



Pyrolysis of *Scenedesmus obliquus* Biomass Following the Treatment of Different Wastewaters

Ana F. Ferreira¹ · Alice Ferreira² · Ana Paula S. Dias¹ · Luísa Gouveia²

© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

The potential of *Scenedesmus obliquus* biomass, after treating several wastewaters (WWs) (urban, dairy and brewery industries, cattle and poultry breeding), was evaluated for the production of bio-char, bio-oil and bio-gas, through a pyrolysis process. The experiments were carried out in a fixed bed reactor at a temperature of 748 K during 15 min. The thermochemical behaviour of each microalga was assessed by thermogravimetry analysis and infrared spectroscopy. The bio-oils obtained were also quantified and characterized. The results reveal that (i) all microalgae show a similar thermal degradation behaviour; (ii) microalga grown in urban WW shows a lower percentage of lipids; in dairy and cattle WWs, it shows a high percentage of both proteins and lipids, and in poultry and brewery WWs, it shows a higher carbohydrates content; (iii) the microalga grown in poultry WW shows the lowest protein content, whereas the urban grown microalga showed the highest protein content; (iv) the bio-oil obtained by pyrolysis process showed yields in the range 30–60% (w/w) and showed a high content of aromatic compounds.

Keywords *Scenedesmus obliquus* · Wastewater · Pyrolysis · Bio-oil · Thermogravimetry analysis

Introduction

Microalgae have shown clear advantages for the production of biofuels when compared with energy crops, especially when the biomass was grown through the recycling of nutrients present in the wastewaters and the CO₂ from the flue gases [1, 2]. The main advantages of microalgae comprise (i) the possibility of being cultivated on non-agricultural land using wastewater, (ii) higher yield per unit of light irradiated area, (iii) the recovery of nutrients from wastewater and CO₂ from flue gases and fossil fuel combustion.

Wastewater (WW) is a dangerous issue to the environment and human health. Many industries, especially the food processing and the ones related to it (e.g. dairy, brewery, poultry and cattle meat production), are intensive water consumers, generating large volumes of wastewater (Table 1). These effluents are rich in organic compounds and nutrients such as

phosphates, ammonia and/or nitrates. Due to their characteristics, these industrial wastes are a suitable cultivation medium for mixotrophic microalgae, presenting carbon/nitrogen (C/N) and nitrogen/phosphorus (N/P) ratios favourable for microalgae growth [7]. Thus, the incorporation of the microalgal systems into conventional wastewater treatment ensures remarkable benefits, such as the following: (1) the oxygen needed for the bacteria is provided through microalgae photosynthesis, avoiding aeration, reducing energy demand and greenhouse gas (GHG) emissions [8]; (2) reduction in hazardous solid sludge formation (e.g. heavy metals, chemicals and pathogens) [9, 10]; (3) reduction of the costs; (4) production of useful algal biomass-energy rich and (5) recycling of the nutrients present in the wastewater [11, 12].

The thermochemical conversion of microalgae biomass into biofuel and high added value products has been gaining significant prominence globally. The combustion, gasification and pyrolysis are the most usual thermochemical conversion processes. However, this last conversion technology produces energy fuels with high fuel-to-feed ratio with the advantage of operating at atmospheric pressure and moderate temperatures. Therefore, pyrolysis has been attracting considerable attention during the past decades as the most promising process for biomass conversion for competing with non-renewable fossil fuel resources [13–17].

✉ Ana F. Ferreira
filipa.ferreira@tecnico.ulisboa.pt

¹ IDMEC, LAETA, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

² LNEG- Laboratório Nacional de Energia e Geologia, I.P./Bioenergy Unit, Estrada do Paço do Lumiar 22, 1649-038 Lisbon, Portugal

Table 1 Wastewater produced in Europe by economic sector and associated nutrient (nitrogen and phosphorus) and chemical oxygen demand (COD) loads [3]

Economic sector	Wastewater flowrate (million m ³ /year)	COD (ton/year)	Nitrogen (ton/year)	Phosphorus (ton/year)
Urban	20,400	8,200,000	816,000	200,000
Dairy	538	8,100,000	322,000	-
Brewery	1051 ¹	4,160,000 ²	54,500 ²	31,200 ²
Slaughterhouse				
Poultry	21	161,000	13,000	-
Cattle	12	92,000	7300	-

¹ Estimated value considering a global beer production (in 2017) of 1.95 billion hL [4] and a wastewater/beer ratio of 5.39 [5]

² Estimated values considering the general composition of brewery wastewater reported by Simate et al. [6]

Pyrolysis involves the treatment of the raw feedstock in an oxygen-free atmosphere (usually nitrogen) in a relatively wide range of temperatures, typically between 573 and 973 K. Under those conditions, the different components present in biomass are decomposed into a variety of compounds that are collected as bio-oil, solid fractions (bio-char) and bio-gas. The pyrolysis gases contain CO, CO₂, light hydrocarbons (C1–C4) and hydrogen, and have a significant high heating value (HHV); hence, they are usually combusted to provide the heat needs of the process itself [18].

Bio-char and bio-oil products are usually used as an alternative to fossil-based fuels. Bio-char, on the other hand, is also used as a carbon source for producing different carbon-based materials and/or to be used as soil amendment [19]. Bio-oil presents properties that allow its use as fuel in simple boilers (for heat production) and turbines (for electricity), although it does not have enough quality to be directly applied in the transport sector [18]. It can also be used as chemical feedstock for valuable chemical products [20].

Several types of biomass have been used in the pyrolysis process, mainly those from lignocellulose. However, bio-oils obtained from microalgae pyrolysis show higher HHV, when compared with the bio-oil from wood [21, 22]. As the bio-oil can be readily stored and transported, much of the present interest in pyrolysis is focused on maximizing bio-oil yields [21].

The thermogravimetric analysis (TGA) and infrared spectroscopy (FTIR) have been the techniques used for the first characterization of microalgae biomass in order to verify its behaviour in pyrolysis atmosphere, giving a fast evaluation of the lipids, proteins, carbohydrates and inorganic contents. In the present work, pyrolysis was chosen as a way to valorize the *Scenedesmus obliquus* biomass that has already successfully treated five different types of wastewater (urban, dairy and brewery industries, cattle and poultry breeding).

The main goal of the present study is to evaluate the influence of the different culture medium in the characteristics of

the microalga biomass and in the properties of the respective bio-oil obtained in pyrolysis process.

Materials and Methods

Microalgae Growth—Wastewater Treatment

The microalgae used in this work were the *Scenedesmus obliquus* (ACOI 204/07) from the Coimbra University Collection of Algae (ACOI), Portugal. The photobioreactors (PBRs) used were bubble column (0.8 L and 150 L), flat panel (2.6 L) and a high rate algal pond (HRAP; area = 2.4 m² with deep of 11 cm, working volume of 365 L and horizontal speed of 0.11 cm/s promoted by paddle wheels) (Table 2). The growth was at autotrophic mode in different effluents as culture medium, such as urban, dairy and brewery industries, cattle and poultry breeding [25].

