Improving bio-oil chemical profile of seaweeds through anaerobic fermentation pre-treatment

Mohamed Ali Wahab[a,b,♣], Katharina Kebelmann[c], Bernhard Schartel[c], Gareth Griffiths[a]

[a]Energy and Bioproducts Research Institute (EBRI), Aston University, Birmingham, United Kingdom

[b]Carthage University, Laboratory of Wastewater Treatment and Valorization, Water Research and Technologies Center, CERTE, Technopark Tourist Route of Soliman Nabeul, Soliman, Tunisia

[c]Bundesanstalt für Materialforschung und -prüfung (BAM) , Berlin, Germany

---

[♣]Corresponding author: CERTE, B.P. 273, 8020 Soliman, Tunisia Tel: +21679325044; Fax. +21679325802
E-mail adresses: mohamedali.wahab@certe.rnrt.tn (M. A. Wahab).

Abstract:

Biomass pre-treatments for bio-oil quality improvement are mainly based on thermal and chemical methods which are costly and hence reduce the sustainability of pyrolysis-based refineries. In this paper, anaerobic digestion (AD) and dark fermentation (DF) are proposed as alternative ‘green’ pre-treatments to improve this situation. For this purpose, three seaweeds namely *Sargassum polycystum*, (Phaephyta), *Gracilaria tenuistipitata*, (Rhodophyta) and *Ulva reticulata*, (Chlorophyta) with high ash and oxygen contents were pre-treated to improve their composition and structure prior to pyrolysis. The results reveal that both biological pre-treatments affected, positively, the composition and structure of the seaweed biomass with AD pre-treatment reducing N and S contents by 86% and 63%, respectively. DF was more efficient in terms of ash and moisture reduction with 25% and 70%, respectively. In addition, oxygen (O) reduction by 27% was observed after DF which was evidenced by FTIR spectroscopy indicating the reduction of most oxygen-containing functional groups in the biomass. On the other hand, the carbon (C) content increased in DF pre-treated seaweeds up to 42%, almost two times higher relative content than C in the raw seaweed. The changes in the composition of pre-treated seaweeds resulted in changes in their thermal degradation and the volatile profiles produced during pyrolysis. Interestingly, anhydrosugars and furans which account for some 70% in raw seaweeds markedly declined or become undetectable after DF pre-treatment and correspondingly more acetic acid and hydrocarbons were produced while after AD more aromatics with high toluene content (*ca.*17%) were generated. The results indicate that bio-oil with profiles more similar to petroleum-based composition i.e. rich in hydrocarbons and low in anhydrosugars, N and S can be generated by AD and DF pre-treatments and opens up the possibility of these approaches to effect cost reduction in the overall generation of bio-based fuels.

Keywords: Anaerobic digestion; dark fermentation; pyrolysis; seaweeds; thermogravimetric analysis; pyrolysis volatiles.

1. Introduction

Over the last decade, thermochemical conversion processes of biomass into bio-oil have attracted increasing attention for the sustainable production of biofuel and high value added chemicals to mitigate environmental and economical issues associated with fossil fuel use [1,2]. In fact, the world's energy supply is largely based on fossil fuel which contributes to environmental pollution and climate change, in addition to the fluctuation of crude oil price which represents a threat to the economic stability of many nations [3,4]. Therefore, bio-oil from pyrolysis could be an interesting alternative to crude oil as well as generating other useful products such as biochar and syngas [5]. Bio-oil is a dark brownish viscous liquid presenting some resemblance to fossil crude oil [6,7]. However, bio-oils have several undesired properties for fuel applications such as high oxygen/water contents, high viscosity, corrosiveness and issues with stability [8]. Thus, the poor quality of bio-oil represents an obstacle to its use as a substitute for petroleum based fuel and its assimilation into existing liquid fuel infrastructures [9].

To overcome these issues, various technologies have been developed for bio-oil upgrading, including hydrocracking, hydrotreating, steam reforming, solvent addition/esterification, emulsification, chemical extraction and supercritical fluids extraction [10–14]. However, the high cost of these upgrading techniques has limited their commercialization [15,16]. In order to reduce costs and to increase their efficiencies, many studies have focused on the pre-treatment of the biomass prior to pyrolysis [17–25]. In fact, the chemical composition of biomass which is characterized by a high moisture, minerals and oxygen content, generates a bio-oil with a lower quality and heating value than crude oil [26, 27]. In the particular case of seaweed, it is characterized by a high ash content, high salinity and associated metal ions and the derived bio-oil often exhibits severe instability as a result of high oxygen content [28–30], in addition to the generation of nitrogen-containing compounds and sulfur responsible of the NOx and SOx emissions. Furthermore, the use of seaweed as a feedstock for bio-oil production is at an early stage of development and needs economically efficient technological solutions [31]. Accordingly, seaweed pre-treatments could be effective in improving the quality of the bio-oil by reducing oxygen and removing alkali and alkaline earth metals.

Among the pre-treatment methods employed to upgrade the quality of bio-oil, torrefaction was found to be beneficial for the deoxygenation of the liquid products by decreasing the oxygen, in addition to reducing nitrogen and sulfur contents of the feedstock [31–33]. On the other hand, pre-treatment of biomass by washing using organic/inorganic acid and base treatment, and ionic liquid treatment allows the removal of water-soluble and non-soluble minerals, producing a pre-treated biomass with lower ash-forming minerals than raw biomass [35–38]. However, these methods have high disposal and recycling costs combined with the use of expensive chemicals and energy considerations.

To address these problems and to promote seaweed pyrolysis efficiency, ‘green’ pre-treatment methods such as biological pre-treatment could be an economical and ecofriendly treatment alternative to chemical and thermal pre-treatment, since it requires no energy or chemical inputs, which will make the process more cost-effective [5]. Thus, some fungal pre-treatment studies revealed biomass composition improvement for the production of high-value-added chemicals and energy-rich pyrolytic products [39–42]. Kumar, et al. [43] recently reviewed biomass pre-treatment methods for bio-oil production and indicated a need to further explore this due to the current lack of information in this area.

Anaerobic fermentation, including dark fermentation (DF) and anaerobic digestion (AD), are biological conversion processes able to treat a wide range of organic wastes while producing biohydrogen and biomethane as biofuel and a nutrient rich digestate. Nonetheless, from an energetic point of view, AD and DF of seaweed biomass are not straight forward due to several technical restraints associated with the low concentration of digestible biodegradable substrate and recalcitrance of some carbohydrate polymers which results in quite limited biodegradability and low biogas production [44–46]. Nevertheless, despite the limitation of anaerobic fermentation to sufficiently extract the energy from seaweed, they could, however, be very efficient green pre-treatment methods to improve bio-oil quality. Furthermore, anaerobic fermentation as a pre-treatment method has the advantage of generating bioenergy as compared to chemical and thermal pre-treatment which consume chemicals and energy.

Currently, integrating anaerobic fermentation processes and pyrolysis is considered as an innovative biorefinery approach and is receiving more attention since it achieves higher energy recovery from biomass [47–54]. However, to date, little information is available about the influence of anaerobic fermentation on the subsequent thermal degradation and composition of chemicals generated during pyrolysis of biologically pre-treated seaweed. Moreover, the vast majority of the studies investigating the use of anaerobic fermentations prior to pyrolysis were only focused on AD while dark fermentation was not generally investigated. In addition, previous studies were mostly focused on increasing the energy recovery from the feedstock and investigating either pyrolysis kinetics, or the characteristics of the derived biochar and its subsequent application as adsorbents. Furthermore, a recent study, integrating AD and pyrolysis was primarily focused on improving gaseous biofuel yield from seaweed [55]. Accordingly, a gap in the state of the art, is in the assessment of the impact of anaerobic fermentation on elemental and proximate composition and the chemical profiles of the resulting pyrolysis products from both raw seaweed and their derived digestates. Thus, the innovation of this study is to investigate, for the first time, the influence of both DF and AD as ‘green’ pre-treatment methods on improving the chemical composition of seaweed prior to their pyrolysis and report on its impact.

Here we report on the effects of DF and AD on modifying the biomass prior to pyrolysis and how this influences the pyrolytic products that are formed. Three macroalgae from different groups namely *Ulva reticulata* (Chlorophyta), *Sargassum polycystum* (Phaeophyta) and *Gracilaria tenuistipitata* (Rhodophyta) were investigated since they contain high ash and sulfur contents and low biodegradability. The impact of pre-treatments on the chemical composition of the derived digestated seaweeds was analyzed using a range of techniques including biochemical characterization, TGA, FT-IR and Pyrolysis Gas Chromatography Mass Spectroscopy (Py-GC/MS).

2. Material and methods

2.1. Algal biomass characterization

Three different seaweeds namely *Ulva reticulata* (green seaweed, Chlorophyta);

Sargassum polycystum (brown seaweed, Phaeophyta) *and Gracilaria tenuistipitata* (red seaweed, Rhodophyta) were used in this study as feedstocks for anaerobic digestion, dark fermentation and pyrolysis experiments and were obtained as a dry milled powder from a commercial supplier in Galway, Ireland.

2.1.1 Elemental analysis (CHNS)

Elemental analysis for carbon, hydrogen, nitrogen and sulfur was carried out using a Thermo Scientific FLASH 2000 organic elemental analyzer. The oxygen content was calculated by difference. The GC column was set at 50˚C and the furnace at 900˚C. Samples were prepared by adding 2 mg of vanadium pentoxide inside a tin capsule followed by approximately 4 mg of each dry algal sample. The capsule was rolled at the ends and placed into the analyzer.

2.1.2 Total Proteins

Ten mg of dry algal samples were weighed and put into Pyrex test tubes followed by the addition of 10 mL of 0.5 M NaOH. The mixture was vortexed for 1 min using a Grant PV-1 Vortex Mixer followed by homogenization for 3 min using an IKA T10 basic ULTRA-TURRAX. The solution was put in an oven for 2 h at 100°C. The solution was then centrifuged (Fisher scientific accuSpin Micro 17) at 6000 rpm for 5 min. Total protein concentration of the supernatant was measured according to the Lowry assay.

2.1.3 Total Carbohydrates

The total carbohydrate concentration of algal samples was determined using the phenol-sulfuric acid method. 10 mg of each dried sample was weighed and transferred to a Pyrex test tube followed by the addition of 10 mL of distilled water and vortexed for 1 minute (Grant PV-1 Vortex Mixer). The suspension was incubated in a water bath at 100°C for 30 min followed by homogenization for 3 min using an IKA T10 basic ULTRA-TURRAX. 5 mL concentrated sulphuric acid followed by 1 mL phenol (5%) were added to 1 mL of each sample. The mixture was further incubated in a water bath at 100°C for 5 min. The concentration of carbohydrate in the samples was determined using a UV-VIS spectrophotometer (Implen C40 Nanophotometer) at 490 nm.

2.1.4 Total Lipids

Total lipids were extracted from whole cells by modification of the method described by Bligh and Dryer [56]. 0.5 g of each sample was weighed and placed in glass Pyrex tubes. Algal cells were acidified with 1 mL 0.15 M acetic acid followed by the addition of 7.5 mL of chloroform/methanol (1:2 v/v). Samples were well mixed by vortexing for 3 min (Grant PV-1 Vortex Mixer). Then 2.25 mL of chloroform was added followed by 2.25 mL of distilled water. The lipids were then gravimetrically determined from the lower chloroform layer.

2.2. Biological Methane Potential (BMP) Test

The biomethane potential of each algal biomass was evaluated by following the methane produced during approximately 2 months under mesophilic conditions at 37°C in a bench top shaking incubator (Incu-Shake MAXI). The biomethane potential of each algal biomass was evaluated by following the methane produced during approximately 2 months under mesophilic conditions incubated at 37°C in a bench top shaking incubator (Incu-Shake MAXI). The inoculum was an industrial sludge sampled in a UASB (Up flow Anaerobic Sludge Blanket) process treating food waste from Severn

Trent plant, Coleshill, UK. The test was performed using a 600 mL flask with a working volume of 400 mL. Each flask contained a reactive medium comprising of the substrate sample, inoculum (sludge) bicarbonate buffer ($NaHCO_3$, 50 g/L), 3.4 mL of a macro elements solution (NH_4Cl, 26.6 g/L; KH_2PO_4, 10 g/L; $MgCl_2$, 6 g/L; $CaCl_2$, 3 g/L), 4 mL of a trace element solution ($FeCl_2$, 2 g/L; $CoCl_2$, 0.5 g/L; $MnCl_2$, 0.1 g/L; $NiCl_2$, 0.1 g/L; $ZnCl_2$, 0.05 g/L; H_3BO_3, 0.05 g/L;Na_2SeO_3, 0.05 g/L; $CuCl_2$, 0.04 g/L; Na_2MoO_4, 0.01 g/L) and water to adjust the volume to 400 mL.

The flasks were sealed and headspaces flushed with nitrogen. One control of the inoculum activity was done with two blanks (no substrate) to measure the endogenous respiration. The biogas production was quantified periodically by pressure measurements. The pressure was measured with a digital manometer LEO 2 (Keller®) with a resolution of 1 mbar. The biogas composition was measured with a micro-gas chromatograph (μGC Varian lGC-CP4900) after an injection of 3 mL of biogas sample. The micro GC was equipped with an injector (100°C), two columns heated at 30 °C for the measurement of CO_2 (HayeSep A) and O_2, N_2, CH_4 and H_2S (Molsieve 5Å PLOT) and a thermal conductivity detector. Finally, the methane production was estimated by subtraction of the average endogenous respiration.

