Article

Valorization of *Chlorella* Microalgae Residual Biomass via Catalytic Acid Hydrolysis/Dehydration and Hydrogenolysis/Hydrogenation

Antigoni G. Margellou [1], Stylianos A. Torofias [1], Georgios Iakovou [1] and Konstantinos S. Triantafyllidis [1,2,*]

[1] Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; amargel@chem.auth.gr (A.G.M.); torofias@gmail.com (S.A.T.)
[2] Centre for Research and Technology Hellas, Chemical Process and Energy Resources Institute, 57001 Thessaloniki, Greece
* Correspondence: ktrianta@chem.auth.gr

Abstract: Microalgal biomass can be utilized for the production of value-added chemicals and fuels. Within this research, *Chlorella vulgaris* biomass left behind after the extraction of lipids and proteins was converted to valuable sugars, organic acids and furanic compounds via hydrolysis/dehydration using dilute aqueous sulfuric acid as a homogeneous catalyst. Under mild conditions, i.e., low temperature and low sulfuric acid concentration, the main products of hydrolysis/dehydration were monomeric sugars (glucose and xylose) and furanic compounds (HMF, furfural) while under more intense conditions (i.e., higher temperature and higher acid concentration), organic acids (propionic, formic, acetic, succinic, lactic, levulinic) were also produced either directly from sugar conversion or via intermediate furans. As a second valorization approach, the residual microalgal biomass was converted to value-added sugar alcohols (sorbitol, glycerol) via hydrogenation/hydrogenolysis reactions over metallic ruthenium catalysts supported on activated carbons (5%Ru/C). It was also shown that a low concentration of sulfuric acid facilitated the conversion of biomass to sugar alcohols by initiating the hydrolysis of carbohydrates to monomeric sugars. Overall, this work aims to propose valorization pathways for a rarely utilized residual biomass towards useful compounds utilized as platform chemicals and precursors for the production of a wide variety of solvents, polymers, fuels, food ingredients, pharmaceuticals and others.

Keywords: *Chlorella* microalgae; hydrolysis; hydrogenolysis; sugars; furans; sugar alcohols

1. Introduction

The projected depletion of fossil fuels has spurred the development of emerging technologies for the conversion of renewable energy sources to value-added chemicals and biofuels. Lignocellulosic and microalgae biomass are recognized as the most promising renewable feedstocks for the production not only biofuels but also a wide variety of platform chemicals. Microalgae biomass has gained significant attention due to its fast growth rate and minimum growth demands without the need for chemicals and energy. Furthermore, their composition, enriched in lipids, proteins, carbohydrates and pigments, provides the potential for valorization towards many chemicals.

Lipids, accounting almost the half of microalgae weight, can be extracted via solvent extraction techniques supported by ultrasonication/microwaves and ionic liquids or solvent free techniques and supercritical carbon dioxide methods [1–3]. Regarding their composition and amount, the microalgae strains and the cultural conditions can determine both the fatty acid profile and the lipid content [4,5]. In general, high carbon-to-nitrogen ratios in cultivation or stress conditions such as nitrogen starvation, high salt concentration, high temperature and high pH favors lipid formation [4,6]. Microalgae lipids are composed of fatty acids with carbon numbers in the range C_{14}–C_{20} and polyunsaturated fatty acids

with carbon numbers higher than C_{20} [5]. The former group of fatty acids is mainly utilized for third-generation biofuel production, as biodiesel or paraffinic hydrocarbons [7,8], while the latter group of polyunsaturated fatty acids, more specifically, eicosapentanenoic (EPA) and docosahexanoic acid (DHA), are used as health and nutritional supplements [9,10]. Taking into consideration that the human body cannot synthesize EPA and DHA, but both are essential fatty acids, oleaginous microalgae could be a good source [10,11].

