample reactor	*Glacilaria gracilis* **(MA)**	500	27.1[a] (68[b])	Present study
Captive sample reactor	*Glacilaria gracilis* **(R)**	500	34.5[a] (65[b])	
Fixed-bed reactor	*E. clathrata*	500	41.2	Wang et al. (2013)
	Sargassum natans	500	33.7	
Continuous fluidized bed reactor	*F. serratus*	500	14	Yanik et al. (2013)
	Mix from Black sea	500	11	
	L. digitata	500	17	

[a] Organic phase of the bio-oil.
[b] Total bio-oil yield (water content + organic phase).

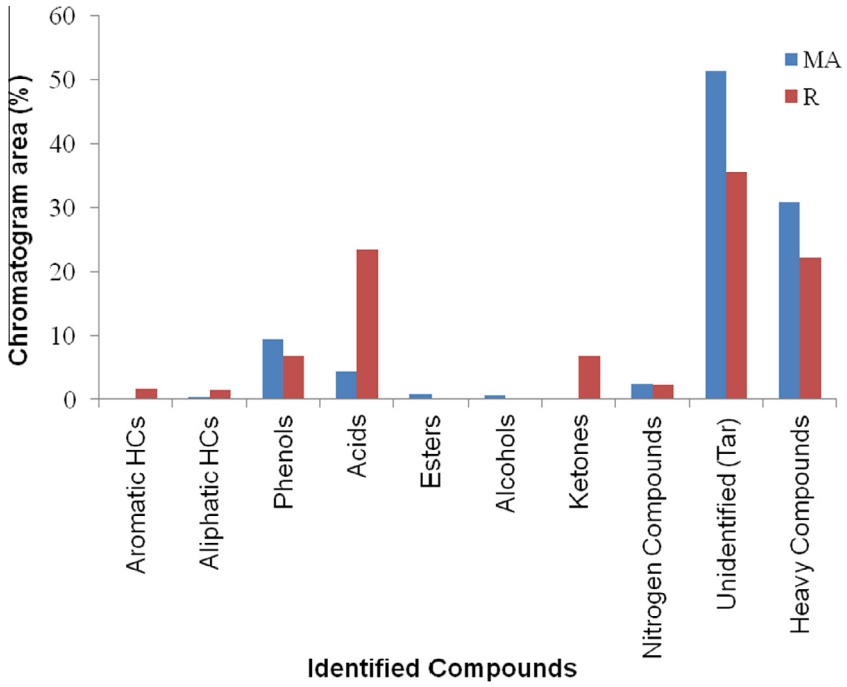


Fig. 4. Pyrolysis bio-oil analysis with GC–MS of *Gracilaria gracilis* biomass before **(MA)** and after **(R)** phycobiliproteins extraction.

Table 6
Elemental composition of bio-oil derived from *Gracilaria gracilis* **(MA)** and its residue **(R)** and comparison with boil derived from other species of macroalgae.

	C[a]	H[a]	N[a]	S[a]	O	H/C[c]	H/O[c]	References
G. gracilis **(MA)**	52.76	8.15	5.32	1.83	31.94[b]	1.85	4.08	Present study
G. gracilis **(R)**	53.34	8.24	2.96	0.57	34.89[b]	1.85	3.78	Present study
Algae meal	60.84	6.05	9.45	0.56	23.1	1.19	4.19	Ferrera-Lorenzo et al. (2014)
Mix of Macroalgae	59.7	7.2	4.2	0.1		1.45		Yanik et al. (2013)
Fucus serratus	68.4	8.9	2.7	0.1		1.56		Yanik et al. (2013)
Laminaria digitata	55.6	7.1	2.2	0.1		1.53		Yanik et al. (2013)
Enteromorpha clathrata	57.8	7.887	9.27		25.03	1.64	5.04	Wang et al. (2013)
Sargassum natans	53.82	8.174	6.54		31.45	1.82	4.16	Wang et al. (2013)

[a] wt%, dry.
[b] wt%, by difference.
[c] Molar ratio.

mainly originated from carbohydrates, proteins, lipids and polyphenolic structures. Generally, pyrolysis oils are mostly highly viscous, acidic and unsaturated. They also contain solid matter as well as dissolved water. To enable the products to be used as fuels, oxygen content must be decreased and the impeding substances removed. This can be done by means of hydrogenation process for example (Wildschut et al., 2010).

Fig. 4 shows the compounds identified in bio-oils produced from the samples of *G. gracilis* **(MA)** and its post extraction residue **(R)** at 500 °C.