2.3. Biological Hydrogen Potential (BHP) Test

Hydrogen production experiments were performed in 600 mL glass bottles with a working volume of 200 mL MES buffer 50 mM and 1 mL of the same trace element solution used for BMP experiments, were added to each flask. The same industrial sludge used in BMP experiments was firstly pre-treated by heat/shock treatment for 15 min and then used as inoculum. The initial pH was adjusted to 5.5 with NaOH 2N or

37% HCl. After inoculation, each bottle was flushed with nitrogen gas for 10 min to ensure anaerobic conditions. The bottles were then capped with a rubber stopper and incubated at 37 °C. The experimental procedure ended when the pressure in the flask headspace started to drop, indicating hydrogen consumption.

Biogas volume was monitored by measuring headspace pressure with a pressure gauge (Mano 2000, Leo 2Keller) and deducted from the Ideal Gas Law. The gas composition was analyzed using a gas chromatograph (Clarus 580, Perkin Elmer) equipped with two columns, a column (RtQBond) and a molecular sieve (Molsieve, 5Å) and a thermal conductivity detector (TCD). One column (RtQBond) was used to separate H_2, O_2, N_2 and CH_4, and the second one (RtMolsieve) was used to separate CO_2 from other gases. The calibration was carried out with a standard gas (Linde TM) composed of 25% CO_2, 2 % O_2, 10 % N_2 and 5 % H_2 and 58 % CH_4.

2.4. Separation and analysis of solid digestates

At the end of the BMP and BHP experiments, the digestates of each replication were filtrated using filter paper to separate the solid and liquid phases of the digestates. In this study, only the solid digestate fraction were recovered and analyzed to be used for pyrolysis experiments. For this purpose, the solid fraction was dried at 40 °C for 24 hours and then their elemental composition were determined using the Thermo Scientific FLASH 2000 organic elemental analyzer.

Fourier transform infrared spectroscopy (FT-IR) was used to characterize the functional groups changes of seaweeds before and after pre-treatment.

2.5. Thermogravimetric Analysis (TGA)

TGA was carried out using a PerkinElmer Pyris 1 analyzer, following the E1131-03 ASTM standard. A sample of approximately 5 mg was pyrolysed with a nitrogen purge. The ash content was investigated in an air atmosphere at a maximum temperature of 620˚C. This method was repeated for the different types of seaweed samples and their derived digestives.

2.6. Pyrolysis Gas Chromatography Mass Spectroscopy (Py-GC/MS)

This experiment consisted of preparing the samples of seaweed followed by running them through the Pyrolysis Gas Chromatography Mass Spectroscopy (Py-GC/MS). A micro-furnace double-shot pyrolyzer (EGA-PY3030iD, Frontier Laboratories, Japan) interfaced to a split-/splitless inlet port of a 7890B Gas chromatograph (Agilent Technologies, USA) combined with a 5977B Mass Selective Detector (EI ionization energy 70 eV, scan range 28–500 amu). Samples of 0.1 mg were inserted by gravimetric fall into the furnace at 550 °C temperature and pyrolysed in helium atmosphere. The pyrolysis products were separated on a DB 1701 (length 60 m, iD 0.25 mm, film thickness 0.25 μm) capillary separation column with a He flow of 1 ml min^{-1}. The GC injector was operated in split mode (20:1) with an inlet temperature of 250 °C. The column temperature was kept at 50 °C for 1 min then increased at 3 °C min^{-1} to 290 °C, held for 10 min. A semi-quantitative analysis was performed on the pyrograms of the seaweeds, by comparing the peak areas in %.

3. Results and discussion

3.1. Anaerobic fermentation pretreatment of seaweed

First, the choice of the three tested seaweeds was based on their chemical composition characterized by a high ash content in order to determine the effectiveness of anaerobic fermentation processes in producing pre-treated seaweed with low ash-forming minerals [38]. In fact, as shown in Table.1, *Gracilaria* and *Sargassum* had high ash content with more than 60 % while *Ulva* ash content was about 47%. Seaweed often have a high ash content due to the large amounts of alkali metals and chlorides [34,56].

Table 1. Proximate, ultimate and biochemical characterization of seaweeds

Parameters (%)	***Sargassum***	***Gracilaria***	***Ulva***
Moisture	6.7	5.9	11.2
Volatile Solids	33.1	32.9	41.7
Ash	60.2	61.2	47.1
C	21.8	21.6	24.3
H	6.2	7.0	6.3
N	2.5	2.1	2.6
S	3.6	4.1	6.9
O	65.9	65.2	59.9
Protein	7.8	6.5	8.5
Carbohydrate	28.2	32.4	34.6
Lipid	2.2	2.4	3.1

Such high ash content will cause slagging and fouling problems which might limit the pyrolysis process of these seaweeds and reducing the bio-oil yield [35].

Furthermore, nitrogen and sulfur were also high as indicated in Table.1. It can be seen that the three seaweeds had similar nitrogen content ranging between 2.1 and 2.6 %, while the sulfur content differed considerably between the three seaweeds. In fact, *Sargassum* had the lowest sulfur content with 3.6 %, followed by *Gracilaria* with 4.1 % and was *ca.*7% in *Ulva.* The high nitrogen and sulfur content in the three seaweeds would limit the application of the derived bio-oil which will produce NOx and SOx emissions that are environmentally harmful. According to a recent review, only catalytic approaches have been applied to algae biocrude upgrading for N and S removal [58]. To date no data are available about the impact of anaerobic fermentation on reducing N and S prior to pyrolysis.

As a result of the high ash and sulfur content, the three seaweeds had low carbon content ranging between 21.5 and 25%, in addition to a low volatile solids ranging between 32 and 44% with carbohydrate as the major component, followed by protein with less than 9% and a very low lipid content ranging between 2.2% and 3.1%.

The low volatile solids and high sulfur could explain the low biohydrogen and biomethane potentials obtained for the three seaweeds. In fact, according to Fig. 1, presenting the biohydrogen and biomethane potentials of the different algal feedstocks tested, the results indicated that the highest biomethane production were obtained for *Sargassum* with 226 mL/g VS followed by *Gracilaria* with 195 mL/g VS with similar CH_4 content of 56.3%. *Sargassum* and *Gracilaria* had also similar biohydrogen production which could be explained by their similar volatile solid and carbon contents.

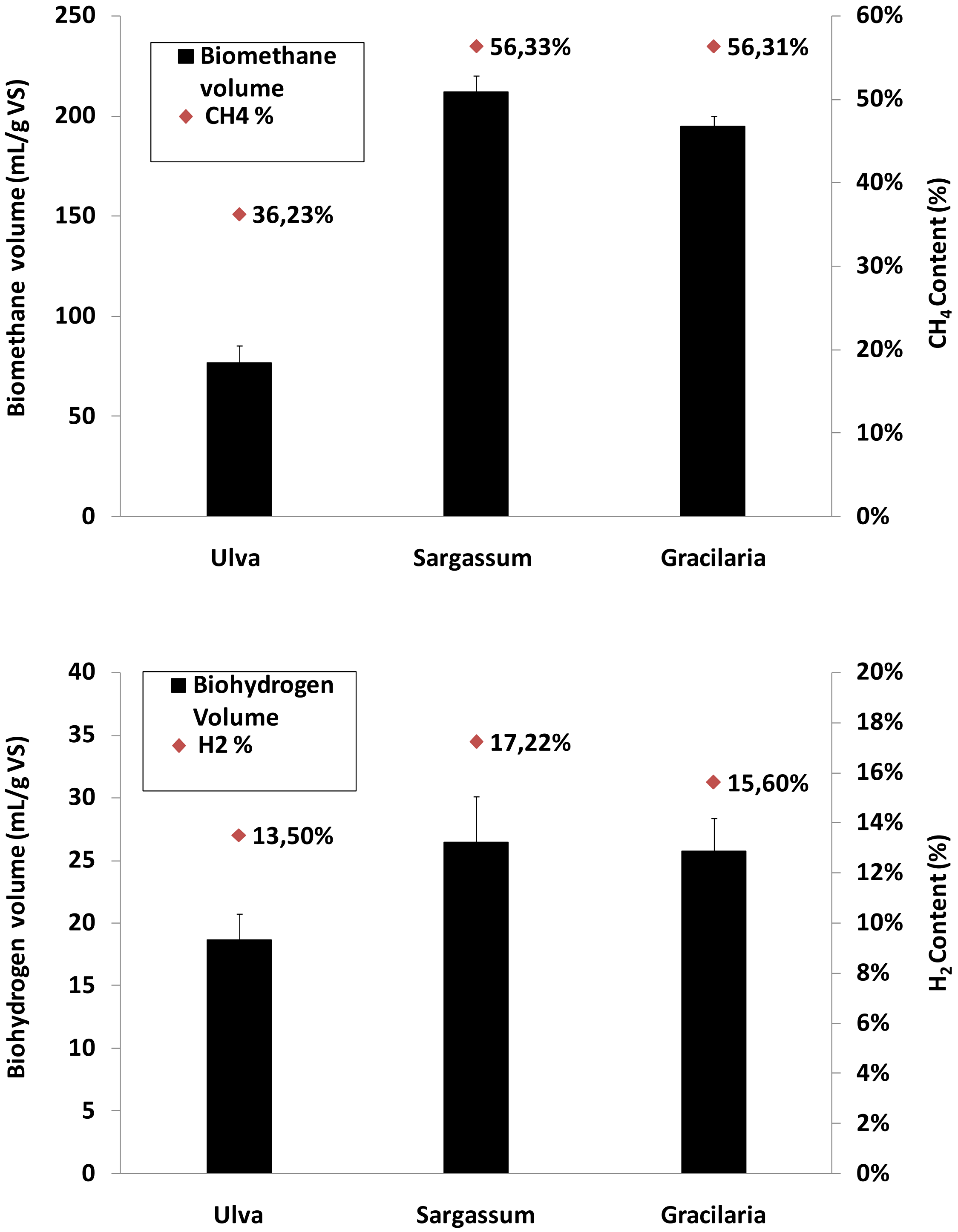


Figure 1. Biomethane and biohydrogen generation during anaerobic fermentation of seaweeds.

The slight difference recorded between the two algal biogas potentials could be attributed to the difference in their sulfur content. In fact, *Gracilaria* had a sulfur content higher than *Sargassum* which could be responsible of the lower biogas potentials produced. Indeed, sulfur is known as an inhibitor of archaeal growth and activity [58,59].

The negative effect of sulfur on biogas production was more important in the case of *Ulva* which had the highest sulfur content (6.9%). In fact, despite its higher volatile solid and carbohydrate contents, *Ulva* produced the lowest biomethane volume with only 80 mL/g VS. Likewise, the CH_4% content in the biogas produced from *Ulva* was very low at 36% indicating that the AD process was inhibited by the high sulfur content in this seaweed. Such low biogas potential revealed the low biodegradability of these seaweeds and the inefficiency of AD and DF to convert the whole volatile solids in seaweed into biogas due to the presence of inhibitors such as sulfur and the high ash content. However, these biological processes could be very interesting pre-treatments to partially degrade carbohydrate in seaweeds to produce more valuable compounds during pyrolysis and to release mineral in the liquid and gas phases to reduce ash, S and N contents in the solid digestate. Furthermore, despite the low biogas produced, AD and DF would have a positive energy balance compared to chemical and thermal pre-treatments.

3.2. Impact of DF and AD on seaweed physicochemical composition and structure

In order to determine the effectiveness of AD and DF in reducing ash, nitrogen and sulfur contents in the three seaweeds, the digestates resulting from the anaerobic fermentation experiments were filtered and the solid fractions were recovered and analyzed for their proximate and elemental composition and compared to raw seaweeds. The results of the proximate analysis are given in Fig. 2. The results clearly showed that the ash contents were reduced in the different digestates especially after DF. In fact, the ash contents were reduced by 25%, 20% and 16% in the dark fermented digestate of *Sargassum*, *Gracilaria* and *Ulva*, respectively, which followed the same order of biodegradability.

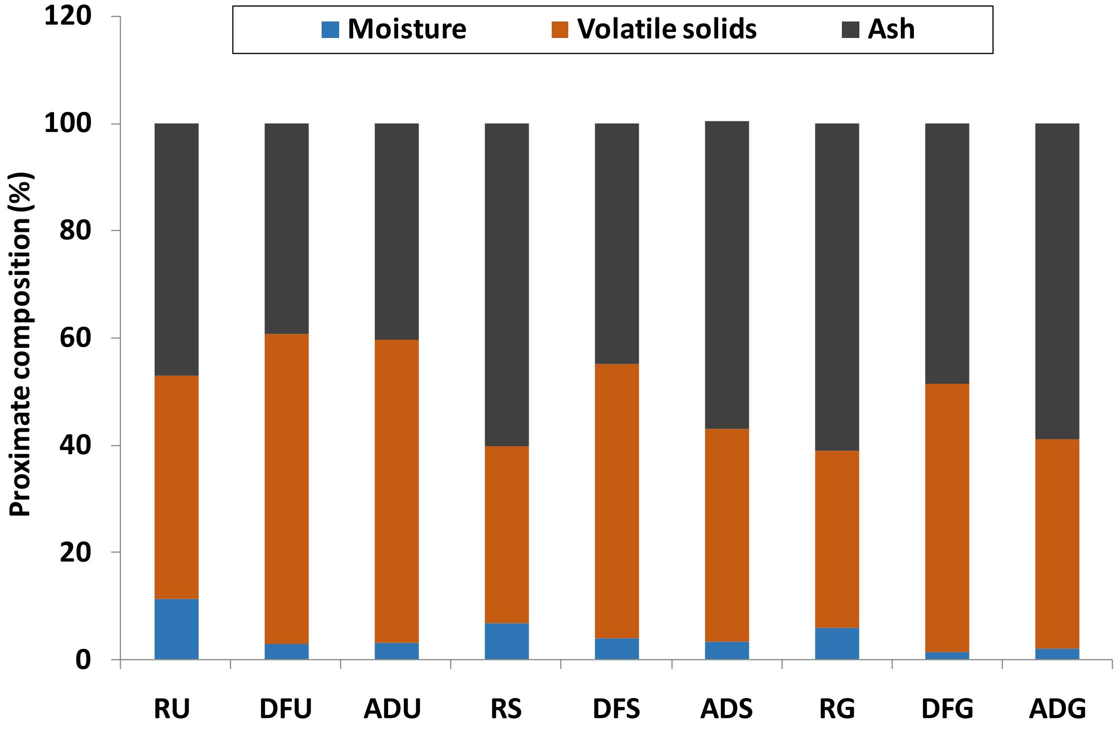


Figure 2. Proximate analysis of raw and pretreated seaweeds. RU: Raw *Ulva*; DFU: Dark fermented *Ulva*; ADU: Anaerobic Digested *Ulva* RS : Raw *Sargassum*; DFS: Dark fermented *Sargassum*; ADS: Anaerobic Digested *Sargassum*; RG : Raw *Gracilaria*; DFG: Dark fermented *Gracilaria*; ADG: Anaerobic Digested *Gracilaria*.