With regard to sugar composition, the main sugars are glucose, identified in high concentrations, while rhamnose, fucose, ribose, xylose, arabinose, mannose and galactose were identified in lower amounts [12]. As in the case of lipids, carbohydrate content and composition exhibit differences between the microalgae species and can be controlled by finetuning the cultivation conditions [13]. In order to increase the carbohydrate content, two stage cultivation has been proposed, comprising a first stage where all the main nutrients are supplied to increase the production of biomass, and a second stage where specific nutrients are supplied to increase the formation of carbohydrates [14]. Microalgal carbohydrates are recognized as promising feedstocks for the production of biofuels and value-added chemicals [15]. After proper treatment, microalgal oligosaccharides can be utilized as a source of prebiotics or converted to bioethanol/biobutanol via fermentation and to biogas via anaerobic digestion [15–18]. Furthermore, microalgae-derived polysaccharides are widely used in the production of packaging materials [19,20], as animal feed providing antibiotic and antibacterial properties [13], and as promoters of plant growth and nutrient uptake [21]. Especially, delipidified microalgal biomass has been utilized in the synthesis of biopolymers, biomethane, biohydrogen and bioethanol [22].

Another major component of microalgae is proteins. Interestingly, some speciescan contain up to 80 wt.% proteins [23]. The proteins after extraction, separation and purification can be consumed by humans as alternative protein sources with valuable effects on human health [24–26]. Other value-added compounds which can be isolated from microalgae are pigments, such as chlorophylls, carotenoids and phycobilins [9]. Carotenoids are responsible for the yellow-red color of biomass and can be categorized in two main groups: xanthophylls and carotenes. Carotenes are linear hydrocarbons with 40 carbon atoms, and the most common compound is β-carotene, while xanthophylls are oxygenated derivatives of them [9]. Among the microalgae with the highest carotene production is *Dunaliella* which belongs to the Chlorophyta species and can be cultivated in highly saline environments [27]. The isolated β-carotene is used in food and pharmaceutical applications, and the foreseen global market is expected to be USD 380 billion by 2028 [28].

In addition to the valorization processes based on selective extraction of the various microalgae fractions, established thermochemical processes, such as pyrolysis, gasification and liquefaction, have also been studied towards the production of bio-oil/biocrude for further upgrading towards biofuels or other bio-based products. Pyrolysis is carried out at relatively high temperatures (400–550 °C) in an inert atmosphere towards bio-oil, char and gases, with typical yields being 50 wt.% bio-oil, 20 wt.% char and 30 wt.% gases [29,30]. With regard to the composition of bio-oil, complex mixtures are obtained that are rich in nitrogen-containing compounds, such as pyrroles, amines, amides and indoles, produced via proteins, carboxylic acids, phenolic compounds, deoxygenated aliphatics and aromatics [30,31]. Removal of nitrogen compounds can be achieved via downstream denitrogenation processes while removal of oxygen can be achieved via in-situ or ex-situ hydrodeoxygenation [31,32]. Heterogeneous catalysts with acid sites induce partial deoxygenation towards monoaromatics (benzene, toluene, etc.) [30,33]. Hydrothermal liquefaction is performed at lower temperatures (200–400 °C), higher residence times and higher pressures, using solvents and catalysts [34,35]. The main products are biocrude, gases and solids.

Within a biorefinery concept, the whole biomass needs to be converted to value-added chemicals and biofuels. Microalgae biorefining is usually based on the primary extraction of lipids and the valorization of delipidified biomass via pyrolysis, resulting, however, in slightly lower yields of bio-oils that do not containing lipid-derived compounds [33,36].

After lipid extraction and carbohydrate removal via saccharification, the residual biomass can yield bio-oil rich in phenolic and nitrogen-containing compounds [37]. Alternatively, after lipid extraction, proteins can be isolated, leaving carbohydrate- and pigment-enriched biomass [38,39]. Delipidified biomass can also be hydrolyzed towards the production of monomeric sugars, with potential for the production of biofuels. Usually, the hydrolysis of the carbohydrates is carried out using inorganic acids, such as sulfuric and hydrochloric acid, at 25–200 °C for between 5 min and 24 h, and the liquid product is rich in monomeric sugars, mainly glucose and xylose [40–42].

A very novel area of microalgal biomass valorization is the production of bio-based plastics. Microalgae-derived plastics can be produced via the extraction of lipids and carbohydrates and their downstream conversion to polymers. Alternatively, polyhydroxyalkanoates can be synthesized in microalgae cells under specified cultivation conditions [43,44].