The brewery and urban wastewater experiments were run outdoors at LNEG Alfragide (38° 43' 54.3" N 9° 12' 41.3" W) and Lumiar (38° 46' 25" N 9° 10' 36" W) *campus*, respectively (Lisbon, Portugal).

All the wastewaters were previously characterized in terms of their total Kjeldahl nitrogen, ammonia nitrogen, phosphorous and COD contents. The characterization was done according to standard methods [27] and it is shown in Table 3. Each sample was analysed at least two times, and the average value was considered.

The cattle wastewater was pre-treated by anaerobic digestion under mesophilic temperature conditions (310 K ± 1) and hydraulic retention time (HRT) of 6 days in a hybrid reactor [24]. The brewery WW was also treated using a BIOPAQ®IC reactor, located in the industry wastewater treatment plant.

Besides the production of the biomass in all effluents, it was also obtained clean water with nutrient contents below the limits imposed by the Portuguese legislation [28] to discharge the effluents into a natural water body

Table 2 Operational conditions used for the cultivation of *Scenedesmus obliquus* in different culture media (poultry, cattle, brewery, dairy, urban and Bristol)

Culture medium	Inlet gaseous mixture and agitation	Light source and intensity ($\mu\text{E}/(\text{m}^2 \text{s}^{-1})$)	Temperature (K)	Reactor type and volume (L)	Operating strategy and cultivation time (days)	References
Poultry	Air diffuser	Artificial (20.2)	298	Bubble column (0.8)	Batch (29)	[23]
Cattle	Air diffuser	Artificial (60.7)	291–294	Flat panel airlift (2.6)	Batch (12)	[24]
Brewery	Paddle wheels	Sunlight	285–286	High rate algal pond (300)	Batch (12)	[25]
Dairy	Air diffuser	Artificial (31.1)	292–295	Flat panel airlift (2.6)	Batch (12)	[25]
Urban	Air diffuser	Sunlight	291	Bubble column (150)	Fed-Batch (13)	[12]
Bristol	Paddle wheels	Sunlight	293	High rate algal pond (300)	Batch (12)	[26]

without potentially harmful consequences to the environment, or re-use in the process. These results are shown elsewhere [25].

In addition, the microalga *Scenedesmus obliquus* grown in synthetic medium (Bristol) was considered for comparison [26, 29].

Microalgae Biomass Characterization—Biochemical Composition

Lipid Content

Lipid extraction from *Scenedesmus* biomass was carried out in a Soxhlet apparatus using 1.0 g of biomass and n-hexane as a solvent, for 6 h. The amount of total lipids was determined gravimetrically, after solvent rotating evaporation.

Sugar Content

The extraction of sugars from the biomass was performed by the addition of H_2SO_4 (2 N) to 500 mg of dried microalgae and autoclaving for 60 min at 394 K. Then the sample was filtered through a 0.2- μm filter [30]. The total sugar content was determined by the phenol-sulphuric reagent method [31]. To 1 mL of sample previously diluted (1:20) was added 1 mL of phenol solution (5% w/v) and 5 mL of 96% sulphuric acid. It rested for 10 min at room temperature and for 15 min in a cold-water bath. Then, it was measured with a spectrophotometer at 490 nm. A calibration curve was prepared using glucose standards and the results of total carbohydrates in the

microalgae samples are expressed in terms of glucose equivalents.

Protein Content

The total nitrogen present in the microalgae biomass was determined using a modified Kjeldahl method [27]. In a Kjeldahl tube, 200 mg of dried microalgae, 5 mL of distilled water and 50 mL of digestion solution (134 g K_2SO_4 + 650 mL H_2O + 200 mL H_2SO_4 + 2gHgO/25 mL H_2SO_4 (6 N)) were mixed together and digested in a Buchi Digestion Unit K-424 for 4 h. Afterwards, it was placed in the distillation device (Buchi Distillation Unit K-350) for 6 min with the reagent sodium hydroxide plus sodium thiosulfate. Thereafter, 50 mL of boric acid indicator solution was added to the distillate. Then it was titrated with a stock solution of H_2SO_4 (0.02 N). Crude protein was calculated by multiplying total nitrogen by the conventional conversion factor of 6.25 [32].

Thermogravimetric Analysis

Thermogravimetric analysis was performed in a TGA apparatus, NETZSCH STA 409 PC/PG, with a weighing precision of $\pm 0.01\%$ and sensitivity in the mass measurements of 0.1 μg . The TGA was carried out using a sample weight of around 5–6 mg, depending on the density of the biomass used. The measurements were carried out at heating rates of 298 K/min, in nitrogen atmosphere for pyrolysis behaviour, respectively. Temperature was measured with an experimental

Table 3 Average composition of the different wastewaters used as culture media for *Scenedesmus obliquus* cultivation [25]. Average values are given, with standard deviation from at least two replicates determinations

Wastewater	N-NH ₃ (mg N/L)	TKN (mg N/L)	PO ₄ ³⁻ (mg P/L)	P-PO ₄ ³⁻ (mg P/L)	COD (g O ₂ /L)	Reference
Poultry	122.7 ± 1.9	-	-	27.9 ± 1.6	3.7 ± 0.7	[23]
Cattle	498 ± 1.2	618 ± 0.9	23.5 ± 0.01	7.8 ± 0.01	2.9 ± 0.02	[24]
Brewery	4.11 ± 2.1	28.0	20.0 ± 0.16	6.5 ± 0.18	0.2 ± 0.0	[25]
Dairy	204 ± 0.1	312 ± 0.03	18 ± 0.2	6 ± 0.2	3.0 ± 0.4	[25]
Urban	175 ± 0.9	-	14.3 ± 0.11	4.6 ± 0.12	0.08 ± 1.5	[12]

uncertainty of ± 1 °C. The TG curve represents the evolution of the mass (weight loss) as a function of the temperature. The DTG is the TG derivative and represents the rate of weight variation (%/min) [33]. The carbohydrates, lipids and protein contents of the biomass grown in synthetic medium and in effluents were assessed by deconvolution of the DTG curves in the range 473–773 K. For each pseudo-component (i), the pyrolysis process was modelled using a symmetric Gaussian curve (DTG_i) as described by Rego et al. [34].

$$DTG_i = a_i \text{EXP} \left(- \left(\frac{T-b_i}{c_i} \right)^2 \right) \quad (1)$$

where T is the temperature (K) and a_i , b_i and c_i are the Gauss curve parameters. The DTG curve was computed adding the contribution of each component i (lipids, proteins and sugars):

$$DTG_{\text{comp}} = \sum i a_i \text{EXP} \left(- \left(\frac{T-b_i}{c_i} \right)^2 \right) \quad (2)$$

The Gauss curves parameters were optimized by minimizing the function F :

$$F = \sum T \left[\left(\frac{DTG - DTG_{\text{comp}}}{DTG} \right)^2 \right]_T \quad (3)$$

where DTG is the experimental curve and DTG_{comp} the computed curve for T in the range 473–773 K.