Contrary to DF, the AD had a lower impact on the ash content and indeed they were only reduced by 4.5 % and 3.5 % for *Sargassum* and *Gracilaria*, respectively. Interestingly, in the case of *Ulva*, ash content reduction was three times higher than the two other seaweeds and reached similar rate of removal (14%) in comparison to dark fermented digestate. These results could be explained by the fact that methanogenesis was inhibited during AD of *Ulva* and the rate of degradation obtained was resulting from the three first stages of the AD process (hydrolysis, acidogenesis and acetogenesis) which are similar to the DF stages. Accordingly, DF was more efficient in term of reducing ash content than AD. Furthermore, moisture was also decreased with about 70% in *Gracilaria* and *Ulva* and 43% in *Sargassum*. This reduction would decrease the water content in bio-oil. Moreover, as a result of ash and water reduction, volatile solids increased in all digestates which could positively influence the yield of bio-oil.

In addition to the positive impact on the proximate composition, anaerobic fermentation pre-treatments also influenced the elemental composition of seaweeds by reducing S, O and N and increasing C contents. Fig.3 shows the impact of AD and DF on the elemental composition of the seaweed and indicated that N was highly removed from the digestates of both AD and DF with a removal rate ranging between 86-90% and 84-87%, respectively. As a consequence, anaerobic fermentation pre-treatments were very effective in reducing N from seaweeds avoiding the production of N compound in the bio-oil and reducing the risk of NOx emission. Furthermore, S was also removed from seaweeds during AD and DF but with lower rate as compared to N (Fig.3). In fact, S reduction was higher in the AD digestate of *Sargassum* and *Gracilaria*, with 70% and 63%, respectively. The same trend was observed during AD of organosulfur compound-rich wastes where 94.5% and 76.2% of sulfur compounds in the fish and pork wastes were converted into volatile sulfur compounds (VSCs) [60]. For DF digestate of *Sargassum* and *Gracilaria*, S removal was 61% and 52%, respectively. In contrast, S removal in *Ulva* was higher in the DF digestate with 52% and with 42% in the AD digestate. This could be explained by the low mineralization rate of *Ulva* and as a consequence the low conversion of S into VSCs in the gas phase. Yang et al. [61] investigated three different species of white-rot fungus (*Pleurotus ostreatus* BP2, *Echinodontiumtaxodii* 2538, and *Irpex lacteus* CD2) to bio-pre-treat corn stover and found that it could effectively decrease the emission of toxic SOx through reduction of the sulfur content in the feedstock by 30–45%.

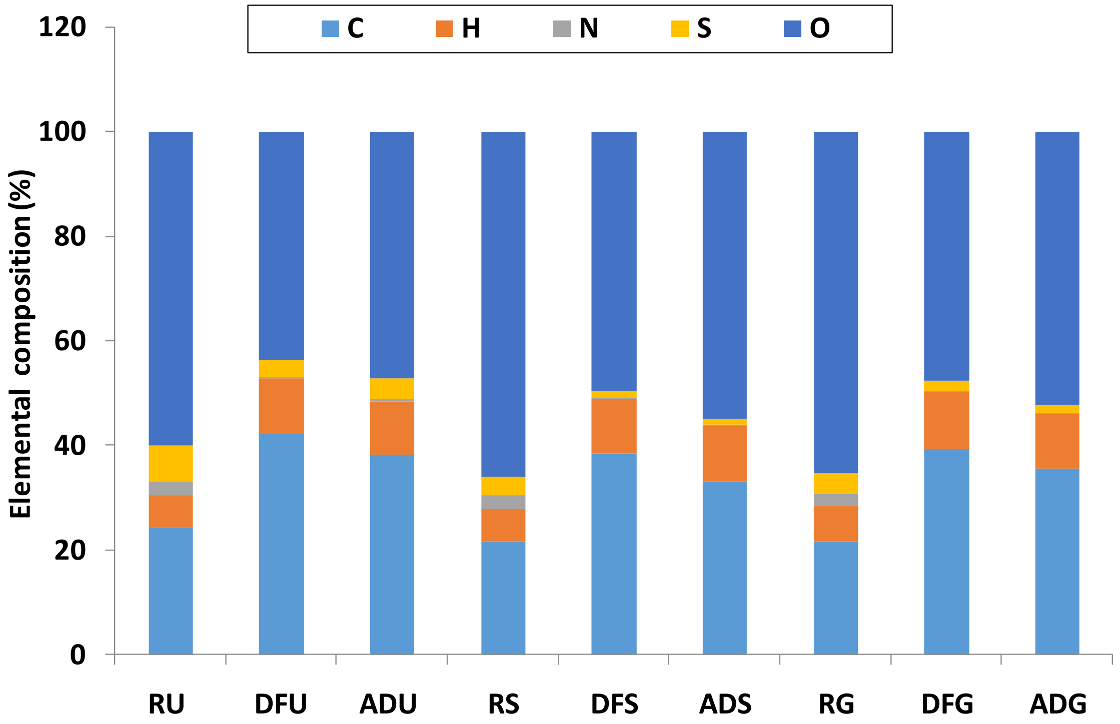


Figure 3. Elemental composition of raw and pretreated seaweed RU: Raw *Ulva*; DFU: Dark fermented *Ulva*; ADU: Anaerobic Digested *Ulva* RS : Raw *Sargassum*; DFS: Dark fermented *Sargassum*; ADS: Anaerobic Digested *Sargassum*; RG : Raw *Gracilaria*; DFG: Dark fermented *Gracilaria*; ADG: Anaerobic Digested *Gracilaria*.

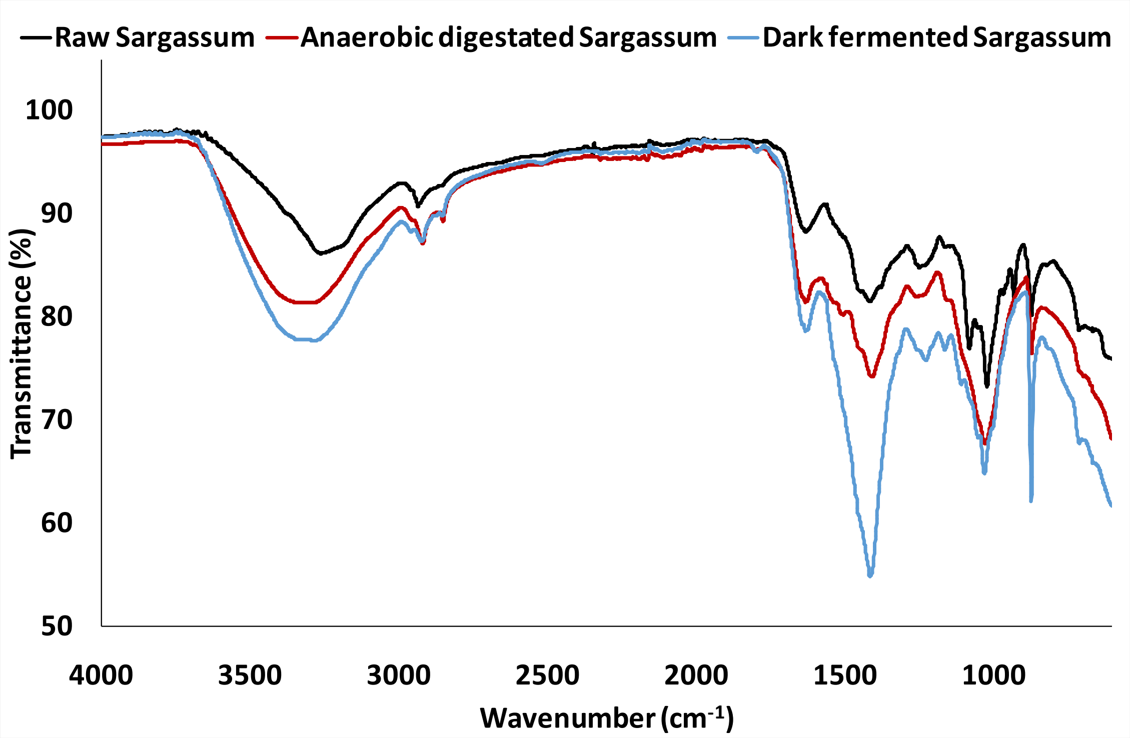
Accordingly, the reduction of N and S through anaerobic fermentation pre-treatments could avoid recourse to denitrogenation and desulphurization of seaweed biocrude.

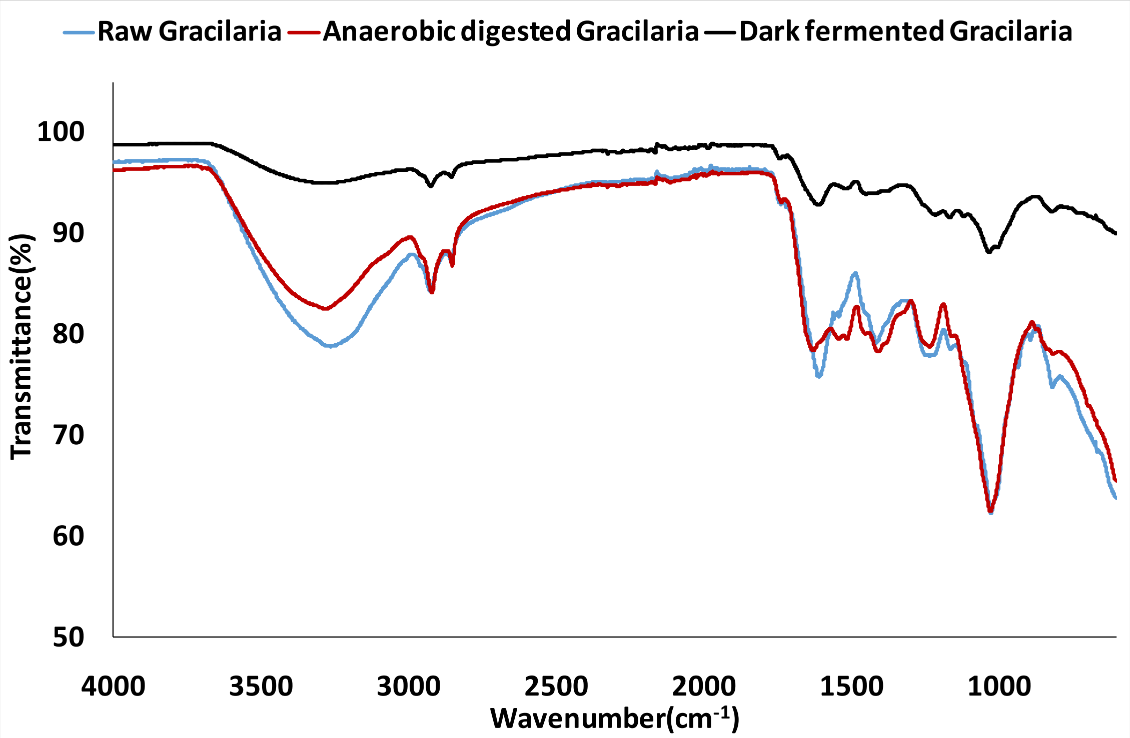
The results of the elemental composition of digestates also reveal the impact of pre-treatment on the carbon content which increased in the different digestates. The carbon content (C) was higher in the DF digestates, ranging between 38 and 42 %, which was almost two times higher than C in the raw seaweed. Moreover, carbon in AD digestate were lower than C in DF digestate but more evident than in raw seaweeds. Indeed, anaerobic fermentation converted the biodegradable fraction of seaweed into biogas which tends to reduce carbon in the feedstock. However, the recalcitrant organic matter remains in the solid fraction of the digestate while the mineral fraction has a tendency to be released in the liquid fraction which could explain the reduction of ash and mineral in solid digestate while volatile solids and C increased.

Another important impact of anaerobic fermentation on seaweeds was the reduction of the oxygen (O) content. In fact, O reduction was more obvious in the DF digestate with about 25- 27.5% for the three seaweeds. AD also reduced O in the digestate with 17% for *Sargassum* and 20 and 21% for *Gracilaria* and *Ulva*, respectively. The reduction of O in the feedstock is very important to upgrade bio-oil. Furthermore, anaerobic fermentation pre-treatments were more efficient in reducing O and increasing C from algal biomass than fungal pre-treatment of other biomasses. In fact, Yu et al. [41] reported that fungal pre-treated corn stover had slightly increased carbon content from 41.5 to 43.8% and decreased oxygen content from 53.0 to 50.4% than untreated control samples. In comparison with chemical and thermal pre-treatment of seaweed, anaerobic fermentation was more efficient in terms of reducing N and S in contrast to acid washing of *Cladophora glomerata* which increased N and S from 5.1% to 8.9% and 2.4

% to 5.2%, respectively [62]. Ross et al, [63] investigated acid washing of three brown algae, found that N content increased in all treatments. N in *Saccharina japonica* was also increased when pre-treated with diluted acid solution [30]. However, acid washing pre-treatmentis more efficient than anaerobic fermentation pre-treatment in terms of reducing ash from seaweed with a removal rate *ca.*50 % [61,62]. In terms of O reduction and C improvement anaerobic fermentation is more effective than both torrefaction and acid washing. Indeed, Hu et al, [64], investigating the effect of torrefaction on the green seaweed *Enteromorpha clathrata*, indicated that increasing torrefaction temperature had positively affected C content which increased from 29.4% to 34.4% at 275°C. The effects of acid washing are unclear with Nikkhah et al.[62] finding that acid washing of green seaweed increased C from 35% to 44% while Ross et al,[63] showed a reduction of C from 52% to 44% in the brown seaweed *F. vesticulosus*.