The aim of this work was the valorization of *Chlorella* microalgae residual biomass via catalytic acid hydrolysis/dehydration and hydrogenolysis/hydrogenation. *Chlorella vulgaris* was subjected to solvent lipid extraction followed by protein removal and the remaining carbohydrate-enriched biomass was converted into sugars, organic acids and furans via hydrolysis/dehydration. Alternatively, hydrogenation/hydrogenolysis using heterogeneous catalysts was applied in-situ to convert sugars to sugar alcohols.

2. Results

2.1. Characterization of Chlorella vulgaris Strains

The composition of the *Chlorella vulgaris* biomass used in this study is shown in Table 1. The high nitrogen concentration in the cultivation of the LL (low lipid) sample led to higher protein (28.1 wt.%) and carbohydrate content (33.6 wt.%) compared to the ML (medium lipid) sample (see experimental section). The commercially available biomass (MF) exhibited the highest protein and lipid content (46.9 wt.% and 30.2 wt.%, respectively). The sample cultivated at pilot scale (AF) exhibited lower protein and lipid content (22.4 wt.% and 21.3 wt.%, respectively). On the other hand, this sample exhibited the lowest carbohydrate content (14.5 wt.%). The detailed analysis of the carbohydrate monomers is shown in Table S1. All the biomass samples contained mainly glucose and xylose, while the concentration of both sugars gradually increased after the extraction of lipids and proteins. It can be noted that the two lab-scale cultivated samples (LL and ML) exhibited significantly higher glucose content (16.8–26.8 wt.%) compared to the commercially available biomass (MF) and the pilot-scale cultivation (AF), which had glucose content of 8.9 and 5.7 wt.%, respectively. Furthermore, based on elemental analysis, all biomass samples exhibited high carbon (43.8–47.6 wt.%), hydrogen (6.6–7.8 wt.%) and nitrogen (6.1–11.7 wt.%) content and were almost sulfur-free. An exception to this trend is the AF samples, which showed the lowest carbon concentration (21.3 wt.%).

Table 1. Biochemical composition and elemental analysis of microalgae feedstocks.

Samples	Composition [a] (wt.%)				Elemental Analysis [a] (wt.%)				
	Lipids	Proteins	Carbohydrates	Ash	C	H	N	S	O
LL	15.0	28.1	33.6	19.3	47.6	6.7	11.7	0.0	14.7
ML	22.6	25.7	25.1	17.6	46.7	6.6	6.1	0.1	23.0
MF	30.2	46.9	14.8	6.2	43.8	7.8	9.8	0.0	32.4
AF	21.3	22.4	14.5	21.4	21.3	6.1	4.9	0.0	46.3

[a] Expressed as wt.% on dry biomass.

2.2. Catalytic Acid Hydrolysis/Dehydration

The hydrothermal hydrolysis of biomass was performed in aqueous solutions with sulfuric acid as an acidic homogeneous catalyst. Preliminary experiments were performed without a catalyst to study the effects of reaction temperature and feedstock composition. The hydrothermal treatment of the LL-Res sample (i.e., the remaining biomass after extraction of lipids from microalgae sample LL; see experimental section) at 175 °C for 15 min led

to 63.7 wt.% solubilization while the extraction of proteins (sample LL-Re-P) enhanced the solubilization to 71.9 wt.%, as shown in Figure S1. The *Chlorella* strain with medium lipid content after lipid extraction (ML-Res sample) exhibited significantly higher solubilization (79.1 wt.%) compared to the strain of low lipid (LL) extracted biomass. An increase in the reaction temperature from 175 °C to 250 °C led to a slight increase in solubilization to 81.7 wt.%. The addition of dilute sulfuric acid (0.25% w/v) at 175 °C did not affect the solubilization (79.7 wt.%), as was shown in the case of ML-Res.