The elemental analysis of the produced bio-oil is reported in Table 6. The observed content in carbon and hydrogen were not significantly different in bio-oil derived from **(MA)** and **(R)**, while nitrogen and sulfur content were higher in bio-oil from MA than R. The content of oxygen was estimated by difference and seemed to be lower for the bio-oil derived from macroalgal biomass **(MA)**. The moisture content was higher in bio-oil obtained from **(MA)** pyrolysis than **(R)** (60.1 wt% and 47.06 wt%, respectively).

The chemical characteristics of pyrolytic oil are strictly dependent on the species of macroalgae (Bae et al., 2011). In contrast to bio-oil obtained by other species of macroalgae, the bio-oil from *G. gracilis* was characterized by higher hydrogen content, while the carbon content was close to the others. As a consequence, the H/C ratio increased (Table 6). The high content of nitrogenous compounds prevents their use as a biofuel, unless some further denitrogenation occurs. Nevertheless, they could be used for the production of other useful high-value chemicals (Ross et al., 2008).

In addition to GC–MS, FTIR analysis was used to identify the functional groups contained in bio-oil derived from pyrolysis of the residue **(R)** of macroalgae *G. gracilis,* at two different pyrolysis temperatures: 500 and 600 °C. The following chemical bonds were identified in bio-oil, obtained at 500 °C pyrolysis (Harman-Ware et al., 2013):

- Around 3400 cm^{-1}, the wide band is assigned to O–H stretching vibrations indicating the presence of water, alcohols, amides and amines. These bands almost disappeared in bio-oil produced at 600 °C.
- The bands at 2854, 2924 and 2966 cm^{-1}, are characteristics of C–H stretching vibrations in acyl chains of methyl and methylene groups (alkanes, alkenes).
- Between 1650 and 1750 cm^{-1}, is the detection area of carbonyl and carboxyl groups.
- The strong band at 1575 cm^{-1} in bio-oil produced at 500 °C is attributed to stretching vibrations of the double bond (–C=C–), revealing the presence of alkenes and/or aromatic

compounds. In bio-oil produced at 600 °C, The presence of aromatic compounds is confirmed by the band at 1493 cm^{-1} (attributed to aromatic C=C ring stretching) and the small brands observed at the area of 700–900 cm^{-1}.

- Finally, given the fact that the bands at 1083, 1130, 1181, 1283 cm^{-1} (in bio-oil at 500 °C) and 1142 cm^{-1} and 1195 cm^{-1} (in bio-oil at 600 °C) are characteristics of the bond (–C–O–), alcohols and ethers (1283 cm^{-1}) are probably identified in the sample.

N-content of bio-oil, as GC–MS (Fig. 4) and FTIR spectra depict, show that bio-oil de-nitrogenation and upgrading are necessary in order to be used as bio-fuel (Ross et al., 2009). Crude bio-oil produced by pyrolysis cannot be used as fuel due to its high water and oxygen contents and the presence of unsaturated and phenolic moieties (Bae et al., 2011). As a result, bio-oils need to be upgraded or pretreated to improve their quality before used for most applications. It was claimed that bio-oil production for various uses is feasible after appropriate pretreatment of the feedstock (Bae et al., 2011). Various pretreatment methods with promising results have been introduced in order to remove the ash content of macroalgae (Ross et al., 2009; Bae et al., 2011; Choi et al., 2014; Ferrera-Lorenzo et al., 2014). As a consequence of pretreatment, losses also of organic compounds might occur.

3.5. Biochar characteristics

Pyrolysis at 500 °C of *G. gracilis* residue **(R)** resulted in ~29 wt% biochar yield. In this study, ICP-OES analyses of the derived biochar from the pyrolysis of **(R)** revealed the presence of inorganic nutrients including P, K, Ca, Fe and Mg (Table 2). The carbon content of the biochar derived from pyrolysis of **(R)** at 500 °C (defined by elemental analysis tests) was higher (~41 wt%) than the carbon content of the biochar derived from **(MA)** at the same pyrolysis temperature (Table 1). These results are in accordance with other studies reporting that macroalgal biochar has properties that provide direct nutrient benefits to soils and thereby to crop productivity; it can be particularly useful for application on acidic soils. However, macroalgal biochar according to the same researchers is less able to provide carbon sequestration benefits compared to ligno-cellulosic biochar, which has high carbon content (Bird et al., 2011).

4. Conclusions

G. gracilis can give phycobiliproteins without affecting the characteristics of residual biomass **(R)**. However, a different thermochemical behavior was found for **(R)** compared to **(MA)**, probably due to the different presence of carbohydrate biopolymer (agar) and alkali earth metals concentration.