In addition to their effects on seaweed physicochemical composition, anaerobic fermentation pre-treatments strongly affected their structure as indicated by FTIR spectroscopy (Fig.4). Anaerobic fermentation pre-treatments had a significant influence on surface functional groups. In *Sargassum*, alginic acid (a linear co-polymer with homopolymeric blocks of(1-4) linkedβ-*D*-mannuronate and α-*L*-guluronate residues), sulphated polysaccharides and peptidoglycans are major cell wall components [65] whereas, in *Gracilaria*, agarose appears to be amajor constituent [66] and in *Ulva*, sulphated polysaccharides and ulvan [67] together with cellulose [68] have been reported. These components are particularly rich in -OH and -COOH and the peptides contain -NH_3 functionalities.

417 
Raw Sargassum
Anaerobic digestated Sargassum
Dark fermented Sargassum
Transmittance (%)
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

418 
Raw Gracilaria
Anaerobic digested Gracilaria
Dark fermented Gracilaria
Transmittance(%)
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
Wavenumber(cm-1)

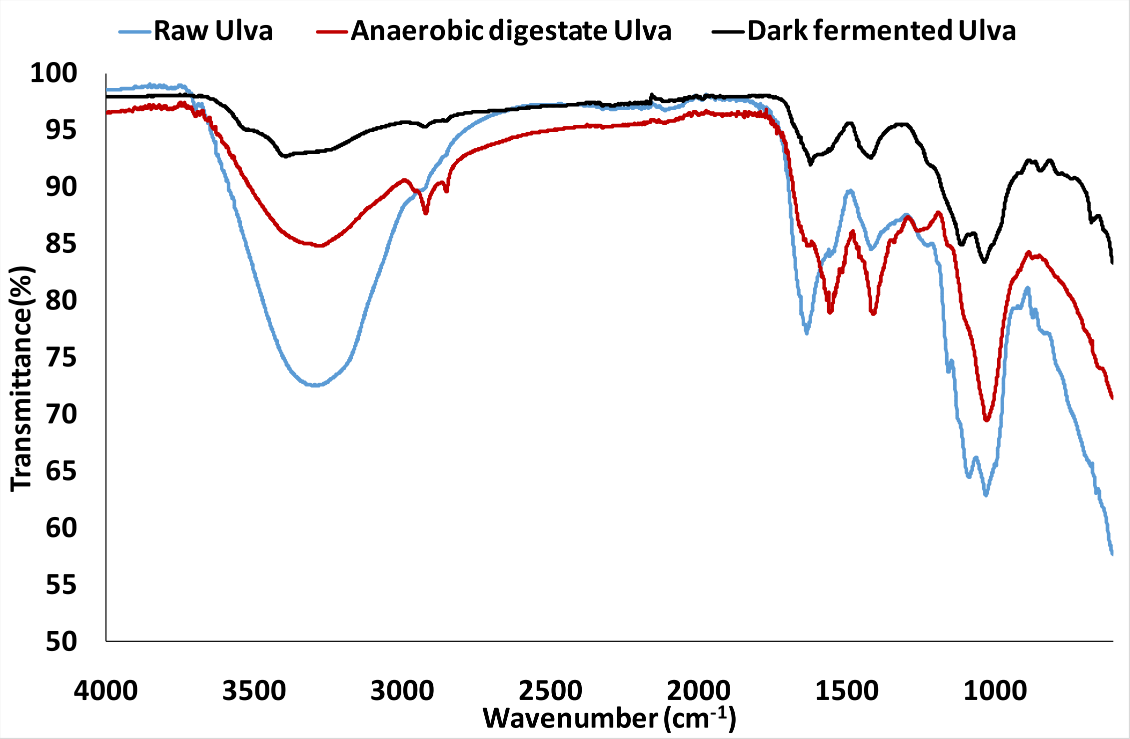
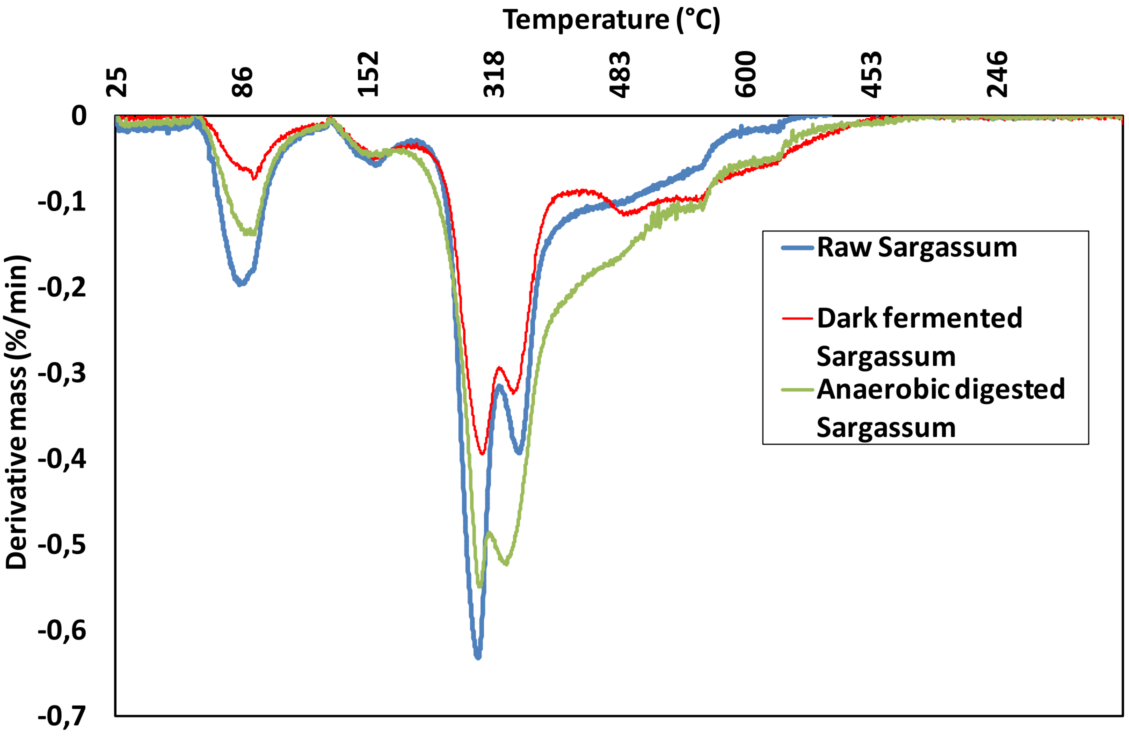


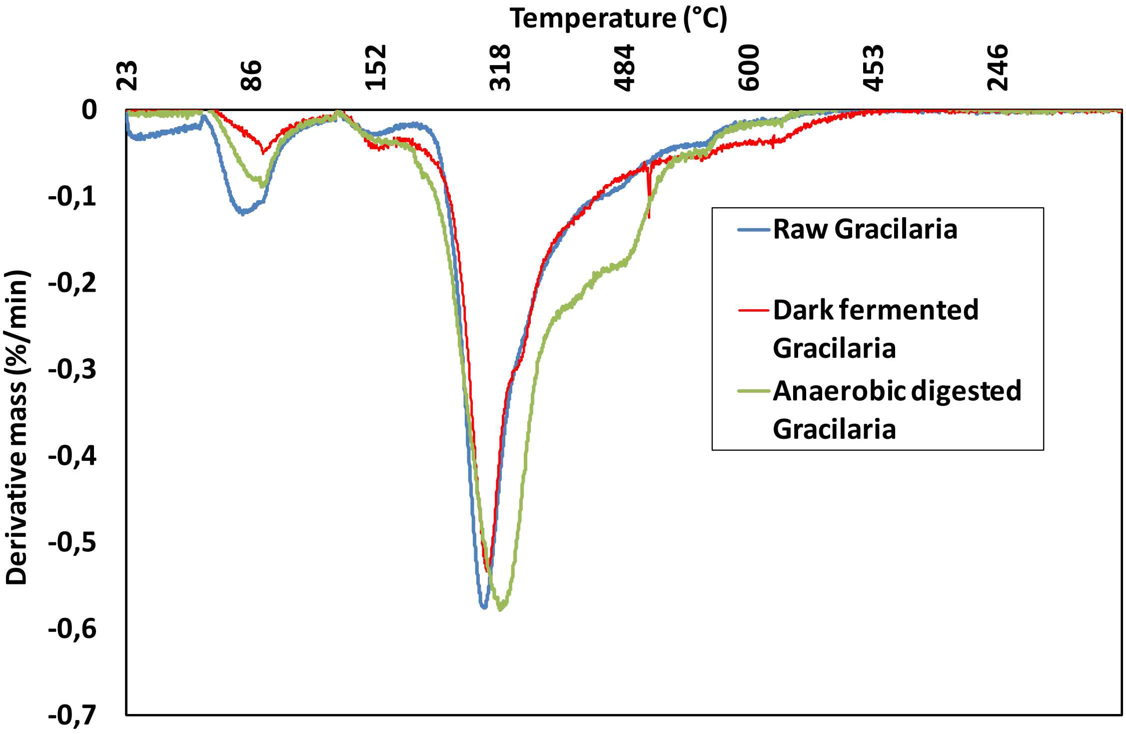
Figure 4. FTIR spectroscopy of raw and pretreated seaweeds

We noted that the intensity of the major peaks located at 3500–3300 cm^{-1} , 2927-2842 cm^{-1} , 1705 cm^{-1} , 1690–1450 cm^{-1} , and 1475-1000 cm^{-1} assigned to the stretching vibration of –OH, C–H (aliphatic $–CH_2$ and$–CH_3$), C=O, C=C, and C–O and C–H (aliphatic $–CH_3$ or phenolic–OH bonds) become weaker after AD. The decrease of the band intensity was more important in the case of dark fermentation and the intensity of band located at 3500–3300 cm^{-1} and at 1035 cm^{-1} decreased sharply and were close to disappearing especially in the case of *Ulva* and *Gracilaria*. The reduction of the intensity of most oxygen-containing functional groups is due to the decarbonization, dehydrogenation and deoxygenation reactions [69]. This could also confirm the reduction of O after anaerobic fermentation pre-treatments.

3.3. Thermogravimetric Analysis

The DTG and TG profiles of raw seaweeds and their derived digestates under N_2 are shown in Fig.5 and Fig.6, respectively, to determine the effect of AD and DF pre-treatment processes on the pyrolysis behavior of seaweeds. During pyrolysis, the mass loss of the three seaweed biomasses was due to the release of vapors and gases. DTG curves can be characterized into three stages (Fig.5) and during the first stage, (25 and 150°C) all the samples had the lowest weight loss which was mainly due to dehydration [70]. At this stage the weight loss was more important in the raw seaweeds which had the highest water content. With anaerobic fermentation the weight loss in this stage tended to decrease in the digestate of the three seaweeds. Thus, DF and AD pre-treatment would reduce water vapor from the seaweeds and as a consequence would reduce the water content in the bio-oil. A similar trend was observed during biomass torrefaction [71].