Regarding the composition of the hydrolysates, the main compounds identified via the HPLC analysis appertain to three main categories: sugars, organic acids and furanic compounds. The solubilization of the LL-Res biomass in neat water yielded a low concentration of monomeric sugars (5 mg/g) due to the partial depolymerization of carbohydrates, while the monomeric sugars were directly degraded to organic acids (Figure 1a). At 175 °C-15 min, LL-Res saw the formation of cellobiose (1.3 mg/g) and glucose (3.8 mg/g). The main organic acids formed were lactic acid (27.9 mg/g), acetic acid (4.6 mg/g) and propionic acid (7.9 mg/g), as can be observed in Figure 1b. A part of the formed organic acids, especially lactic acid and propionic acid, may also be attributed to protein conversion. More specifically, proteins can be converted to lactic acid via deamination, which is further dehydrated towards propionic acid [45]. Based on the analysis of the hydrolysate obtained via hydrothermal treatment at 190 °C-15 min (in neat water) of LL-Res-P (biomass derived after the extraction of lipids and proteins from the LL sample), it can be seen that the extraction of proteins led to a higher concentration of propionic acid (27.2 mg/g) but a significantly lower concentration of lactic acid (2.4 mg/g), proving that part of the lactic acid is derived from protein conversion but propionic acid is derived from carbohydrate conversion. Both LL-Res and LL-Res-P yielded low levels of furanic compounds.

The treatment of biomass remaining after the extraction of lipids from a microalgae sample with increased lipids (i.e., ML-Res) in neat water 175 °C-15 min) yielded a higher sugar concentration (15.5 mg/g), lower organic acids (29.9 mg/g) and an almost similar concentration of furans (0.9 mg/g). The high sugar content is mainly attributed to glucose, whose concentration is equal to 11.7 mg/g, and to a lesser extent to galactose, arabinose and mannose, whose concentrations add up to 3.9 mg/g. Also, more organic acids were identified, such as succinic acid (6.1 mg/g), formic acid (8.5 mg/g) and propionic acid (15.2 mg/g) compared to those from LL-Res. Both succinic acid and formic acid are formed as intermediate products during the dehydration of sugars into furans. Raising the reaction temperature from 175 °C to 250 °C induced more severe reaction conditions, which resulted in a slight increase in biomass solubilization and to a completely different composition of the hydrolysates. Under the more severe conditions, fewer sugars were obtained (8.4 mg/g) due to their conversion into organic acids (144 mg/g) and furanic compounds (2.6 mg/g), as can be observed in Figure 1a. Furthermore, the higher temperature yielded a lower glucose content (6.3 mg/g) but enhanced depolymerization of xylooligosaccharides towards xylose monomers (2.2 mg/g). In addition, the more severe conditions increased the concentration of formic acid to 22.4 mg/g and of propionic acid to 89.3 mg/g and further enhanced the formation of acetic acid (15.3 mg/g), lactic acid (11.3 mg/g) and levulinic acid (2.6 mg/g), either via direct conversion of sugars or via rehydration of HMF (i.e., in the case of formic and levulinic acids). The addition of sulfuric acid as a homogeneous acid catalyst in the hydrothermal treatment (175 °C-15 min, 0.25% w/v) of ML-Res, induced increased depolymerization of oligosaccharides to monomeric sugars (32.5 mg/g). i.e., containing glucose (26.3 mg/g) and xylose (6.3 mg/g). The acidic conditions facilitated the dehydration of sugars to furanic compounds (29.1 mg/g) and their further conversion to organic acids (98.5 mg/g). The dehydration of glucose led to the formation of HMF (21.3 mg/g), while the dehydration of xylose yielded furfural (7.9 mg/g), along with the subsequent formation of succinic acid (8.7 mg/g), lactic acid (7 mg/g), formic acid (23.1 mg/g), acetic acid (14.1 mg/g) and propionic acid (45.6 mg/g).
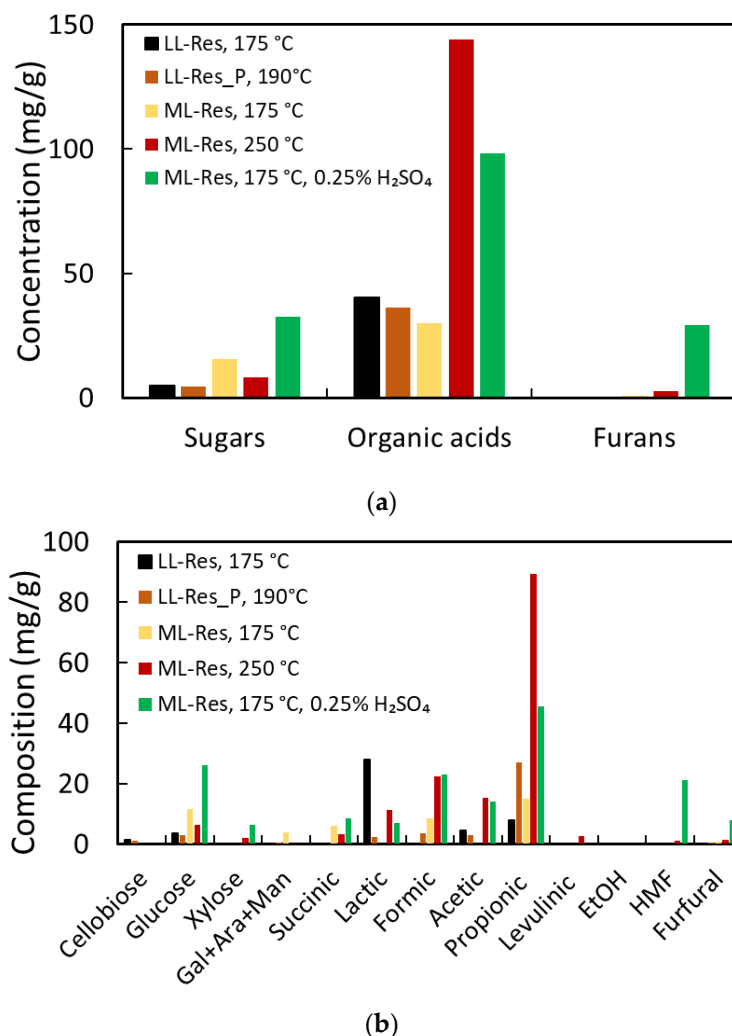
Figure 1. (**a**) Categories of the compounds and (**b**) composition of the hydrolysates of LL-Res and ML-Res *Chlorella vulgaris* hydrolysates.