The computation method was implemented using the SOLVER routine of EXCEL software [35]. To discriminate the three components (carbohydrates, lipids and proteins), data from previous studies [33, 36, 37] were used instead of model compounds. In fact, the variability of such natural compounds makes the selection of standard molecules difficult. Each sample was analysed at least four times, and the average value was considered.

Infrared Spectroscopy

The microalga biomass was characterized by infrared spectroscopy (HATR-FTIR). The infrared spectra were obtained using a spectrophotometer BOMEN FTLA200-100 from ABB using a reflectance mode. This equipment has a horizontal total attenuated reflection accessory (HATR), from PIKE Technologies, with a ZnSe crystal. The IR spectra were recorded at room temperature in the range 3725–725 cm^{-1} .

Sixty-four scans were accumulated for each spectrum to obtain an acceptable signal-to-noise ratio. In addition, the reflectance signal (R) was corrected using the Kubelka-Munk (KM) function in Eq. (1):

$$KM = (1 - R)^2 / 2R \text{ Eq. 1.}$$

Pyrolysis Experiments

The pyrolysis experiments were performed in a quartz fixed bed reactor in a nitrogen atmosphere during 15 min. The used reactor had an internal diameter of 16 mm and a length of 150 mm. The quartz reactor was filled with carborundum. The reactor was externally heated using a circular electric furnace equipped with a PID controlled to ensure the complete pyrolysis. Each pyrolysis test was started by placing a certain amount of raw microalga (about 2.5 g) into the quartz reactor. The reactor was placed in the circular furnace pre-heated to 748 K. Nitrogen with purity of 99.99% was used as the carrier gas and its flow (200 mL/min) was monitored using a mass flow controller. A condenser coupled to the reactor was used immersed in an ice-water bath to obtain the liquid product. The uncondensed gas was not collected. The pyrolysis liquid amount was determined by the mass difference of the condensers before and after a pyrolysis test. The bio-oil was recovered by washing the reactor with acetone followed by rotating evaporation of acetone under a reduced pressure. The used acetone could be recycled for further tests. The weighted mass of char and bio-oil allowed calculating the yield of such products. The gas yield was computed from a mass balance.

Results and Discussion

Characterization of the Microalga Biomass

Thermogravimetric Analysis

The *Scenedesmus obliquus* biomass obtained after the WW treatment and grown in synthetic medium (Bristol) was characterized by TGA to evaluate the differences between their behaviour under inert (N_2 flow) conditions.

Figure 1 shows the results of thermogravimetric analysis for the microalga *Scenedesmus obliquus* biomass grown in urban, poultry, brewery, dairy and cattle WWs and Bristol medium under inert conditions, using nitrogen, for a heating rate of 298 K/min, respectively.

The mass loss profiles (TG) were differentiated (DTG) in order to distinguish processes with different decomposition rates. The thermal degradation profiles obtained under N_2 (Fig. 1) show decomposition processes similar to those reported in the literature for microalgae biomass [38]. All the characterized biomass showed similar thermal degradation profiles with minor dissimilarities related with their different contents of carbohydrates, lipids, proteins and ash (Table 4). The main mass loss processes occurred in the 473–873 K range, been preceded by low temperature process related with biomass drying (moisture loss centred at 423 K). The major mass loss corresponds to the overlap of devolatilization of carbohydrates, proteins and lipids. For temperatures higher than

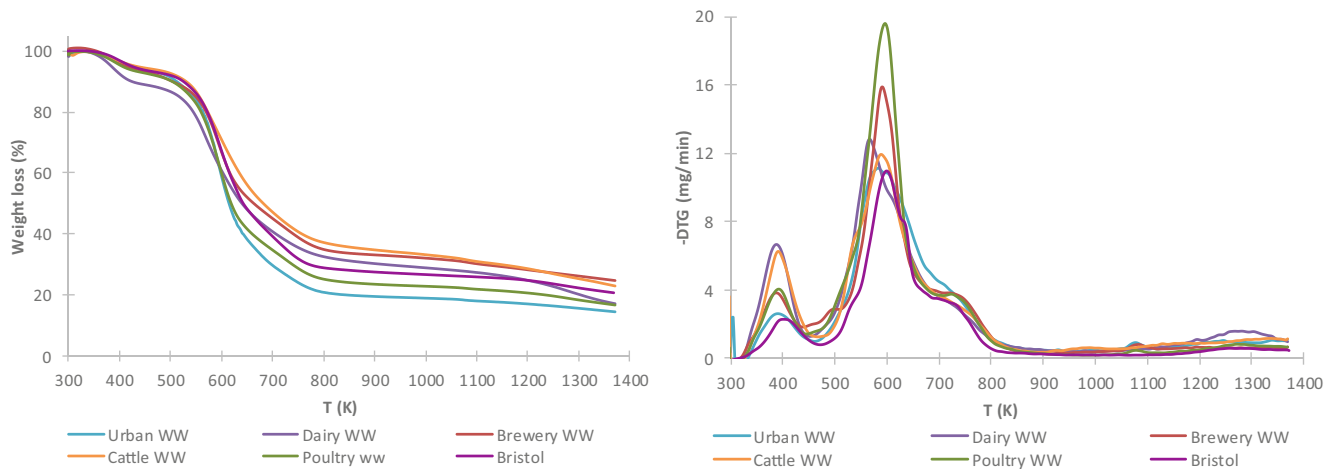


Fig. 1 Thermograms of microalga *Scenedesmus obliquus* grown in synthetic medium (Bristol) and wastewaters (urban, dairy, brewery, cattle and poultry) under nitrogen atmosphere (pyrolysis) at a heating rate of 298 K/min

873 K, the bio-char, previously formed during pyrolysis of carbohydrates, proteins and lipids, is composed mainly by mineral and metal components [39]. The residual mass at 1473 K contains biomass inorganic salts (ashes) and fixed carbon of the biomass. These occur since the thermobalance crucible geometry allied to the laminar flow of gases in the thermobalance oven favour the existence of mass transfer limitations.

The DTG profiles under N₂ flow were used to quantify carbohydrates, proteins and lipids in microalgae biomass using the procedure described by Ferreira et al. [35]. The maximum located around 573 K for all biomass is associated with the carbohydrate and protein decomposition. The maximum located near 723 K revealing the decomposition of the fatty acids present in this microalgal. These results are in agreement with the study reported by López-González et al. [37] and Kebelmann et al. [40]. The maximum observed at 1073–1173 K is related to the volatile metal loss and carbonate decomposition.

The biomass from dairy WW treatment presented the lowest residual mass at 1473 K probably due to the highest proteins content, for growing in wastewaters, as cultivation media with a high percentage of ammonia nitrogen. In fact, during thermal degradation, proteins release gaseous N-containing compounds in parallel with char formation [41].