Pyrolysis of **(R)** at 500 °C resulted in a maximum bio-oil yield equal to 65 wt% and ~29 wt% biochar yield. Bio-oil production is feasible but further up-grading is required, such as de-nitrogenation.

Finally, pyrolysis of **(R)** can provide a bio-char of high inorganic nutrients content, emerging an innovative approach for recovering natural minerals from the oceanic reservoir.

Acknowledgements

Acknowledgements are expressed to European Commission, Directorate-General for Research & Innovation, SP4 – Capacities, Coordination and Support Action, (FP7), Regpot 2011-1, Grant Agreement N° 286269". The study consists also a collaborative work of Biomass Group at Aristotle University of Thessaloniki, Greece with Capacity Center of Foggia University, Italy.

References

Alvarado-Morales, M., Boldrin, A., Karakashev, D.B., Holdt, S.L., Angelidaki, I., Astrup, T., 2013. Life cycle assessment of biofuel production from brown seaweed in Nordic conditions. Bioresour. Technol. 129, 92–99.
Anderson, D.M., 1997. Turning back the harmful red tide. Nature 388, 513–514.
Bae, Y.J., Ryu, C., Jeon, J., Park, J., Suh, D.J., Suh, Y., Chang, D., Park, Y., 2011. The characteristics of bio-oil produced from the pyrolysis of three marine, macroalgae. Bioresour. Technol. 102, 3512–3520.
Bermejo, R., Ruiz, E., Acien, F.G., 2007. Recovery of B-phycoerythrin using expanded bed adsorption chromatography: scale-up of the process. Enzyme Microb. Technol. 40, 927–933.
Bird, M.I., Wurster, C.M., Silva, P.H.D., Bass, A.M., de Nys, R., 2011. Algal biochar – production and properties. Bioresour. Technol. 102, 1886–1891.
Borines, M.G., de Leon, Rizalinda L., Cuello, J.L., 2013. Bioethanol production from the macroalgae *Sargassum* spp.. Bioresour. Technol. 138, 22–29.
Capo, T.R., Jaramillo, J.C., Boyd, A.E., Lapointe, B.E., Serafy, J.E., 1999. Sustained high yields of *Gracilaria* (Rodophyta) grown in intensive large-scale culture. J. Appl. Phycol. 11, 143–147.
Choi, J., Choi, J., Suh, D.J., Ha, J., Hwang, J.W., Jung, H.W., Lee, K., Woo, H., 2014. Production of brown algae pyrolysis oils for liquid biofuels depending on the chemical pretreatment methods. Energy Convers. Manage. 86, 371–378.
Ferrera-Lorenzo, N., Fuente, E., Suαrez-Ruiz, I., Ruiz, B., 2014. Pyrolysis characteristics of a macroalgae solid waste generated by the industrial production of Agar-Agar. J. Anal. Appl. Pyrol. 105, 209–216.
Francavilla, M., Franchi, M., Monteleone, M., Caroppo, C., 2013a. The red seaweed *Gracilaria gracilis* as a multi products source. Mar. Drugs 11 (10), 3754–3776.
Francavilla, M., Pineda, A., Lin, C.S.K., Franchi, M., Trotta, P., Romero, A.A., Luque, R., 2013b. Natural porous agar materials from macroalgae. Carbohydr. Polym. 92 (2), 1555–1560.
Francavilla, M., Pineda, A., Romero, A.A., Colmenares, J.C., Vargas, C., Monteleone, M., Luque, R., 2014. Efficient and simple reactive milling preparation of photocatalytically active porous ZnO nanostructures using biomass derived polysaccharides. Green Chem. 16 (5), 2876–2885.
Harman-Ware, A.E., Morgan, T., Wilson, M., Crocker, M., Zhang, J., Liu, K., Stork, J., Debolt, S., 2013. Microalgae as a renewable fuel source: fast pyrolysis of *Scenedesmus* sp. Renew. Energy 60, 625–632.
Kan, T., Grierson, S., de Nys, R., Strezov, V., 2014. Comparative assessment of the thermochemical conversion of freshwater and marine micro and macroalgae. Energy Fuel 28, 104–114.
Kebelmann, K., Hornung, A., Karsten, U., Griffiths, G., 2013. Thermo-chemical behaviour and chemical product formation from *Polar seaweeds* during intermediate pyrolysis. J. Anal. Appl. Pyrol. 104, 131–138.
Kerton, F.M., Liu, Y., Omaria, K.W., Hawboldt, K., 2013. Green chemistry and the ocean-based biorefinery. Green Chem.. http://dx.doi.org/10.1039/c3gc36994c.
Kursar, T.A., Van Der Meer, J., Alberte, R.S., 1983. Light harvesting system of red alga *Gracilaria tikvahiae* I. biochemical analyses of pigment mutations. Plant Physiol. 73, 353–360.
Li, D., Chen, L., Yi, X., Zhang, X., Ye, N., 2010a. Pyrolytic characteristics and kinetics of two brown algae and sodium alginate. Bioresour. Technol. 101, 7131–7136.
Li, D., Chen, L., Zhao, J., Zhang, X., Wang, Q., Wang, H., Ye, N., 2010b. Evaluation of the pyrolytic and kinetic characteristics of *Enteromorpha prolifera* as a source of renewable bio-fuel from the Yellow Sea of China. Chem. Eng. Res. Des. 88, 647–652.
Li, D., Chen, L., Zhang, X., Ye, N., Xing, F., 2011. Pyrolytic characteristics and kinetic studies of three kinds of red algae. Biomass Bioenergy 35, 1765–1772.
Lordan, S., Ross, R.P., Stanton, C., 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar. Drugs 9, 1056–1100.
Niu, J.F., Wang, G.C., Tseng, C.K., 2006. Method for large-scale isolation and purification of R-phycoerythrin from red alga *Polysiphonia urceolata* Grev. Protein Expr. Purif. 49, 23–31.
Pathak, T.S., Yun, J.H., Lee, J., Paeng, K.J., 2010. Effect of calcium ion (cross-linker) concentration on porosity, surface morphology and thermal behavior of calcium alginates prepared from algae (*Undaria pinnatifida*). Carbohydr. Polym. 81, 633–639.
Rees, D.A., 1970. Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. In: Melville, L., Wolfrom, R.S.T., Derek, H. (Eds.), Advances in Carbohydrate Chemistry and Biochemistry. Academic Press, Amsterdam, The Netherlands, pp. 267–332.
Ross, A.B., Jones, J.M., Kubacki, M.L., Bridgeman, T., 2008. Classification of macroalgae as fuel and its thermochemical behaviour. Bioresour. Technol. 99, 6494–6504.
Ross, A.B., Anastasakis, K., Kubacki, M., Jones, J.M., 2009. Investigation of the pyrolysis behaviour of brown algae before and after pre-treatment using PY-GC/MS and TGA. J. Anal. Appl. Pyrol. 85, 3–10.
Ross, A.B., Hall, C., Anastasakis, K., Westwood, A., Jones, J.M., Crewe, R.J., 2011. Influence of cation on the pyrolysis and oxidation of alginates. J. Anal. Appl. Pyrol. 91, 344–351.
Sahoo, D., Elangbam, G., Devi, S.S., 2012. Using algae for carbon dioxide capture and biofuel production to combat climate change. Phykos 42, 32–38.