A more detailed investigation of the effects of the process parameters (reaction temperature, time and acid catalyst concentration) was performed using the MF-Res biomass. Under the milder reaction conditions (190 °C, 15 min, 0.25% w/v H_2SO_4), the solubilization of the biomass was 84.0 wt.%, as shown in Figure S2. An increase in the reaction temperature from 190 °C to 230 °C enhanced the solubilization (90.0 wt.%), but a further increase to 250 °C led to slightly lower solubilization (87.0 wt.%), probably due to the formation of humins. Humins in the recovered solids were indirectly determined via TGA analysis. As can be observed in Table S2, the recovered solids exhibited two distinct weight-loss steps. The first step was in the range of 25–120 °C and corresponds to the evaporation of the remaining water, while the second, most dominant step was in the range 120–550 °C, corresponding to the decomposition of the remaining biomass. Above this temperature, the residual mass stabilized. An increase in the hydrolysis reaction temperature from 190 to 250 °C led to an increase in the residual mass from 41.6% to 50.6%, probably due to the formation of humins, which are more stable and resilient when heated in an inert atmosphere (TGA) and convert further to char. Unlike the reaction temperature, the reaction time did not significantly influence the solubilization of biomass. A prolonged reaction time (60 min) maintained the solubilization at 84.0 wt.%, equal to a shorter reaction time (15 min). The most profound impact was that of the concentration of sulfuric acid. A higher concentration (0.5% w/v) improved the solubilization from 84.0 wt.% to 93.0 wt.%. The sequential extraction of proteins (MF-Res-P) led to a partial decrease in the solubilization

(81.4 wt.% of the respective biomass sample). All the hydrolysates derived via the hydrolysis/dehydration of the MF-Res biomass are shown in Figure 2. All samples exhibited a light brown color, and changes in the severity of the treatment conditions did not influence the color of the product.
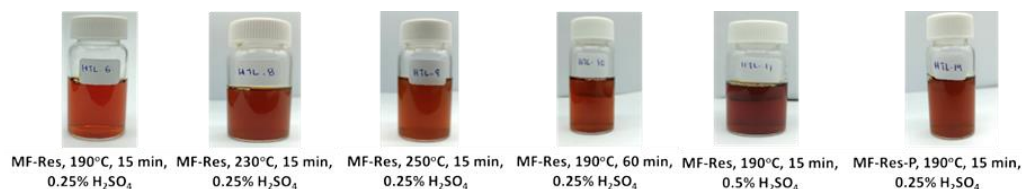

MF-Res, 190°C, 15 min, 0.25% H₂SO₄　　MF-Res, 230°C, 15 min, 0.25% H₂SO₄　　MF-Res, 250°C, 15 min, 0.25% H₂SO₄　　MF-Res, 190°C, 60 min, 0.25% H₂SO₄　　MF-Res, 190°C, 15 min, 0.5% H₂SO₄　　MF-Res-P, 190°C, 15 min, 0.25% H₂SO₄

Figure 2. Hydrolysates obtained via the hydrolysis/dehydration of MF-Res *Chlorella vulgaris* under different reaction conditions.