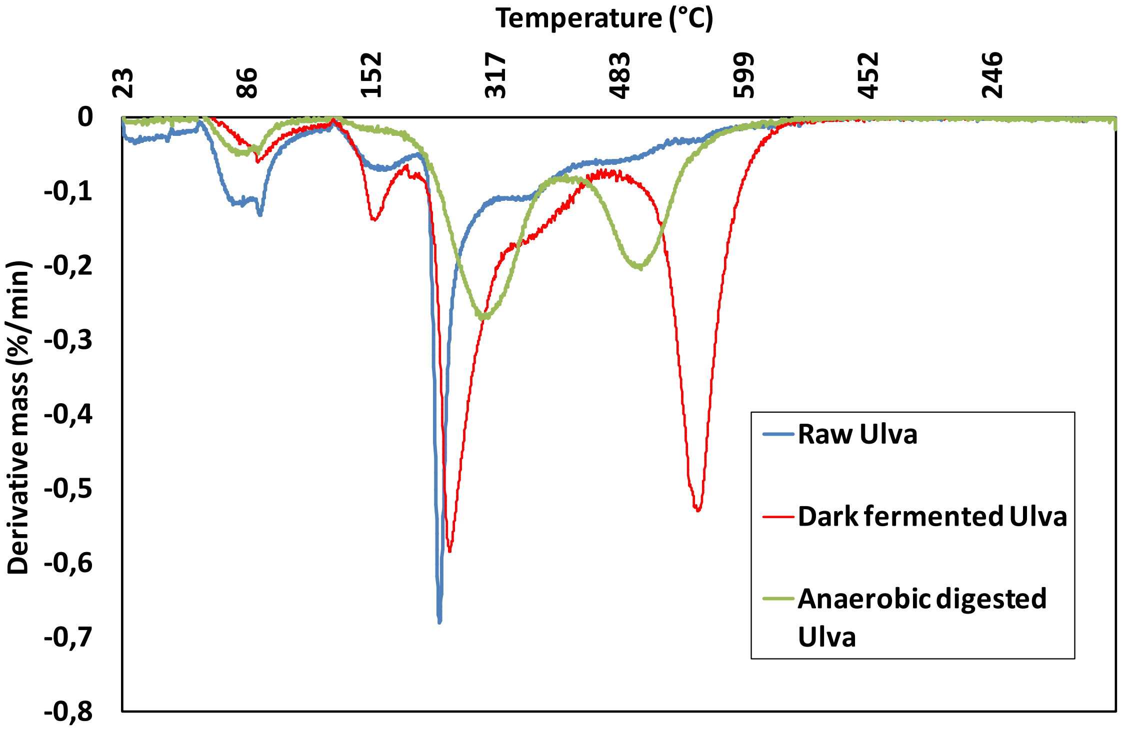
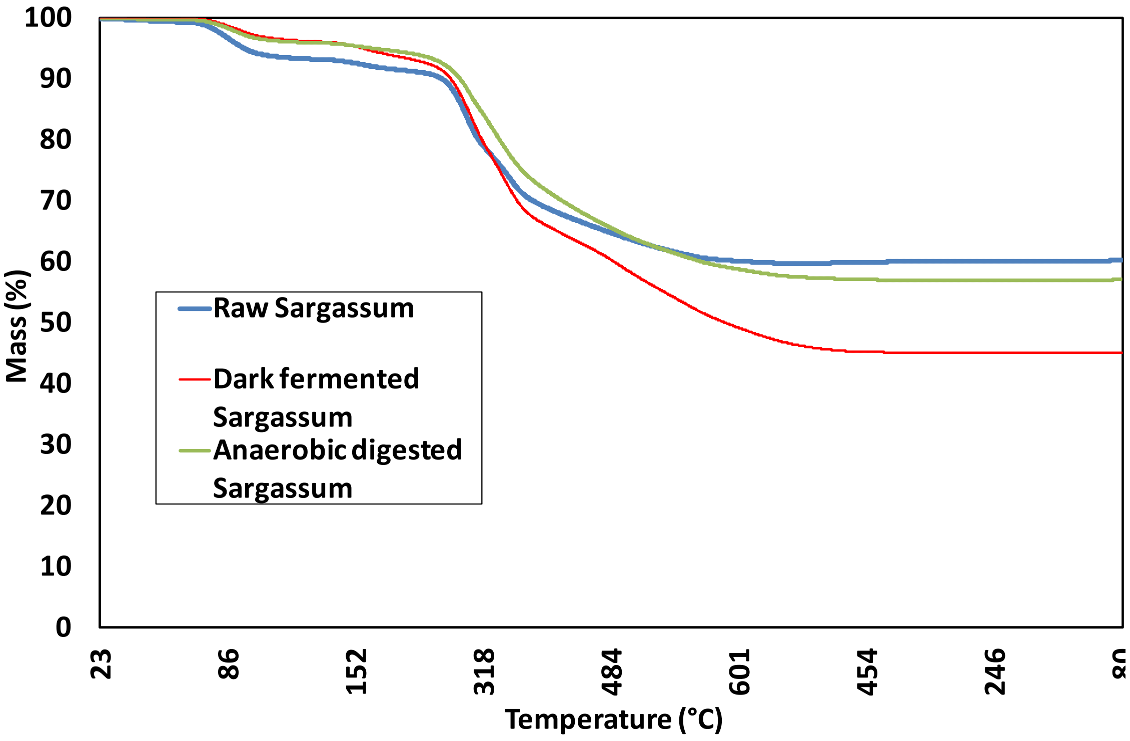
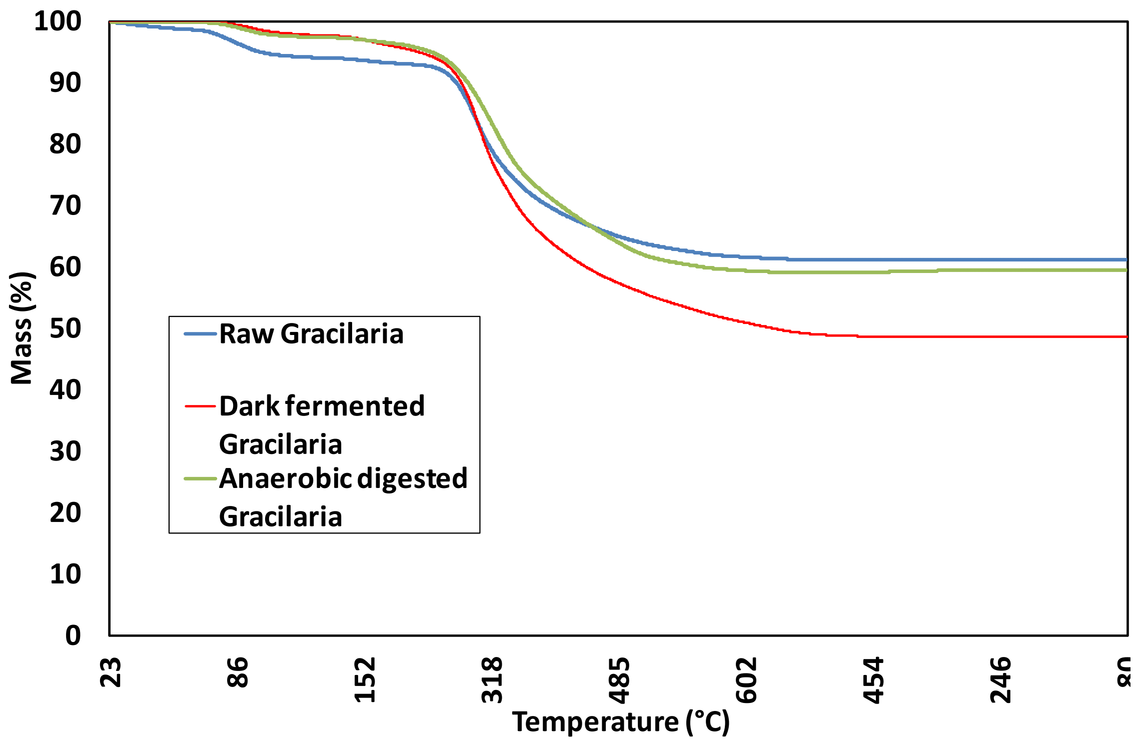


Figure 5. Differential Thermogravimetric profiles of raw seaweeds and their derived AD and DF digestate.

The second stage represented the main pyrolysis stage occurring in the temperature range 150- 600 °C, where the major weight loss occurred. In fact, the three raw seaweeds had their highest peaks between 240-350˚C which corresponded to the decomposition of carbohydrates and proteins [62]. Accordingly, the three seaweeds had their highest peaks in this range of temperature because they were rich in carbohydrate. On the other hand, the digestates presented different thermal behaviors as compared to their raw seaweeds. In fact, the impact of anaerobic fermentation pre-treatments was different for the three species. For *Sargassum* digestates, despite having the same peaks as raw *Sargassum* (Fig.5), however, the weight loss rate was not the same. In fact, according to Fig.5 showing the TG of *Sargassum* and its derived digestate it is clearly seen that after anaerobic fermentation the weight loss increased, indicating the presence of more volatile matter in the digestate in comparison to raw *Sargassum*. This could be due to the breakdown of carbohydrates in the raw seaweed during hydrolysis into monosaccharides and leaching of minerals. The same behavior was noticed for *Gracilaria* (Fig.6). For both seaweeds, this effect was more relevant after DF, while after AD the changes in TG was less important. In fact, during DF biopolymers such as carbohydrate are hydrolyzed producing a range of oligo and mono-saccharides derivatives and lipids generating VFA and others metabolites [72], while, during AD the majority of these metabolites are converted into methane by methanogenic community. Thus, an important part of these volatile molecules was lost in the gas phase as CH_4 and CO_2. This impact was clearer in the case of *Ulva* which had a very low production of biomethane with <90mL/g VS and a very low methane content in the biogas 36% (as shown previously in section Fig.1). In fact, TG profiles of *Ulva* digestate after DF and AD (Fig. 6) were very similar and had almost the highest weight loss at 600°C indicating that metabolites resulting from the hydrolysis stage were not converted into methane due to the presence of inhibitors such as sulfur.

DTG profiles of raw *Ulva* and its digestate indicated significant changes after anaerobic fermentation by the presence of two wide peaks in the digestates instead of one narrow peaks in raw *Ulva* (Fig.5). Indeed, raw *Ulva* was characterized by a main peak at 246°C. After anaerobic fermentation, this peak in both digestates resulting from DF and AD shifted to 256°C and 295°C, respectively. These shifts were attributed to the loss of some biodegradable matter during DF and especially after AD. In addition, a second important peak appeared at 500°C and 587 °C after AD and DF, respectively. These peaks appeared only in the digestate of *Ulva* which could result from the degradation of macromolecules during anaerobic fermentation into monomers. Accordingly, the anaerobic fermentation of the seaweeds might result in increasing volatiles in the digestate and as a consequence, produce higher bio-oil yield.





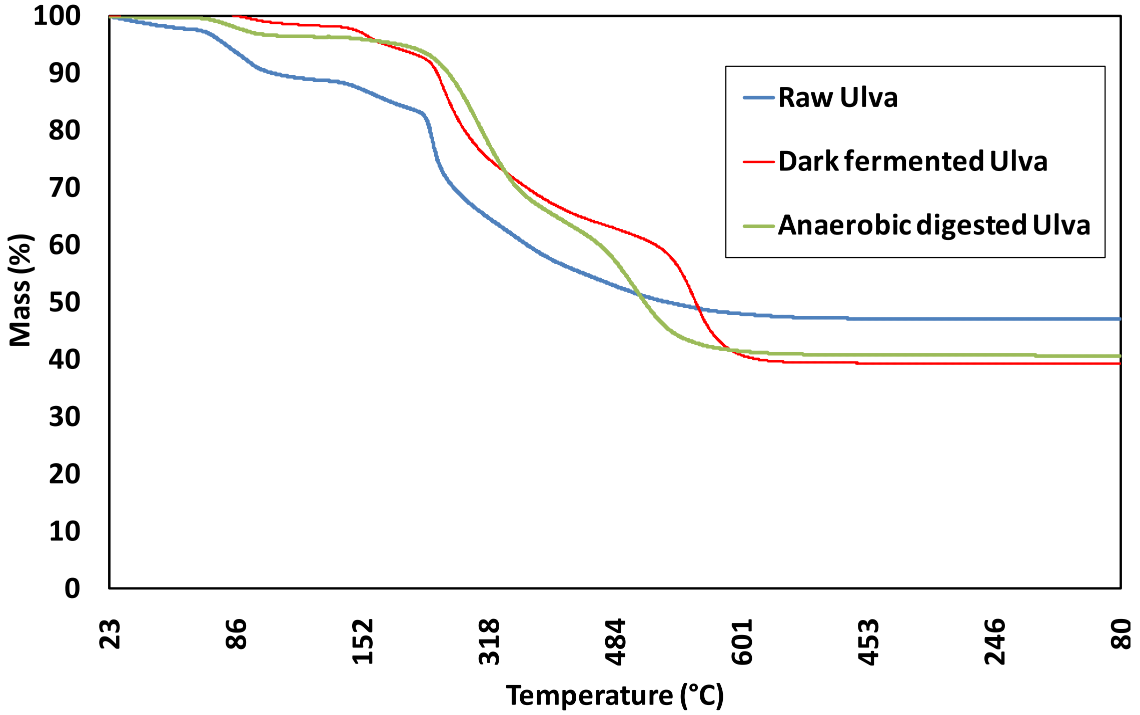
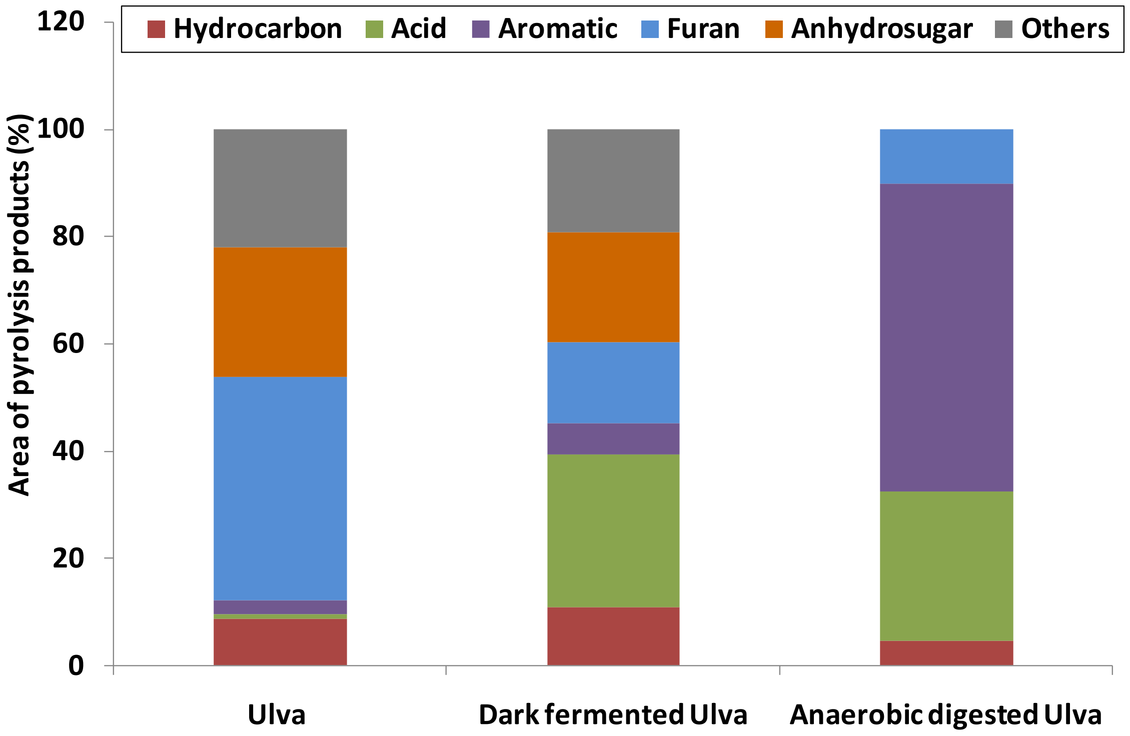