Deconvolution of the DTG Curves

Thermogravimetric quantifications were accomplished by DTG profiles deconvolution using a pseudo-component model [34]. Figure 2 a–f show the deconvolution of the DTG curves of *Scenedesmus obliquus* biomass after treating several wastewaters and grown in Bristol medium. In this process, it is possible to calculate relative values for each compound (lipids, carbohydrates and proteins) in each biomass (Table 4).

Gauss symmetric curves were fitted, and compositions were computed considering the areas under each gauss curve (Fig. 3). Moisture, volatiles, carbohydrates, proteins and lipids

Table 4 Biochemical composition (wt%, dry basis) of the microalga *Scenedesmus obliquus* grown in different wastewaters and synthetic medium (Bristol), using classic techniques and thermogravimetry (Nitrogen; 298 K/min). The data for Bristol medium was obtained from Batista et al. [26]

	Culture medium	Protein	Lipids	Sugars	Ash	Moisture
Classic	Urban WW	32.7 ± 0.1	8.1 ± 0.1	11.7 ± 3.7	14.1 ± 0.5	n.d.
	Dairy WW	53 ± 0.1	18.0 ± 0.9	14 ± 0.6	8.2 ± 0.1	7.1 ± 0.1
	Brewery WW	31.4 ± 0.04	17.9 ± 0.6	30.2 ± 0.5	15.5 ± 0.0	3.1 ± 0.3
	Cattle WW	42 ± 0.02	18.0 ± 0.2	22 ± 0.01	14.2 ± 0.1	8.6 ± 0.0
	Poultry WW	n.d.	19.8 ± 0.3	36.2 ± 0.2	4.2 ± 0.1	2.7 ± 0.1
	Bristol	20.4 ± 0.02	17.1 ± 0.2	30.7 ± 0.8	20.2 ± 0.5	5.4 ± 0.1
Thermogravimetry	Urban WW	37.2 ± 0.1	8.8 ± 0.1	13.4 ± 0.1	16.9 ± 0.1	9.7 ± 0.1
	Dairy WW	46.0 ± 0.1	16.9 ± 0.1	12.1 ± 0.1	5.0 ± 0.1	8.8 ± 0.1
	Brewery WW	30.2 ± 0.1	17.2 ± 0.1	30.8 ± 0.1	17.7 ± 0.1	6.6 ± 0.1
	Cattle WW	41.2 ± 0.1	21.3 ± 0.1	19.0 ± 0.1	7.9 ± 0.1	8.9 ± 0.1
	Poultry WW	19.1 ± 0.1	18.5 ± 0.1	35.8 ± 0.1	14.3 ± 0.1	7.3 ± 0.1
	Bristol	21.6 ± 0.1	18.4 ± 0.1	32.5 ± 0.1	18.2 ± 0.1	5.5 ± 0.1

n.d., not determined

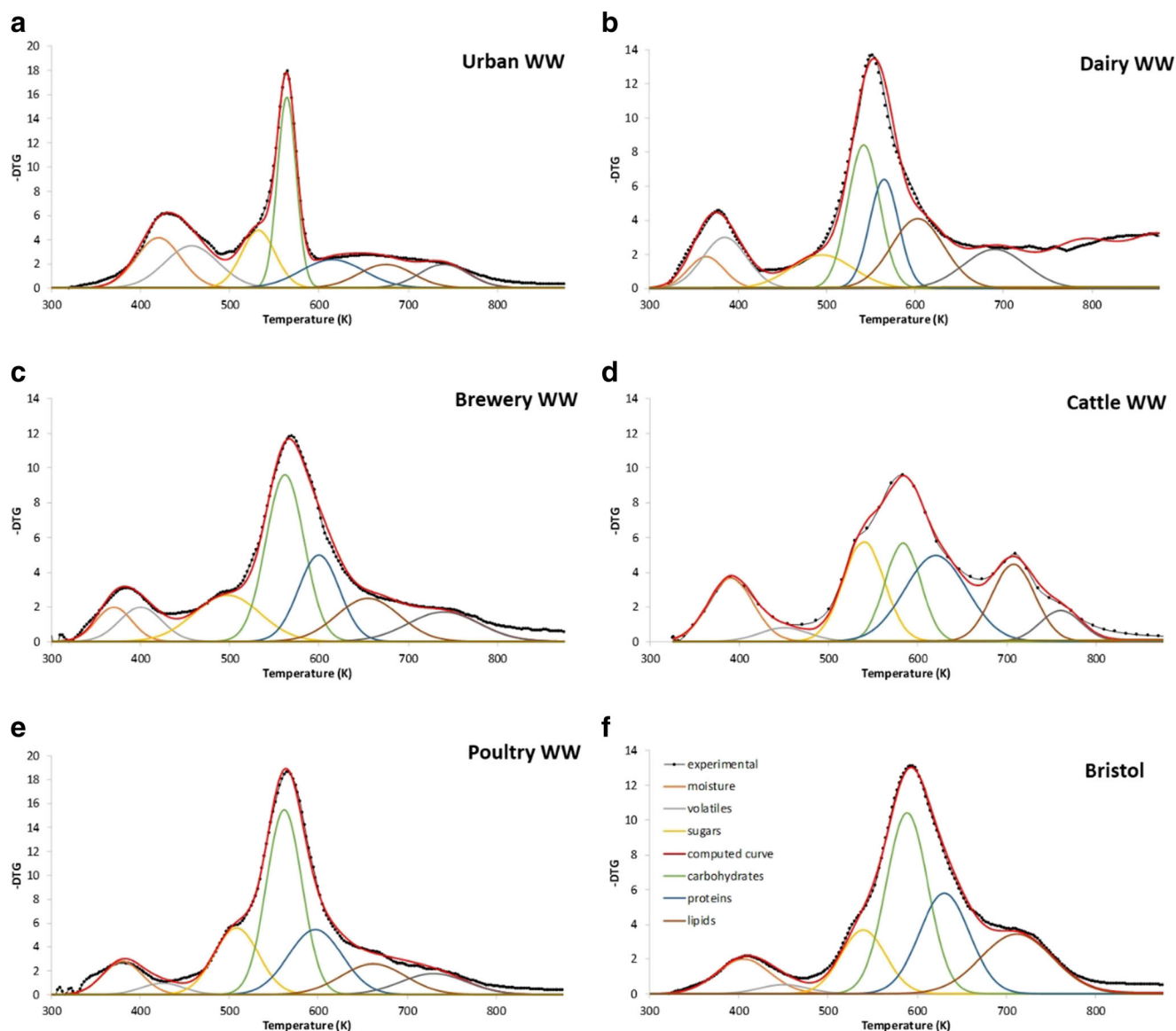
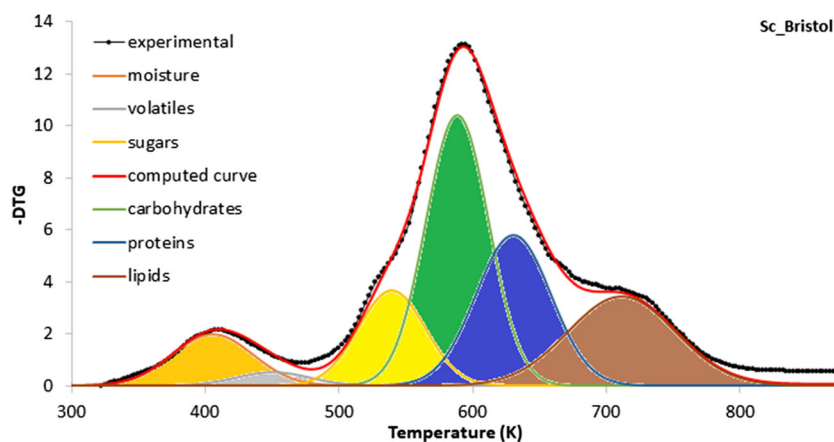