Despite the similar levels of solubilization, reaction conditions did tailor the composition of the hydrolysates, as can be observed in Figure 3a,b. The least severe conditions (190 °C, 15 min, 0.25% w/v H_2SO_4) yielded the highest concentration of sugars (32.3 mg/g) and the lowest concentration of organic acids (91.4 mg/g). Surprisingly, these conditions also enhanced the dehydration of sugars towards furanic compounds, whose concentration was 27.8 mg/g. An increase in the reaction temperature from 190 °C to 230 °C yielded fewer sugars (23.0 mg/g), more organic acids (106.0 mg/g) and significantly lower furanic compounds (3.0 mg/g). A further increase in the reaction temperature to 250 °C proved to enhance further the formation of organic acids. This hydrolysate exhibited the lowest concentration of sugars (15.9 mg/g) and furanic compounds (3.1 mg/g), while organic acids exhibited the highest concentration (121 mg/g). To give more details regarding the content of individual compounds in the hydrolysates, it was observed that under the milder conditions, glucose was the main sugar formed (22.4 mg/g), with xylose and arabinose/galactose/mannose exhibiting lower concentrations (5.5 and 4.4 mg/g, respectively). The most abundant organic acid was propionic acid (46.8 mg/g), while succinic acid (9.2 mg/g), lactic acid (8.4 mg/g), formic acid (12.8 mg/g) and acetic acid (14.2 mg/g) were at substantially lower concentrations. The dehydration of glucose led to the formation of HMF (23.6 mg/g), while the dehydration of C_5 sugars led to the formation of furfural (4.3 mg/g). An increase in the reaction temperature from 190 °C to 230 °C yielded a lower glucose concentration (17.3 mg/g) as well as lower concentrations of xylose (3.5 mg/g) and other sugars (2.3 mg/g). A higher reaction temperature enhanced the formation of propionic acid, whose concentration increased to 80.5 mg/g, instead of the formation of other organic acids or furanic compounds. The highest reaction temperature (250 °C) induced further conversion of sugars towards organic acids. Glucose and xylose content were 12.3 mg/g and 3.5 mg/g, respectively, while none of the other sugars (arabinose, galactose and mannose) was identified. An increase in the reaction time from 15 to 60 min had a less profound effect on the hydrolysis/dehydration of sugars. The total sugar concentration decreased from 32.3 mg/g to 22.7 mg/g, mainly attributed to glucose, whose concentration was 18.6 mg/g, and xylose, with concentration 4.1 mg/g. The most profound effect was on the formation of organic acids, whose concentration increased from 91.4 mg/g to 121 mg/g. The main organic acid formed was propionic acid (75.4 mg/g). The acetic acid concentration also increased from 14.2 mg/g to 16.6 mg/g, while all the other acids were formed at lower concentrations. The concentration of the furanic compounds was low (2.0 mg/g), due to their subsequent conversion to organic acids as well as their condensation to humins. Characterization of the recovered solids via TGA showed that an increase in the hydrolysis reaction time from 15 to 60 min led to an increase in the residual mass from 41.6% to 50.7%, which may also be considered as indication of humin formation, as discussed above for the effect of higher hydrolysis temperature. Generally, harsher conditions in terms of reaction temperature and time are considered to decrease the carbohydrate yield in favor of furans and/or acids, while mild conditions can enhance the sugar yield [46].
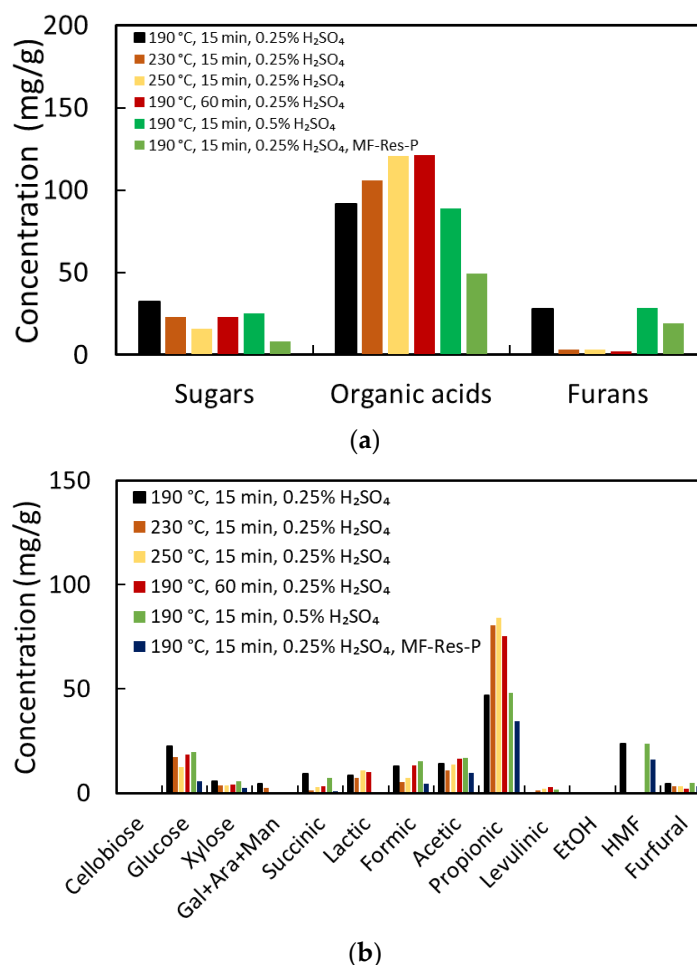
Figure 3. (**a**) Categories of the compounds and (**b**) composition of the MF-Res *Chlorella vulgaris* hydrolysates.