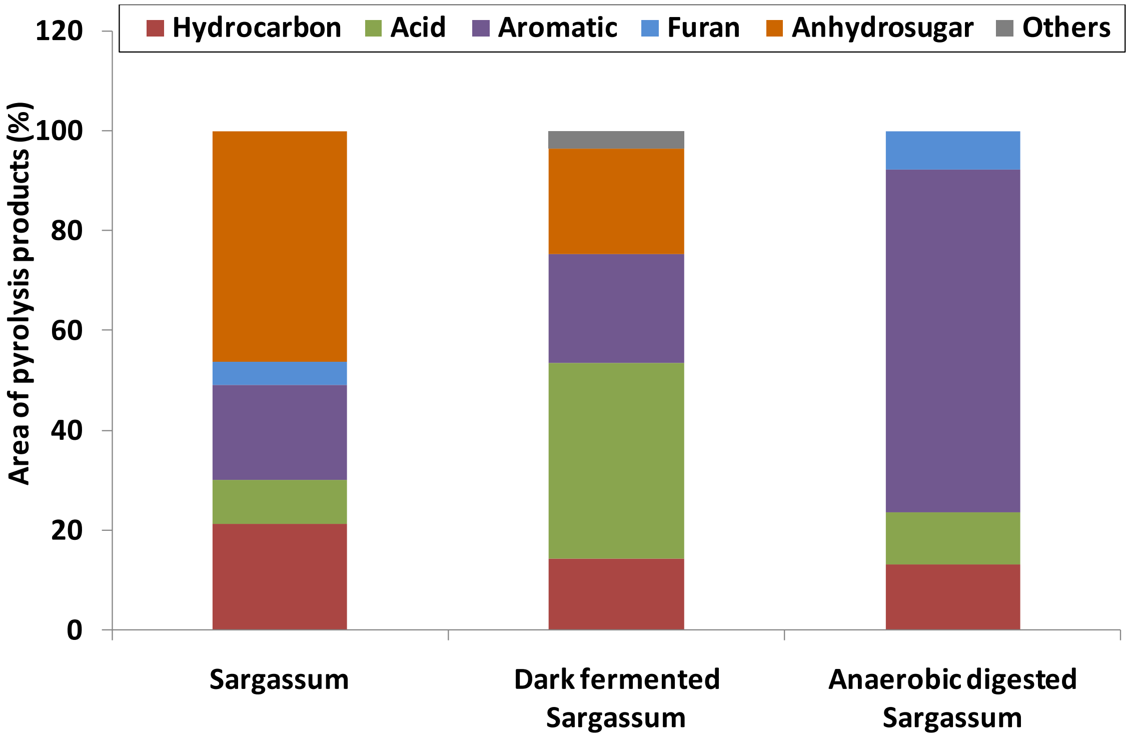
Figure 6. Thermogravimetric analysis of raw seaweeds and their derived AD and DF digestate.

This finding is consistent with the results of the proximate and elemental composition indicating the increase in volatile solids and carbon contents after anaerobic fermentation pre-treatments.

3.4. Impact of anaerobic fermentation on volatile profiles generated during pyrolysis of raw and pre-treated seaweeds

The composition of volatiles obtained from the pyrolysis of raw and pre-treated seaweeds was analyzed using Py-GC/MS and the products obtained were expressed as area percentage of the chromatogram (Fig.7 and Table.2). The results indicate that the products obtained from raw seaweeds and their derived digestates were markedly different. In fact, DF and AD affected strongly the pyrolysis products in term of composition and number of components which was not the case observed for fungal treatment of corn stover biomass reported by Yu et al., 2013 [41] that showed only differences in relative abundance. Anhydrosugars and furans were the main pyrolysis products of raw seaweeds, representing together 51%, 66% and 75% of the total components produced during the pyrolysis of *Sargassum*, *Ulva* and *Gracilaria*, respectively (Fig.7). The highest proportion of anhydrosugars were produced from raw *Gracilaria* with 62% followed by *Sargassum* with 46% and *Ulva* with 24%. *D*-Allose is the main anhydrosugar produced in the three raw seaweeds (Table 2) and the levels of anhydrosugars was strongly affected by the anaerobic fermentation pre-treatments. In fact, after DF, they decreased significantly in the case of *Gracilaria and Sargassum* by 54% and 37%, respectively and after AD, were depleted to below detection levels. By contrast, fungal pre-treatment was found to increase anhydrosugars in the corn stover [41]. Interestingly, in a previous study, we only detected *D*-allose in one species of Chlorophyte, namely, *Prasiolacrispa*, out of 12 macroalgae that were analyzed following intermediate pyrolysis [73].





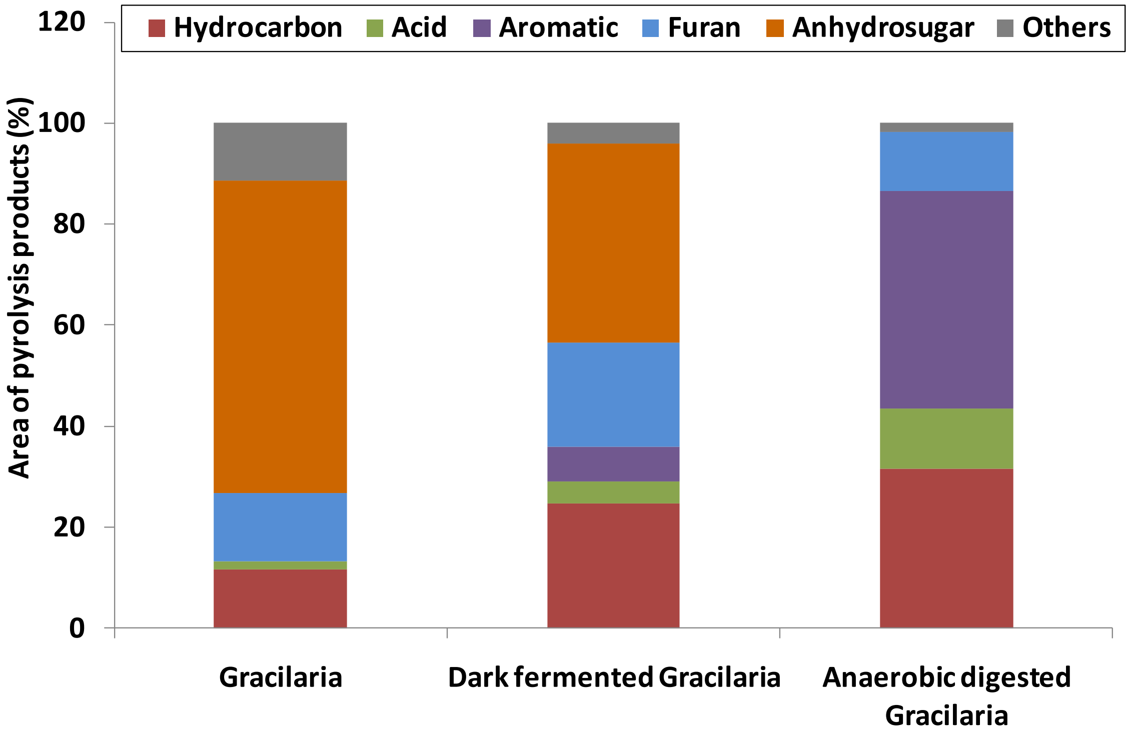


Figure 7. Major classes of products generated from pyrolysis of raw and pre-treated seaweeds.

The same pattern was observed for furans as for anhydrosugars, which were affected by the anaerobic fermentation pre-treatment especially in the case of *Ulva* where furans composition declined in the raw seaweed by 64% and 75%, following DF and AD, respectively. Interestingly, after AD, benzofuran, which did not appear in the composition of furans produced from raw seaweed or even DF digestate, was the only furan produced from *Sargassum* and *Ulva* AD digestate and the main furan in *Gracilaria* digestate. The reduction of anhydrosugars and furans in the pyrolysis products of the digestate could be explained by the fact that during anaerobic fermentation polysaccharide and protein were hydrolyzed thus decreasing both the number and abundance of components derived from them.

Anaerobic pre-treatment promoted the production of carboxylic acids, hydrocarbon and particularly, aromatics (Table 2).

Table 2. Composition of the main pyrolysis volatiles(area %) of the different seaweeds and their derived digestates (S: *Sargassum*; G: *Gracilaria*; U: *Ulva*)

		Raw Seaweeds			Dark fermented seaweeds			Anaerobic digested seaweeds		
Compounds		**S**	**G**	**U**	**S**	**G**	**U**	**S**	**G**	**U**
Anhydro-sugars	***D*-Allose**	33.8	53.2	24.2	15.4	39.4	20.5	-	-	-
	Anhydrosugar	12.4	4.5	-	5.7	-	-	-	-	-
	Dianhydro-mannitol	-	4.3	-	-	-	-	-	-	-
Furans	**Furfural**	-	3.2	11.4	-	5.2	-	-	4.0	-
	2-Furan Carboxaldehyde, 5-methyl-	4.6	-	30.2	-	-	15.1	-	-	-
	Ethanone, 1-(2-furanyl)-	-	2.1	-	-	4.0	-	-	1.7	-
	5-Hydroxymethyl-furfural	-	9.3	8.5	-	-	-	-	-	-
	Methyl 2-furoate	-	-	-	-	15.5	-	-	-	-
	Benzofuran	-	-	-	-	-	-	7.6	7.7	10.3
Acids	**Acetic acid**	8.9	1.6	1.0	33.7	4.5	28.4	10.5	12.0	27.9
	Propanoic acid, 2-oxo-, methyl ester	-	-	-	5.5	-	-	-	-	-
Hydro-carbons	**Aliphatic hydrocarbon**	-	10.0	20.7	3.5	16.1	24.3	6.8	-	-
	2-Propanone, 1-hydroxy-	21.3	2.3	1.3	14.4	7.4	4.2	6.2	11.8	4.4
	1,2-Cyclopentanedione	-	-	-	-	-	1.6	-	5.0	-
Aromatics	**Toluene**	7.7	-	2.7	10.2	-	5.8	17.6	-	13.9
	Styrene	8.1	-	-	-	-	-	11.1	6.6	4.9
	Phenol	3.3	-	-	5.1	3.5	-	5.5	6.4	5.6
	Indole	-	-	-	6.4	3.3	-	8.1	-	7.6
	Phenol, 2-methoxy-4-(1-propenyl)-	-	-	-	-	-	-	4.9	6.9	-
	1H-Indole, 2-methyl-	-	-	-	-	-	-	4.5	-	-
	p-Cresol	-	-	-	-	-	-	6.9	-	6.5
	Phenol, 2-methoxy-	-	-	-	-	-	-	-	8.6	6.3
	2-Methoxy-4-vinylphenol	-	-	-	-	-	-	10.0	14.3	12.5

In fact, raw seaweeds produced acetic acid as the main components with 9% for *Sargassum* and <2% in *Gracilaria* and *Ulva*. After DF, the digestate produced acids with >40% for *Sargassum* and about 29% for *Ulva.* AD digestate also produced more acetic acid than raw seaweed. In contrast to AD, DF pre-treatment promoted significantly the production of aliphatic hydrocarbon which reach 24.3% in *Ulva* DF digestate against 7.2% in raw *Ulva* and 16% in *Gracilaria* DF digestate against 9.5% in raw *Gracilaria* and appear in *Sargassum* DF digestate (3.5%) while it was absent in raw *Sargassum.*

The most important impact of anaerobic fermentation pre-treatments was noticed in the content of aromatics. In fact, high content of aromatics was produced from AD digestate of *Sargassum* and *Ulva* with 68.7% and 57.3%, respectively. Aromatics such as toluene and ethylbenzene are the main components present in gasoline representing 35 % with 15 % being toluene [74]. Aromatics are key components of gasoline fuel used for the internal combustion engines of automotive vehicles because of its high heating value and superior capability of mixing with air [8]. From Table 2, we can distinguish the positive impact of anaerobic fermentation on toluene which increased in the pre-treated seaweeds and reached 17% in *Sargassum* AD digestate and 14% *Ulva* AD digestate. On the other hand, *Gracilaria* and its derived digestates did not produced toluene. In fact, raw *Gracilaria* did not produced aromatics but when pre-treated, small amounts of aromatics appeared in the DF digestate (6.8%) and increased in the AD digestate reaching 43%. Most of these aromatics were phenols with 2-methoxy-4-vinylphenol as the main components (14.3%). Moreover, fungal pre-treatment was also found to increase aromatics by 14 %. However, the sum of aromatics produced was about 12% [41], which is 4 to 5 times proportionally lower than aromatics produced in the anaerobically pre-treated seaweeds reported here. The increased production of aromatics could be attributed to the enhanced deoxygenation reactions, such as dehydration, decarboxylation, decarbonylation, aromatization and rearrangement reactions [43]. Aromatics may arise from the side chains (R-groups) of specific amino acids such as phenylalanine and tyrosine present in proteins [56].

According to these results, anaerobic fermentation pre-treatments were as effective as acid washing in increasing toluene. In fact, Cao et al. [35], investigating the impact of acid washing on the relative contents of pyrolysis volatiles of pre-treated *Enteromorpha clathrata*, revealed the increase of toluene between 5.4 and 15.5 % as compared to raw seaweed with only 3.3%. In addition, it has been shown that catalytic pyrolysis of the acid-washed *Saccharina japonica* using HZSM-5 catalyst results in an increase in aromatic compounds such as phenol, indole, and naphthalene-derivatives [30].

As compared to torrefaction, anaerobic pre-treatments were more efficient in terms of increasing aromatics. Dong et al. [75] have revealed that the relative contents of phenols increased from 28 to 42 % in the bio-oils from the pyrolysis of the torrefied rice which is lower than aromatics produced from anaerobically pre-treated seaweed reported here.