Fig. 2 Deconvolution of the DTG curves for *Scenedesmus obliquus* grown in urban (a), dairy (b), brewery (c), cattle (d), poultry (e) WWs and Bristol medium (f), in pyrolysis conditions

Fig. 3 DTG deconvolution using pseudo-component model and Gauss curves for each component for *Scenedesmus obliquus* grown in Bristol medium, in pyrolysis conditions



are considered, taking into account the different decomposition processes occurring in the 300–800 K temperature range [33].

It is possible to verify that the *Scenedesmus obliquus* grown in urban WW shows a lower percentage of lipids, compared with all the other WWs. In dairy and cattle WWs, the microalga has a high percentage of both proteins and lipids. The higher percentage of protein, when compared with the microalga grown in synthetic medium (Bristol), were probably due to the high availability of ammonia nitrogen in the wastewaters used as cultivation media (Table 3).

The microalga grown in poultry WWs has a higher carbohydrate/sugar content, while in brewery WW, the percentage of carbohydrates is similar to that of microalga grown in Bristol medium. Moreover, microalga grown in cattle and brewery WW and Bristol medium has a high lipid content, comparable with urban WWs, maybe due to the lower light intensity applied since this factor (light) was shown to have a greater impact on lipid content for *Scenedesmus obliquus* [25, 42]. These results are according to biochemical analysis.

Biochemical Composition

The biochemical composition of the biomass is closely linked to the strain and cultivation conditions, namely wastewater composition, stress caused by nutrient limitations [43], light intensity and regime, agitation, salinity and pH. The biochemical composition of *Scenedesmus obliquus* microalga cultivated in synthetic medium (Bristol) and different wastewater was evaluated by classic techniques and by thermogravimetry under nitrogen flow (Table 4).

The microalgae composition obtained by classic techniques and by thermogravimetry was comparable, with a coefficient of determination of 0.946. The results showed that thermogravimetry is a fast and accurate technique, comparable with classic techniques.

Infrared Spectroscopy

The microalga was characterized by ATR-FTIR (Fig. 4) and the IR bands were attributed to species (lipids, proteins and sugars) using data from Driver et al. [44]. A slight displacement, between observed IR bands and data [44], toward lower wavenumbers, was considered due to the ATR acquisition mode. For all the characterized samples, the main IR features are in the range 1800–600 cm^{-1} maybe due to all of them being lyophilized which drastically decreases the broad band around 3600–3000 cm^{-1} characteristic of –OH vibration modes.

Taking into account the spectra in Fig. 4, the main components of all the analysed microalgae are proteins (1750–1500 cm^{-1}) and carbohydrates (1200–900 cm^{-1}). The intensity of proteins IR features are dependents on the microalga

culture medium. The poultry sample showed the lowest protein content, whereas the microalga grown in urban and dairy WW showed the highest protein content. The microalga grown in brewery and poultry WWs showed the highest carbohydrate content. These findings can be related to nutrients availability (Table 3) [45] and biochemical analysis (Table 4).

The band centred around 1745 cm^{-1} belonging to lipids is absent for all the spectra because ATR mode used for spectra acquisition only accounts the surface species and oil species are encapsulated by the cell walls which are mainly composed by glycoproteins [46]. In fact, intense bands around 1639 cm^{-1} and 1534 cm^{-1} with variable intensities are characteristic of –NH and –C=O of amides associated with proteins [44]. A second group of intense reflectance bands appears, for all the samples, in the range 1200–900 cm^{-1} and belongs to polysaccharides of cell walls. Prior disruption of microalgae cell walls seems to be essential to characterize by ATR-FTIR the intracellular components.

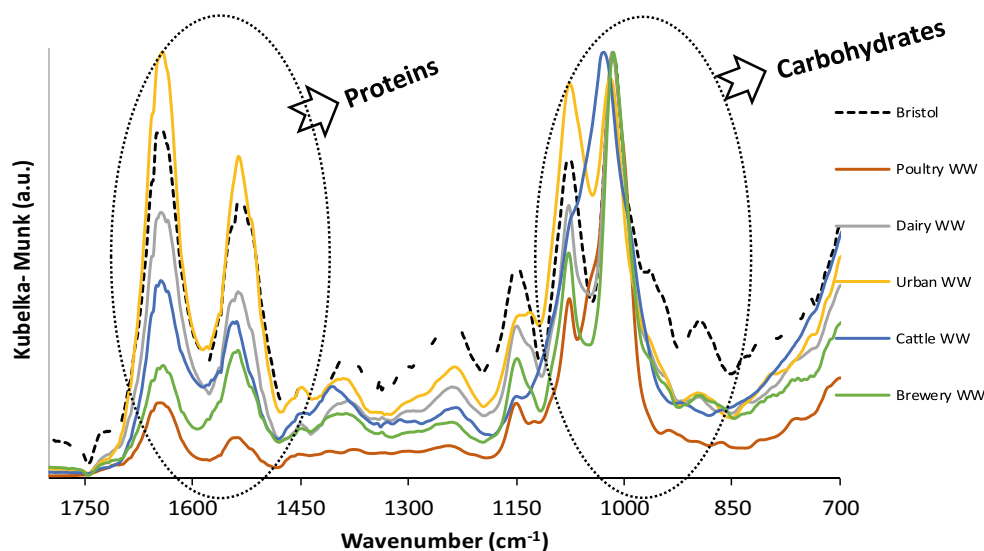
Pyrolysis Products

The pyrolysis of microalga biomass was carried out at 748 K in a fixed bed reactor. The temperature of 748 K was chosen from the thermal degradation profiles as the temperature corresponding to the maximum rate of thermal degradation. A relatively low pyrolysis temperature was used in order to minimize energy consumption and emissions [36]. Figure 5 shows the pyrolysis product yields (wt%) of *Scenedesmus obliquus* biomass grown in synthetic medium (Bristol) at different temperatures (648 and 748 K) and N_2 flow rate (83, 200 and 300 mL/min) during 30 min.