Regarding the effect of the acidic catalyst, a higher acid concentration, i.e., from 0.25 to 0.5% *w/v* H_2SO_4, did not significantly change the products' distribution (Figure 3a,b). More specifically, the higher acid concentration yielded slightly lower total sugars, 25.1 mg/g, compared to 32.3 mg/g formed when using 0.25% *w/v* H_2SO_4. Both glucose and xylose were formed at lower concentrations, 19.6 and 5.5 mg/g, respectively, and none of the other sugars was identified. Additionally, the total concentration of organic acids decreased from 91.4 mg/g to 88.9 mg/g. The higher acid concentration enhanced the formation of formic and acetic acids, whose content increased from 12.8 mg/g to 15.4 mg/g and from 14.2 mg/g to 16.7 mg/g, respectively. The succinic acid concentration decreased to 7.2 mg/g, while the propionic acid concentration remained almost the same at 48.0 mg/g. Furfural and HMF were measured at 4.9 and 23.7 mg/g, respectively. The lower formation of sugars and the higher concentration of acids and furanic compounds when using higher concentrations of sulfuric acid is in accordance with similar results from the literature for other microalgae [47].

A similar study was performed using the AF microalgae derived via pilot-scale cultivation. Regarding the solubilization of microalgae residual biomass (Figure S3), the hydrolysis of AF-Res (biomass obtained after lipid extraction from AF) at 190 °C, 15 min, 0.25% *w/v* H_2SO_4 yielded 91.7 wt.% solubilization, slightly higher than the 84 wt.% obtained during the hydrolysis of lab-scale cultivated samples (LL, ML) and the commercially (MF) available microalgae. Treatment of the AF-Res-P (lipid- and protein-extracted biomass) led to lower solubilization (83.4 wt.%) under the same hydrolysis conditions. An increase in the reaction time from 15 to 60 min led to slightly higher solubilization 85.7 wt.%, while

higher sulfuric acid concentration further improved the solubilization to 90.2 wt.%. The obtained hydrolysates are shown in Figure 4. All hydrolysates exhibited a brown color, while increase of reaction time or of the sulfuric acid concentration turned the color to dark brown, possibly due to the formation of humins.
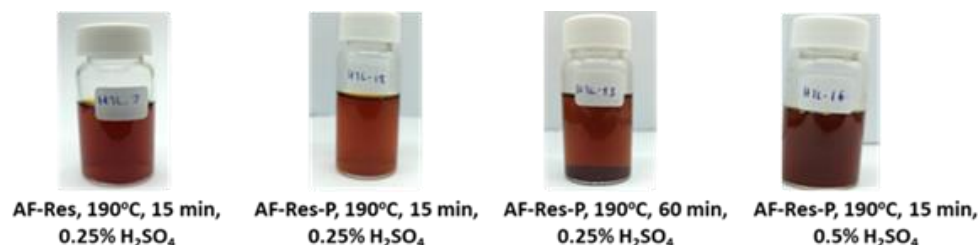