Trinh, T.N., Jensen, P.A., Dam-Johansen, K., Knudsen, N.O., Sørensen, H.R., Hvilsted, S., 2013. Comparison of lignin, macroalgae, wood, and straw fast pyrolysis. Energy Fuels 27, 1399–1409.
Wang, S., Wang, Q., Jiang, X., Han, X., Ji, H., 2013. Compositional analysis of bio-oil derived from pyrolysis of seaweed. Energy Convers. Manage. 68, 273–280.
Wargacki, A.J., Leonard, E., Win, M.N., Regitsky, D.D., Santos, C.N.S., Kim, P.B., Cooper, S.R., Raisner, R.M., Herman, A., Sivitz, A.B., Lakshmanaswamy, A., Kashiyama, Y., Baker, D., Yoshikuni, Y., 2012. An engineered microbial platform for direct biofuel production from brown macroalgae. Science 335, 308–313.
Wei, N., Quarterman, J., Jin, Y.S., 2013. Marine macroalgae: an untapped resource for producing fuels and chemicals. Trends Biotechnol. 31 (2), 70–77.
Wildschut, J., Melián-Cabrera, I., Heeres, H.J., 2010. Catalyst studies on the hydrotreatment of fast pyrolysis oil. Appl. Catal. B 99 (1–2), 298–306.
Yanik, J., Stahl, R., Troeger, N., Sinag, A., 2013. Pyrolysis of algal biomass. J. Anal. Appl. Pyrol. 103, 134–141.
Zhao, H., Yan, H., Liu, M., Zhang, C., Qin, S., 2011. Pyrolytic characteristics and kinetics of the marine green tide macroalgae, *Enteromorpha prolifera*. Chin. J. Oceanol. Limnol. 29 (5), 996–1001.