Increasing the N_2 flow, for the same temperature, tends to decrease the oil outcome. Jourabchi et al. [47] showed that the influence of nitrogen sweeping gas on biomass pyrolysis decreases the bio-oil outcome for the same temperature [36]. It was possible to analyse that the best operational conditions to obtain a higher amount of bio-oil were 748 K with 200 mL/min of N_2 for 30 min.

Table 5 presents the yields (%) of products (bio-oil, char and gas) obtained on the pyrolysis process from the *Scenedesmus obliquus* biomass grown in different wastewaters (urban, dairy and brewery industries, cattle and poultry breeding) and in Bristol culture medium. Table 5 shows bio-oil yields in the range 30–60% (w/w) with the brewery WW microalga giving the highest bio-oil yield (64%). The lowest bio-oil yield was obtained using poultry WW microalga, which presents the highest content of carbohydrates. It seems that carbohydrates are more easily gasified in the pyrolysis conditions, possibly due to the high inorganic content, which acts as a gasification catalyst [48]. A simple correlation between bio-oil yield and microalga composition cannot be inferred due to the high variability of lipids, carbohydrates, proteins and ashes compositions (Table 4) of the pyrolysed

Fig. 4 HATR-FTIR spectra of *Scenedesmus obliquus* biomass grown in different wastewaters (Bristol culture medium was used as reference)



biomass. Globally, the pyrolysis yields (bio-char, bio-oil and bio-gas products) are in the range of those previously published for fixed bed reactor microalgae pyrolysis [49].

The produced bio-oil fractions presented a brownish dark colour, high viscosity and pungent odour. They were characterized by FTIR in order to identify organic functional groups of the major compounds [50, 51], and the FTIR spectra for bio-oils obtained from *Scenedesmus obliquus* grown in WWs and Bristol medium are shown in Fig. 6. FTIR bands of the produced bio-oils (Fig. 6) were compared with data collected from FTIR bands of bio-oil functional organic groups [50, 51]. The bio-oil FTIR spectra do not differ substantially from that of lignocellulosic-derived bio-oils, showing, mainly, reflectance bands belonging to carboxylic groups and aromatic containing compounds [52]. The major spectral difference lies in the presence of $-NH$ species vibration modes arising from proteins, which are dominant in the microalgae biomass. The presence of such N-containing compounds difficult the biorefinery bio-oil upgrading into fuel because it will require

an additional denitrogenation step. All the produced bio-oils showed a complex band in the range $3700-3000\text{ cm}^{-1}$ attributed to $-OH$ and $-NH$ species. According to Aboulkas et al. [50], the presence of heteroatoms (O and N) in bio-oil is related to the bands intensity in the range $1730-1150\text{ cm}^{-1}$. Thus, the bio-oil produced from dairy WW microalga presents the highest content of heteroatoms, which is compatible with its higher proteins content. This bio-oil also shows the highest intensity bands in the $3050-2800\text{ cm}^{-1}$ range attributed to alkanes.

The Bristol-derived bio-oil shows intense bands centred at 1072 cm^{-1} and 1100 cm^{-1} belonging to alcohols, ethers and esters and a band centred at 1550 cm^{-1} belonging to alkanes. The presence of alcohols ($-OH$ species) is also pointed by the broad band, and intense, in the range $3600-3100\text{ cm}^{-1}$. Bio-oils from WW-grown microalga biomass have IR bands centred at 1045 and 1088 cm^{-1} also belonging to alcohols, ethers and esters and, a band around 878 cm^{-1} of aromatics. This band is absent for Bristol-derived bio-oil spectrum. IR bands of carboxylic species

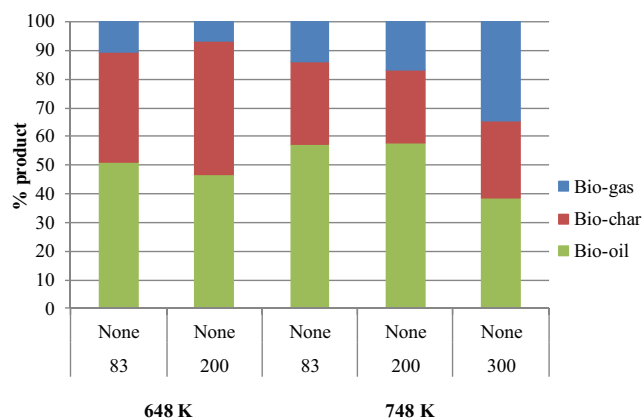


Fig. 5 Pyrolysis product yields (wt%) of *Scenedesmus obliquus* biomass, grown in Bristol medium, at 648 and 748 K; 30 min; N₂ flow rate, 83, 200 and 300 mL/min

Table 5 Yields (% w/w) of products (bio-oil, bio-char and bio-gas) obtained on the pyrolysis process from the *Scenedesmus obliquus* biomass grown in different wastewaters (urban, dairy and brewery industries, cattle and poultry breeding) and in Bristol culture medium

Culture medium	Yield (% w/w)		
	Bio-oil	Bio-char	Bio-gas
Urban WW	38.4 ± 0.1	21.6 ± 0.1	40.1 ± 0.1
Dairy WW	45.3 ± 0.1	36.0 ± 0.1	18.7 ± 0.1
Brewery WW	64.1 ± 0.1	29.8 ± 0.1	6.1 ± 0.1
Cattle WW	60.8 ± 0.1	37.2 ± 0.1	2.0 ± 0.1
Poultry WW	32.3 ± 0.1	19.5 ± 0.1	48.3 ± 0.1
Bristol	57.6 ± 0.1	25.6 ± 0.1	16.8 ± 0.1

Fig. 6 FTIR of bio-oil obtain from pyrolysis of microalga that treat the different wastewaters (urban, dairy and brewery industries, cattle and poultry breeding) and after growth in Bristol culture medium. The FTIR spectrum was divided in two wavenumber ranges to give a clearer view of the peaks at these ranges



that are visible in the spectra of bio-oils from WW-grown microalga are also absent for Bristol-derived bio-oil. The lower protein content, in parallel with the highest inorganic content, which can act as gasification catalysts [53], seems to contribute to the better quality of the Bristol-grown microalga bio-oil, when compared with that of the bio-oils produced using WW-grown microalga.

Conclusions

Biomass for green energy production can be obtained growing microalga using wastewater nutrients thus allowing a double benefit: green energy production and wastewater treatment.

Scenedesmus obliquus microalga was successfully used to treat urban, dairy, brewery, cattle and poultry wastewaters.

The grown biomass showed carbohydrate, protein and lipid contents dependent on the nutrients availability in wastewater. The following conclusions can be summarized from the present results:

- all microalgae show a similar thermal degradation behaviour;
- microalga grown in urban WW shows a lower percentage of lipids; in dairy and cattle WWs shows a high percentage of both proteins and lipids, and in poultry and brewery WWs shows a higher carbohydrates content;
- the microalga grown in poultry shows the lowest protein content, whereas the urban microalga showed the highest protein content;
- the bio-oil obtained by pyrolysis process showed yields in the range 30–60% (w/w) and showed a high content of aromatic compounds.