4. Conclusion

In this study we have investigated the impact of anaerobic fermentation pre-treatments on three seaweeds composition and structure prior to pyrolysis. The findings of this investigation revealed the potential of the anaerobic fermentation to be used as an effective ‘green’ pre-treatment to improve seaweed characteristics as compared to thermal and chemical pre-treatments. In fact, AD and DF pre-treatments reduced N and S contents by more than 80% which avoids the production of N and S compound in the bio-oil and reducing the risk of NOx and SOx emissions. DF was more efficient in terms of reducing ash content than AD resulting in the production of fermented seaweeds with higher volatile solids than digested seaweeds. Moreover, DF strongly affected the structure of seaweed by reducing the intensity of most oxygen-containing functional groups. As a result, DF pre-treated seaweeds had a lower O content which will improve the quality of bio-oil. Furthermore, the pre-treated seaweeds presented different thermal behaviors and produced different pyrolysis volatiles as compared to raw seaweeds. Anhydrosugars, which were the main components of the pyrolysis volatiles produced from raw seaweeds, decreased sharply after DF pretreatment while acetic acid and aliphatic hydrocarbons increased. The generation of pyrolysis oil demonstrated here that is rich in aromatics, particularly toluene, at levels similar to that found in petroleum-based fuel and largely devoid of anhydrosugars with low N and S content makes it a fuel source with a closer match to fossil fuel than previous methods have achieved. It should also be pointed out that the minerals released from the algal biomass during anaerobic pre-treatments into the liquid phase of the digestate could be recovered and used as culture media for algae growth and thereby improve the efficiency of such biorefinery configurations.

Acknowledgement

The authors acknowledge the European Union and Horizon 2020 for the financial support of the action H2020-MSCA-IF, TASAB, Grant 706642.

Reference

[1] Y. K. Park, J. M. Ha, S. Oh, and J. Lee, “Bio-oil upgrading through hydrogen

transfer reactions in supercritical solvents," Chem. Eng. J., vol. 404, p. 126527, Jan. 2021, doi: 10.1016/j.cej.2020.126527.

[2] M. Patel, X. Zhang, and A. Kumar, "Techno-economic and life cycle assessment on lignocellulosic biomass thermochemical conversion technologies: A review," Renew. Sustain. Energy Rev., vol. 53, pp. 1486–1499, Jan. 2016, doi: 10.1016/j.rser.2015.09.070.

[3] J. Nicoletti and F. You, "Multiobjective economic and environmental optimization of global crude oil purchase and sale planning with noncooperative stakeholders," Appl. Energy, vol. 259, p. 114222, Feb. 2020, doi: 10.1016/j.apenergy.2019.114222.

[4] K. Lang and B. R. Auer, "The economic and financial properties of crude oil: A review," North Am. J. Econ. Finance, vol. 52, p. 100914, Apr. 2020, doi: 10.1016/j.najef.2019.01.011.

[5] T. Kan, V. Strezov, and T. J. Evans, "Lignocellulosic biomass pyrolysis: A review of product properties and effects of pyrolysis parameters," Renew. Sustain. Energy Rev., vol. 57, pp. 1126–1140, May 2016, doi: 10.1016/j.rser.2015.12.185.

[6] X. Hu and M. Gholizadeh, "Progress of the applications of bio-oil," Renew. Sustain. Energy Rev., vol. 134, p. 110124, Dec. 2020, doi: 10.1016/j.rser.2020.110124.

[7] Z. Yang, A. Kumar, and R. L. Huhnke, "Review of recent developments to improve storage and transportation stability of bio-oil," Renew. Sustain. Energy Rev., vol. 50, pp. 859–870, Oct. 2015, doi: 10.1016/j.rser.2015.05.025.

[8] M. Gholizadeh, X. Hu, and Q. Liu, "A mini review of the specialties of the bio-oils produced from pyrolysis of 20 different biomasses," Renew. Sustain. Energy Rev.,

vol. 114, p. 109313, Oct. 2019, doi: 10.1016/j.rser.2019.109313.

[9] G. Kabir and B. H. Hameed, “Recent progress on catalytic pyrolysis of lignocellulosic biomass to high-grade bio-oil and bio-chemicals,” Renew. Sustain. Energy Rev., vol. 70, pp. 945–967, Apr. 2017, doi: 10.1016/j.rser.2016.12.001.

[10] L. Fan, Y. Zhang, S. Liu, N. Zhou, P. Chen, Y. Cheng, M. Addy, Q. Lu, M. M. Omar, Y. Liu, Y.Wang, L. Dai, E. Anderson, P. Peng, H. Lei and R. Ruan, “Bio-oil from fast pyrolysis of lignin: Effects of process and upgrading parameters,” Bioresour. Technol., vol. 241, pp. 1118–1126, Oct. 2017, doi: 10.1016/j.biortech.2017.05.129.

[11] S. Gupta, P. Mondal, V. B. Borugadda, and A. K. Dalai, “Advances in upgradation of pyrolysis bio-oil and biochar towards improvement in bio-refinery economics: A comprehensive review,” Environ. Technol. Innov., vol. 21, p. 101276, Feb. 2021, doi: 10.1016/j.eti.2020.101276.

[12] M. Zhang, Y. Hu, H. Wang, H. Li, X. Han, Y. Zeng and C.C. Xu, “A review of bio-oil upgrading by catalytic hydrotreatment: Advances, challenges, and prospects,” Mol. Catal., vol. 504, p. 111438, Mar. 2021, doi: 10.1016/j.mcat.2021.111438.

[13] S. Hansen, A. Mirkouei, and L. A. Diaz, “A comprehensive state-of-technology review for upgrading bio-oil to renewable or blended hydrocarbon fuels,” Renew. Sustain. Energy Rev., vol. 118, p. 109548, Feb. 2020, doi: 10.1016/j.rser.2019.109548.

[14] L. Leng, H. Li, X. Yuan, W. Zhou, and H. Huang, “Bio-oil upgrading by emulsification/microemulsification: A review,” Energy, vol. 161, pp. 214–232, Oct. 2018, doi: 10.1016/j.energy.2018.07.117.

[15] H. A. Baloch, S. Nizamuddin, M.T.H. Siddiqui, S. Riaz, A. S. Jatoi, D. K. Dumbre,

N.M. Mubarak, M.P. Srinivasan and G.J. Griffin, “Recent advances in production and upgrading of bio-oil from biomass: A critical overview,” J. Environ. Chem. Eng., vol. 6, no. 4, pp. 5101–5118, Aug. 2018, doi: 10.1016/j.jece.2018.07.050.

[16] T. Shan Ahamed, S. Anto, T. Mathimani, K. Brindhadevi, and A. Pugazhendhi, “Upgrading of bio-oil from thermochemical conversion of various biomass – Mechanism, challenges and opportunities,” Fuel, vol. 287, p. 119329, Mar. 2021, doi: 10.1016/j.fuel.2020.119329.

[17] S. Zhou, Y. Xue, J. Cai, C. Cui, Z. Ni, and Z. Zhou, “An understanding for improved biomass pyrolysis: Toward a systematic comparison of different acid pretreatments,” Chem. Eng. J., vol. 411, p. 128513, May 2021, doi: 10.1016/j.cej.2021.128513.

[18] X. Lu, T. Han, J. Jiang, K. Sun, Y. Sun, and W. Yang, “Comprehensive insights into the influences of acid-base properties of chemical pretreatment reagents on biomass pyrolysis behavior and wood vinegar properties,” J. Anal. Appl. Pyrolysis, vol. 151, p. 104907, Oct. 2020, doi: 10.1016/j.jaap.2020.104907.

[19] K. Wu, H. Wu, H. Zhang, B. Zhang, C. Wen, C. Hu, C. Liu and Q. Liu, “Enhancing levoglucosan production from waste biomass pyrolysis by Fenton pretreatment,” Waste Manag., vol. 108, pp. 70–77, May 2020, doi: 10.1016/j.wasman.2020.04.023.

[20] A. Trubetskaya, V. Budarin, M. Arshadi, D. Magalhães, F. Kazanç, and A. J. Hunt, “Supercritical extraction of biomass as an effective pretreatment step for the char yield control in pyrolysis,” Renew. Energy, vol. 170, pp. 107–117, Jun. 2021, doi: 10.1016/j.renene.2021.01.116.

[21] X. Xin, S. Pang, F. de Miguel Mercader, and K. M. Torr, “The effect of biomass

pretreatment on catalytic pyrolysis products of pine wood by Py-GC/MS and principal component analysis," J. Anal. Appl. Pyrolysis, vol. 138, pp. 145–153, Mar. 2019, doi: 10.1016/j.jaap.2018.12.018.

[22] Z. Ma,Y. Zhang, C. Li, Y. Yang, W. Zhang, C. Zhao, and S. Wang, "N-doping of biomass by ammonia (NH3) torrefaction pretreatment for the production of renewable N-containing chemicals by fast pyrolysis," Bioresour. Technol., vol. 292, p. 122034, Nov. 2019, doi: 10.1016/j.biortech.2019.122034.

[23] K. Cen, J. Zhang, Z. Ma, D. Chen, J. Zhou, and H. Ma, "Investigation of the relevance between biomass pyrolysis polygeneration and washing pretreatment under different severities: Water, dilute acid solution and aqueous phase bio-oil," Bioresour. Technol., vol. 278, pp. 26–33, Apr. 2019, doi: 10.1016/j.biortech.2019.01.048.

[24] D. Chen, K. Cen, X. Jing, J. Gao, C. Li, and Z. Ma, "An approach for upgrading biomass and pyrolysis product quality using a combination of aqueous phase bio-oil washing and torrefaction pretreatment," Bioresour. Technol., vol. 233, pp. 150–158, Jun. 2017, doi: 10.1016/j.biortech.2017.02.120.

[25] O. Das and A. K. Sarmah, "Value added liquid products from waste biomass pyrolysis using pretreatments," Sci. Total Environ., vol. 538, pp. 145–151, Dec. 2015, doi: 10.1016/j.scitotenv.2015.08.025.

[26] B. J. Alvarez-Chavez, S. Godbout, J. H. Palacios-Rios, É. Le Roux, and V. Raghavan, "Physical, chemical, thermal and biological pre-treatment technologies in fast pyrolysis to maximize bio-oil quality: A critical review," Biomass Bioenergy, vol. 128, p. 105333, Sep. 2019, doi: 10.1016/j.biombioe.2019.105333.

[27] W. Yi , X. Wang, K. Zeng, H. Yang, J. Shao, S. Zhang and H. Chen "Improving

bio-oil stability by fractional condensation and solvent addition," Fuel, vol. 290, p. 119929, Apr. 2021, doi: 10.1016/j.fuel.2020.119929.

[28] X. J. Lee, H. C. Ong, Y. Y. Gan, W.-H. Chen, and T. M. I. Mahlia, "State of art review on conventional and advanced pyrolysis of macroalgae and microalgae for biochar, bio-oil and bio-syngas production," Energy Convers. Manag., vol. 210, p. 112707, Apr. 2020, doi: 10.1016/j.enconman.2020.112707.

[29] R. Verma , S. K.Verma, V. Verma, S. Verma, Y. Vaishnav, V. Jena, A. Kumar and K.P. Rakesh, "Catalytic pyrolysis of ulva lactuca macroalgae: Effects of mono and bimetallic catalysts and reaction parameters on bio-oil up-gradation," Bioresour. Technol., vol. 324, p. 124594, Mar. 2021, doi: 10.1016/j.biortech.2020.124594.

[30] H. V. Ly, J. H. Choi, H. C. Woo, S.-S. Kim, and J. Kim, "Upgrading bio-oil by catalytic fast pyrolysis of acid-washed Saccharina japonica alga in a fluidized-bed reactor," Renew. Energy, vol. 133, pp. 11–22, Apr. 2019, doi: 10.1016/j.renene.2018.09.103.

[31] S. Wang , S. Zhao, B. B. Uzoejinwa, A. Zheng, Q. Wang, J. Huang and A. E.-F. Abomohra "A state-of-the-art review on dual purpose seaweeds utilization for wastewater treatment and crude bio-oil production," Energy Convers. Manag., vol. 222, p. 113253, Oct. 2020, doi: 10.1016/j.enconman.2020.113253.

[32] Y. Uemura, R. Matsumoto, S. Saadon, and Y. Matsumura, "A study on torrefaction of Laminaria japonica," Fuel Process. Technol., vol. 138, pp. 133–138, Oct. 2015, doi: 10.1016/j.fuproc.2015.05.016.

[33] P. Das, C. V.p., T. Mathimani, and A. Pugazhendhi, "A comprehensive review on the factors affecting thermochemical conversion efficiency of algal biomass to energy," Sci. Total Environ., vol. 766, p. 144213, Apr. 2021, doi:

10.1016/j.scitotenv.2020.144213.

[34] W.-H. Chen , B.-J. Lin , Y.-Y. Lin, Y.-S. Chu, A. T. Ubando, P. L. Show, H. C. Ong, J.-S. Chang, S.-H. Ho , A.B. Culaba , A. Pétrissans and M. Pétrissans, “Progress in biomass torrefaction: Principles, applications and challenges,” Prog. Energy Combust. Sci., vol. 82, p. 100887, Jan. 2021, doi: 10.1016/j.pecs.2020.100887.

[35] B. Cao , S. Wang, Y. Hu, A.E.F. Abomohra, L. Qian, Z. He, Q. Wang, B. B. Uzoejinwa and S. Esakkimuthu, “Effect of washing with diluted acids on Enteromorpha clathrata pyrolysis products: Towards enhanced bio-oil from seaweeds,” Renew. Energy, vol. 138, pp. 29–38, Aug. 2019, doi: 10.1016/j.renene.2019.01.084.