Acknowledgements The authors would like to thank Dr. Paula Assemany, Dr. Henrique Vieira de Mendonça MSc Ana Barata for microalga biomass production; Graça Gomes (LNEG) and Natércia Santos (LNEG) for microalgae culture maintenance and laboratory assistance.

Funding Information The study was financially supported by the Project ALGAVALOR - MicroALGAs: produção integrada e VALORização da biomassa e das suas diversas aplicações, from the Portugal 2020 programme (grant agreement no. POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234); GreenCoLab – Green Ocean Technologies and Products Collaborative Laboratory, no. ALG-05-3559-FSE-000010, Algarve 2020 Operational Regional Program, funded by European Social Fund and Portuguese Government budget; Biomass and Bioenergy Research Infrastructure (BBRI)-LISBOA-01-0145-FEDER-022059, supported by Operational Programme for Competitiveness and Internationalization (PORTUGAL2020), by Lisbon Portugal Regional Operational Programme (Lisboa 2020) and by North Portugal Regional Operational Programme (Norte 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Ana F. Ferreira and Ana Paula Dias would like to thank the financial support by the Fundação para a Ciência e a Tecnologia (FCT), through IDMEC, under LAETA, project UID/EMS/50022/2019. Alice Ferreira is pleased to acknowledge her PhD grant no. SFRH/BD/144122/2019 awarded by Fundação para a Ciência e Tecnologia.

References

- Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources. *Bioresour Technol* 102:17–25. <https://doi.org/10.1016/j.biortech.2010.06.035>
- Lundquist T, Woertz I, Quinn N, Benemann J (2010) A realistic technology and engineering assessment of algae biofuel production. *Energy Biosciences Institute, University of California* p 1–178
- Eurostat Eurostat 2013-2015. <https://ec.europa.eu/eurostat/>
- Barth-Haas Group (2019) Beer production worldwide from 1998 to 2018 (in billion hectoliters). In: *Statistics. Hops 2018/2019*, p 9
- Brewers Association (2013) *Wastewater Management Guidance Manual*. 1–47
- Simate GS, Cluett J, Iyuke SE et al (2011) The treatment of brewery wastewater for reuse: state of the art. *Desalination* 273:235–247. <https://doi.org/10.1016/j.desal.2011.02.035>
- Maroneze MM, Barin JS, De Menezes CR et al (2014) Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. *Sci Agric* 4:521–524
- Oswald WJ (2003) My sixty years in applied algology. *J Appl Phycol* 15:99–106. <https://doi.org/10.1023/A:1023871903434>
- Craggs RJ, Adey WH, Jenson KR et al (1996) Phosphorus removal from wastewater using an algal turf scrubber. *Water Sci Technol* 33: 191–198. [https://doi.org/10.1016/0273-1223\(96\)00354-X](https://doi.org/10.1016/0273-1223(96)00354-X)
- Green FB, Lundquist TJ, Oswald WJ (1995) Energetics of advanced integrated wastewater pond systems. *Water Sci Technol* 31:9–20. [https://doi.org/10.1016/0273-1223\(95\)00488-9](https://doi.org/10.1016/0273-1223(95)00488-9)
- Batista AP, Ambrosano L, Graça S, Sousa C, Marques PASS, Ribeiro B, Botrel EP, Castro Neto P, Gouveia L (2015) Combining urban wastewater treatment with biohydrogen production – an integrated microalgae-based approach. *Bioresour Technol* 184:230–235. <https://doi.org/10.1016/j.biortech.2014.10.064>
- Gouveia L, Graça S, Sousa C et al (2016) Microalgae biomass production using wastewater: treatment and costs: scale-up considerations. *Algal Res* 16:167–176. <https://doi.org/10.1016/j.algal.2016.03.010>
- Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sust Energ Rev* 14:557–577. <https://doi.org/10.1016/j.rser.2009.10.009>
- Demirbaş A (2001) Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Convers Manag* 42:1357–1378. [https://doi.org/10.1016/S0196-8904\(00\)00137-0](https://doi.org/10.1016/S0196-8904(00)00137-0)
- Demirbas A (2008) Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Convers Manag* 49: 2106–2116. <https://doi.org/10.1016/j.enconman.2008.02.020>
- Marcilla A, Catalá L, García-Quesada JC et al (2013) A review of thermochemical conversion of microalgae. *Renew Sust Energ Rev*. <https://doi.org/10.1016/j.rser.2013.06.032>
- Chen WH, Lin BJ, Huang MY, Chang JS (2014) Thermochemical conversion of microalgal biomass into biofuels: a review. *Bioresour Technol*. <https://doi.org/10.1016/j.biortech.2014.11.050>
- Fermoso J, Hernando H, Jiménez-Sánchez S et al (2017) Bio-oil production by lignocellulose fast-pyrolysis: isolating and comparing the effects of indigenous versus external catalysts. *Fuel Process Technol*. <https://doi.org/10.1016/j.fuproc.2017.08.009>
- Vereš J, Kolonicný J, Ochodek T (2014) Biochar status under international law and regulatory issues for the practical application. *Chem Eng Trans*. <https://doi.org/10.3303/CET1437134>
- Özçimen D (2013) An approach to the characterization of biochar and bio-oil. In: Lohani SP (ed) *Renewable energy for sustainable future*, pp 41–58
- Miao X, Wu Q, Yang C (2004) Fast pyrolysis of microalgae to produce renewable fuels. *J Anal Appl Pyrolysis* 71:855–863. <https://doi.org/10.1016/j.jaap.2003.11.004>
- Yang C, Li R, Cui C et al (2016) Catalytic hydroprocessing of microalgae-derived biofuels: a review. *Green Chem* 18:3684–3699. <https://doi.org/10.1039/c6gc01239f>
- Oliveira AC, Barata A, Batista AP, Gouveia L (2019) *Scenedesmus obliquus* in poultry wastewater bioremediation. *Environ Technol* 40:3735–3744. <https://doi.org/10.1080/09593330.2018.1488003>
- Mendonça HV, Ometto JPHB, Otenio MH et al (2018) Microalgae-mediated bioremediation and valorization of cattle wastewater previously digested in a hybrid anaerobic reactor using a photobioreactor: comparison between batch and continuous operation. *Sci Total Environ* 633:1–11. <https://doi.org/10.1016/J.SCITOTENV.2018.03.157>
- Ferreira A, Marques P, Ribeiro B, Assemany P, de Mendonça HV, Barata A, Oliveira AC, Reis A, Pinheiro HM, Gouveia L (2018) Combining biotechnology with circular bioeconomy: from poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. *Environ Res* 164:32–38. <https://doi.org/10.1016/J.ENVRES.2018.02.007>
- Batista AP, Moura P, Marques PASS et al (2014) *Scenedesmus obliquus* as feedstock for biohydrogen production by *Enterobacter aerogenes* and *Clostridium butyricum*. *Fuel* 117: 537–543. <https://doi.org/10.1016/j.fuel.2013.09.077>
- American Public Health Association (2005) *Standard methods for the examination of water and wastewater*, 23th ed. <https://doi.org/10.2105/AJPH.51.6.940-a>
- Decree-Law No 236/98 (1998) Decree-Law 236/98 of the Portuguese Ministry of the Environment of 1 August establishing water quality standards. *Diário da República: I Série-A* 3676–3722
- Molina Grima E, Belarbi E-H, Ación FF et al (2003) Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 20:491–515. [https://doi.org/10.1016/S0734-9750\(02\)00050-2](https://doi.org/10.1016/S0734-9750(02)00050-2)
- Hoebler C, Barry JL, David A, Delort-Laval J (1989) Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-

- liquid chromatography. *J Agric Food Chem* 37:360–367. <https://doi.org/10.1021/jf00086a020>
31. DuBois M, Gilles KA, Hamilton JK et al (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356. <https://doi.org/10.1021/ac60111a017>
 32. Jones DB (1941) Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. US Department of Agriculture-circ. 183, Washington, DC
 33. Ferreira AF, Soares Dias AP, Silva CM, Costa M (2015) Evaluation of thermochemical properties of raw and extracted microalgae. *Energy* 92:365–372. <https://doi.org/10.1016/j.energy.2015.04.078>
 34. Rego F, Soares Dias AP, Casquilho M et al (2019) Fast determination of lignocellulosic composition of poplar biomass by thermogravimetry. *Biomass Bioenergy* 122:375–380. <https://doi.org/10.1016/j.biombioe.2019.01.037>
 35. Ferreira AF, Dias APS, Silva CM, Costa M (2016) Effect of low frequency ultrasound on microalgae solvent extraction: analysis of products, energy consumption and emissions. *Algal Res* 14:9–16. <https://doi.org/10.1016/j.algal.2015.12.015>
 36. Silva CM, Ferreira AF, Dias AP, Costa M (2016) A comparison between microalgae virtual biorefinery arrangements for bio-oil production based on lab-scale results. *J Clean Prod* 130:58–67. <https://doi.org/10.1016/j.jclepro.2015.09.053>
 37. López-González D, Fernandez-Lopez M, Valverde JL, Sanchez-Silva L (2014) Kinetic analysis and thermal characterization of the microalgae combustion process by thermal analysis coupled to mass spectrometry. *Appl Energy* 114:227–237. <https://doi.org/10.1016/j.apenergy.2013.09.055>
 38. Ranjith Kumar R, Ramesh D, Mutanda T et al (2015) Thermal behavior and pyrolytic characteristics of freshwater *Scenedesmus* sp. biomass. *Energy Sour Part A* 37:1383–1391. <https://doi.org/10.1080/15567036.2011.605428>
 39. Vassilev SV, Vassileva CG (2016) Composition, properties and challenges of algae biomass for biofuel application: an overview. *Fuel* 181:1–33. <https://doi.org/10.1016/j.fuel.2016.04.106>
 40. Kebeilmann K, Hornung A, Karsten U, Griffiths G (2013) Intermediate pyrolysis and product identification by TGA and Py-GC/MS of green microalgae and their extracted protein and lipid components. *Biomass Bioenergy* 49:38–48. <https://doi.org/10.1016/j.biombioe.2012.12.006>
 41. Yu J, Maliutina K, Tahmasebi A (2018) A review on the production of nitrogen-containing compounds from microalgal biomass via pyrolysis. *Bioresour Technol* 270:689–701. <https://doi.org/10.1016/j.biortech.2018.08.127>
 42. Álvarez-Díaz PD, Ruiz J, Arbib Z et al (2015) Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. *Bioresour Technol* 181:90–96. <https://doi.org/10.1016/j.biortech.2015.01.018>
 43. Sun XM, Ren LJ, Zhao QY, Ji XJ, Huang H (2018) Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. *Biotechnol Biofuels* 11:1–16. <https://doi.org/10.1186/s13068-018-1275-9>
 44. Driver T, Bajhaiya AK, Allwood JW, Goodacre R, Pittman JK, Dean AP (2015) Metabolic responses of eukaryotic microalgae to environmental stress limit the ability of FT-IR spectroscopy for species identification. *Algal Res* 11:148–155. <https://doi.org/10.1016/j.algal.2015.06.009>
 45. Thomas WH, Krauss RW (1955) Nitrogen metabolism in *Scenedesmus* as affected by environmental changes. *Plant Physiol* 30:113–122. <https://doi.org/10.1104/pp.30.2.113>
 46. Voigt J, Stolarczyk A, Zych M, Malec P, Burczyk J (2014) The cell-wall glycoproteins of the green alga *Scenedesmus obliquus*. The predominant cell-wall polypeptide of *Scenedesmus obliquus* is related to the cell-wall glycoprotein gp3 of *Chlamydomonas reinhardtii*. *Plant Sci* 215–216:39–47. <https://doi.org/10.1016/j.plantsci.2013.10.011>
 47. Jourabchi SA, Gan S, Ng HK (2014) Pyrolysis of *Jatropha curcas* pressed cake for bio-oil production in a fixed-bed system. *Energy Convers Manag* 78:518–526. <https://doi.org/10.1016/j.enconman.2013.11.005>
 48. Klinghoffer NB, Castaldi MJ, Nzihou A (2015) Influence of char composition and inorganics on catalytic activity of char from biomass gasification. *Fuel* 157:37–47. <https://doi.org/10.1016/j.fuel.2015.04.036>
 49. Yang C, Li R, Zhang B et al (2019) Pyrolysis of microalgae: a critical review. *Fuel Process Technol* 186:53–72. <https://doi.org/10.1016/j.fuproc.2018.12.012>
 50. Aboulkas A, Hammani H, El Achaby M et al (2017) Valorization of algal waste via pyrolysis in a fixed-bed reactor: production and characterization of bio-oil and bio-char. *Bioresour Technol* 243:400–408. <https://doi.org/10.1016/j.biortech.2017.06.098>
 51. Peng X, Ma X, Lin Y (2016) Investigation on characteristics of liquefied products from solvolysis liquefaction of *Chlorella pyrenoidosa* in ethanol-water systems. *Energy Fuels* 30:6475–6485. <https://doi.org/10.1021/acs.energyfuels.6b01103>
 52. Uzun BB, Apaydin-Varol E, Ateş F et al (2010) Synthetic fuel production from tea waste: characterisation of bio-oil and bio-char. *Fuel* 89:176–184. <https://doi.org/10.1016/j.fuel.2009.08.040>
 53. Debiagi PEA, Trinchera M, Frassoldati A et al (2017) Algae characterization and multistep pyrolysis mechanism. *J Anal Appl Pyrolysis* 128:423–436. <https://doi.org/10.1016/j.jaap.2017.08.007>