[36] Y. Hu , W. Shuang, W. Qian, H. Zhixia, L. Xiaochi, X. Shannan, J. Hengsong and L. Yun, “Effect of different pretreatments on the thermal degradation of seaweed biomass,” Proc. Combust. Inst., vol. 36, no. 2, pp. 2271–2281, Jan. 2017, doi: 10.1016/j.proci.2016.08.086.

[37] J. Choi ,J.W. Choi, D. J. Suh, J. M. Ha, J. W. Hwang, H. W. Jung, K.Y. Lee and H.C. Woo, “Production of brown algae pyrolysis oils for liquid biofuels depending on the chemical pretreatment methods,” Energy Convers. Manag., vol. 86, pp. 371–378, Oct. 2014, doi: 10.1016/j.enconman.2014.04.094.

[38] J. H. Choi, S.-S. Kim, H. V. Ly, J. Kim, and H. C. Woo, “Effects of water-washing Saccharina japonica on fast pyrolysis in a bubbling fluidized-bed reactor,” Biomass Bioenergy, vol. 98, pp. 112–123, Mar. 2017, doi: 10.1016/j.biombioe.2017.01.006.

[39] T. You, X. Li, R. Wang, X. Zhang, and F. Xu, “Effects of synergistic fungal pretreatment on structure and thermal properties of lignin from corncob,”

Bioresour. Technol., vol. 272, pp. 123–129, Jan. 2019, doi: 10.1016/j.biortech.2018.09.145.

[40] X. Yang, F. Ma, H. Yu, X. Zhang, and S. Chen, “Effects of biopretreatment of corn stover with white-rot fungus on low-temperature pyrolysis products,” Bioresour. Technol., vol. 102, no. 3, pp. 3498–3503, Feb. 2011, doi: 10.1016/j.biortech.2010.11.021.

[41] Y. Yu , Y. Zeng, J. Zuo, F. Ma, X. Yang, X. Zhang and Y. Wang, “Improving the conversion of biomass in catalytic fast pyrolysis via white-rot fungal pretreatment,” Bioresour. Technol., vol. 134, pp. 198–203, Apr. 2013, doi: 10.1016/j.biortech.2013.01.167.

[42] K. Yan, F. Liu, Q. Chen, M. Ke, X. Huang, W. Hu, B. Zhou, X. Zhang and H. Yu, “Pyrolysis characteristics and kinetics of lignin derived from enzymatic hydrolysis residue of bamboo pretreated with white-rot fungus,” Biotechnol. Biofuels, vol. 9, no. 1, p. 76, Mar. 2016, doi: 10.1186/s13068-016-0489-y.

[43] R. Kumar V. Strezov, H. Weldekidan, J. He, S. Singh, T. Kan and B.Dastjerdi, “Lignocellulose biomass pyrolysis for bio-oil production: A review of biomass pre-treatment methods for production of drop-in fuels,” Renew. Sustain. Energy Rev., vol. 123, p. 109763, May 2020, doi: 10.1016/j.rser.2020.109763.

[44] E. Allen, D. M. Wall, C. Herrmann, A. Xia, and J. D. Murphy, “What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?,” Energy, vol. 81, pp. 352–360, Mar. 2015, doi: 10.1016/j.energy.2014.12.048.

[45] A. J. Guneratnam, A. Xia, and J. D. Murphy, “Comparative study of single- and two-stage fermentation of the brown seaweed Laminaria digitata,” Energy Convers. Manag., vol. 148, pp. 405–412, Sep. 2017, doi:

10.1016/j.enconman.2017.06.013.

[46] M. P. Sudhakar, B. R. Kumar, T. Mathimani, and K. Arunkumar, “A review on bioenergy and bioactive compounds from microalgae and macroalgae-sustainable energy perspective,” J. Clean. Prod., vol. 228, pp. 1320–1333, Aug. 2019, doi: 10.1016/j.jclepro.2019.04.287.

[47] Y. Cao and A. Pawłowski, “Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: Brief overview and energy efficiency assessment,” Renew. Sustain. Energy Rev., vol. 16, no. 3, pp. 1657–1665, Apr. 2012, doi: 10.1016/j.rser.2011.12.014.

[48] Y. Wen, Z. Shi, S. Wang, W. Mu, P. G. Jönsson, and W. Yang, “Pyrolysis of raw and anaerobically digested organic fractions of municipal solid waste: Kinetics, thermodynamics, and product characterization,” Chem. Eng. J., vol. 415, p. 129064, Jul. 2021, doi: 10.1016/j.cej.2021.129064.

[49] V. S. Sikarwar, M. Pohořelý, E. Meers, S. Skoblia, J. Moško, and M. Jeremiáš, “Potential of coupling anaerobic digestion with thermochemical technologies for waste valorization,” Fuel, vol. 294, p. 120533, Jun. 2021, doi: 10.1016/j.fuel.2021.120533.

[50] Z. Yang ,Y. Liu, J. Zhang, K. Mao, M. Kurbonova, G. Liu, R. Zhang and W. Wang “Improvement of biofuel recovery from food waste by integration of anaerobic digestion, digestate pyrolysis and syngas biomethanation under mesophilic and thermophilic conditions,” J. Clean. Prod., vol. 256, p. 120594, May 2020, doi: 10.1016/j.jclepro.2020.120594.

[51] H. Li and K. Feng, “Life cycle assessment of the environmental impacts and energy efficiency of an integration of sludge anaerobic digestion and pyrolysis,” J.

Clean. Prod., vol. 195, pp. 476–485, Sep. 2018, doi: 10.1016/j.jclepro.2018.05.259.

[52] C. A. Salman, S. Schwede, E. Thorin, and J. Yan, “Enhancing biomethane production by integrating pyrolysis and anaerobic digestion processes,” Appl. Energy, vol. 204, pp. 1074–1083, Oct. 2017, doi: 10.1016/j.apenergy.2017.05.006.

[53] Q. Feng and Y. Lin, “Integrated processes of anaerobic digestion and pyrolysis for higher bioenergy recovery from lignocellulosic biomass: A brief review,” Renew. Sustain. Energy Rev., vol. 77, pp. 1272–1287, Sep. 2017, doi: 10.1016/j.rser.2017.03.022.

[54] X. Li, Q. Mei, X. Dai, and G. Ding, “Effect of anaerobic digestion on sequential pyrolysis kinetics of organic solid wastes using thermogravimetric analysis and distributed activation energy model,” Bioresour. Technol., vol. 227, pp. 297–307, Mar. 2017, doi: 10.1016/j.biortech.2016.12.057.

[55] C. Deng, R. Lin, X. Kang, B. Wu, R. O’Shea, and J. D. Murphy, “Improving gaseous biofuel yield from seaweed through a cascading circular bioenergy system integrating anaerobic digestion and pyrolysis,” Renew. Sustain. Energy Rev., vol. 128, p. 109895, Aug. 2020, doi: 10.1016/j.rser.2020.109895.

[56] K. Kebelmann, A. Hornung, U. Karsten, and G. Griffiths, “Intermediate pyrolysis and product identification by TGA and Py-GC/MS of green microalgae and their extracted protein and lipid components,” Biomass Bioenergy, vol. 49, pp. 38–48, Feb. 2013, doi: 10.1016/j.biombioe.2012.12.006.

[57] T. N. Trinh, P. A. Jensen, K. Dam-Johansen, N. O. Knudsen, H. R. Sørensen, and S. Hvilsted, “Comparison of Lignin, Macroalgae, Wood, and Straw Fast Pyrolysis,” Energy Fuels, vol. 27, no. 3, pp. 1399–1409, Mar. 2013, doi:

10.1021/ef301927y.

[58] F. Obeid, T. Chu Van, R. Brown, and T. Rainey, “Nitrogen and sulphur in algal biocrude: A review of the HTL process, upgrading, engine performance and emissions,” Energy Convers. Manag., vol. 181, pp. 105–119, Feb. 2019, doi: 10.1016/j.enconman.2018.11.054.

[59] X. Song, W. Luo, J. McDonald, S. J. Khan, F. I. Hai, W. Guo, H. H. Ngo and L. D. Nghiem “Effects of sulphur on the performance of an anaerobic membrane bioreactor: Biological stability, trace organic contaminant removal, and membrane fouling,” Bioresour. Technol., vol. 250, pp. 171–177, Feb. 2018, doi: 10.1016/j.biortech.2017.11.021.

[60] R. He , X.Z. Yao, M. Chen, R.C. Ma, H.J. Li, C.Wang and S.H, Ding “Conversion of sulfur compounds and microbial community in anaerobic treatment of fish and pork waste,” Waste Manag., vol. 76, pp. 383–393, Jun. 2018, doi: 10.1016/j.wasman.2018.04.006.

[61] X. Yang, Y. Zeng, F. Ma, X. Zhang, and H. Yu, “Effect of biopretreatment on thermogravimetric and chemical characteristics of corn stover by different white-rot fungi,” Bioresour. Technol., vol. 101, no. 14, pp. 5475–5479, Jul. 2010, doi: 10.1016/j.biortech.2010.01.129.

[62] H. Nikkhah, A. Tavasoli, and S. Jafarian, “Investigating the influence of acid washing pretreatment and Zn/activated biochar catalyst on thermal conversion of Cladophora glomerata to value-added bio-products,” Energy Convers. Manag., vol. 225, p. 113392, Dec. 2020, doi: 10.1016/j.enconman.2020.113392.

[63] A. B. Ross, K. Anastasakis, M. Kubacki, and J. M. Jones, “Investigation of the pyrolysis behaviour of brown algae before and after pre-treatment using PY-

GC/MS and TGA," J. Anal. Appl. Pyrolysis, vol. 85, no. 1, pp. 3–10, May 2009, doi: 10.1016/j.jaap.2008.11.004.

[64] Y. Hu, S. Wang, Q. Wang, Z. He, A. E.-F. Abomohra, and B. Cao, "Influence of torrefaction pretreatment on the pyrolysis characteristics of seaweed biomass," Cellulose, vol. 26, no. 15, pp. 8475–8487, Oct. 2019, doi: 10.1007/s10570-019-02595-7.

[65] O. Raize, Y. Argaman, and S. Yannai, "Mechanisms of biosorption of different heavy metals by brown marine macroalgae," Biotechnol. Bioeng., vol. 87, no. 4, pp. 451–458, 2004, doi: https://doi.org/10.1002/bit.20136.

[66] M. C. Rodríguez, M. C. Matulewicz, M. D. Noseda, D. R. B. Ducatti, and P. I. Leonardi, "Agar from Gracilaria gracilis (Gracilariales, Rhodophyta) of the Patagonic coast of Argentina – Content, structure and physical properties," Bioresour. Technol., vol. 100, no. 3, pp. 1435–1441, Feb. 2009, doi: 10.1016/j.biortech.2008.08.025.

[67] J. T. Kidgell, M. Magnusson, R. de Nys, and C. R. K. Glasson, "Ulvan: A systematic review of extraction, composition and function," Algal Res., vol. 39, p. 101422, May 2019, doi: 10.1016/j.algal.2019.101422.

[68] N. Wahlström, U. Edlund, H. Pavia, G. Toth, A. Jaworski, A. J. Pell, F. X. Choong, H. Shirani, K.P.R. Nilsson and A. Richter-Dahlfors "Cellulose from the green macroalgae Ulva lactuca: isolation, characterization, optotracing, and production of cellulose nanofibrils," Cellulose, vol. 27, no. 7, pp. 3707–3725, May 2020, doi: 10.1007/s10570-020-03029-5.

[69] S. Zhang, S. Zhu, H. Zhang, T. Chen, and Y. Xiong, "Catalytic fast pyrolysis of rice husk: Effect of coupling leaching with torrefaction pretreatment," J. Anal.

Appl. Pyrolysis, vol. 133, pp. 91–96, Aug. 2018, doi: 10.1016/j.jaap.2018.04.016.

[70] Z. Hu , Z. Chen, G. Li, X. Chen, M. Hu, M. Laghari, X. Wang and D. Guo,“Characteristics and kinetic studies of Hydrilla verticillata pyrolysis via thermogravimetric analysis,” Bioresour. Technol., vol. 194, pp. 364–372, Oct. 2015, doi: 10.1016/j.biortech.2015.07.007.

[71] X. Xu, R. Tu, Y. Sun, Z. Li, and E. Jiang, “Influence of biomass pretreatment on upgrading of bio-oil: Comparison of dry and hydrothermal torrefaction,” Bioresour. Technol., vol. 262, pp. 261–270, Aug. 2018, doi: 10.1016/j.biortech.2018.04.037.

[72] P. T. Sekoai,A. Ghimire, O.T. Ezeokoli, S. Rao, W.Y. Ngan, O. Habimana, Y. Yao, P. Yang, A.H.Y. Fung, K. O. Yoro, M. O .Daramola and C.H. Hung, “Valorization of volatile fatty acids from the dark fermentation waste Streams-A promising pathway for a biorefinery concept,” Renew. Sustain. Energy Rev., vol. 143, p. 110971, Jun. 2021, doi: 10.1016/j.rser.2021.110971.

[73] K. Kebelmann, A. Hornung, U. Karsten, and G. Griffiths, “Thermo-chemical behaviour and chemical product formation from Polar seaweeds during intermediate pyrolysis,” J. Anal. Appl. Pyrolysis, vol. 104, pp. 131–138, Nov. 2013, doi: 10.1016/j.jaap.2013.08.012.

[74] J. G. Speight, The Chemistry and Technology of Petroleum, Fifth Edition. CRC Press, 2014.

[75] Q. Dong, S. Zhang, K. Ding, S. Zhu, H. Zhang, and X. Liu, “Pyrolysis behavior of raw/torrefied rice straw after different demineralization processes,” Biomass Bioenergy, vol. 119, pp. 229–236, Dec. 2018, doi: 10.1016/j.biombioe.2018.09.032.

903