AF-Res, 190°C, 15 min, 0.25% H₂SO₄   **AF-Res-P, 190°C, 15 min, 0.25% H₂SO₄**   **AF-Res-P, 190°C, 60 min, 0.25% H₂SO₄**   **AF-Res-P, 190°C, 15 min, 0.5% H₂SO₄**

Figure 4. Hydrolysates obtained via the hydrolysis/dehydration of AF-Res and AF-Res-P *Chlorella vulgaris* under different reaction conditions.

The composition of the hydrolysates is shown in Figure 5a,b. The pilot-scale cultivation of *Chlorella* microalgae followed by lipid extraction (sample AF-Res) and hydrolysis at 190 $^\circ$C, 15 min, 0.25% w/v H_2SO_4 yielded 27.4 mg/g sugars, 74.3 mg/g organic acids and 26.9 mg/g furanic compounds, which were slightly lower compared to the composition of the MF-Res hydrolysate under the same treatment conditions. Glucose (21.9 mg/g) and xylose (5.5 mg/g) were the only sugars formed. As can be observed in Figure 5b, propionic acid was the main organic acid formed (35.7 mg/g). Formic acid (17.8 mg/g), acetic acid (11.9 mg/g) and succinic acid (8.8 mg/g) were formed at lower concentrations, while appreciable amounts of furfural and HMF were also determined (ca. 7.6 mg/g and 19.4 mg/g, respectively). The subsequent extraction of proteins and the hydrothermal treatment of the respective sample (AF-Res-P) under the same conditions yielded a slightly lower concentration of sugars (18.7 mg/g) and organic acids (69.4 mg/g) but facilitated the in-situ dehydration of sugars to furanic compounds, whose concentrations amounted to 35.1 mg/g (HMF and furfural concentrations were 25.2 and 9.9 mg/g, respectively). Furthermore, the extraction of proteins did not affect the propionic acid and succinic acid concentrations but slightly reduced the concentrations of formic acid and acetic acid to 14.6 and 8.9 mg/g, respectively.

A higher residence time (60 min) at 190 $^\circ$C for the AF-Res-P treatment enhanced the dehydration of sugars to furanic compounds as well as the conversion to organic acids. The concentration of sugars was found to be 13.4 mg/g, organic acids 85.9 mg/g and furanic compounds 39.5 mg/g. The prolonged reaction time yielded lower concentrations of glucose (9.4 mg/g) and xylose (6.7 mg/g). On the other hand, formic acid concentration increased from 14.6 to 17.4 mg/g, and acetic and propionic acid concentrations increased from 8.9 to 10.2 mg/g and from 37.0 to 55.5 mg/g, respectively. The main difference between the two reaction times is that levulinic acid (2.8 mg/g) was formed at 60 min, while succinic was not detected at all. Additionally, ethanol was produced at 60 min with a concentration of 35.7 mg/g.

On the other hand, higher sulfuric acid concentration facilitated the depolymerization of carbohydrates towards monomeric sugars, whose concentration increased to 23.5 mg/g (from 18.7 mg/g). Both glucose and xylose concentrations increased to 14.2 and 9.3 mg/g, respectively. Furthermore, furanic compounds increased to 42.0 mg/g while organic acid concentration decreased to 58.9 mg/g. Based on the above results, the higher sulfuric acid concentration enhanced the in-situ dehydration of sugars to furanic compounds without subsequent conversion to organic acids. Indeed, formic, acetic and succinic acid concentrations decreased to 6.7, 7.3 and 2.3 mg/g, respectively. On the other hand, HMF concentration increased from 25.2 to 29.8 mg/g and furfural concentration from 9.9 to 12.3 mg/g.
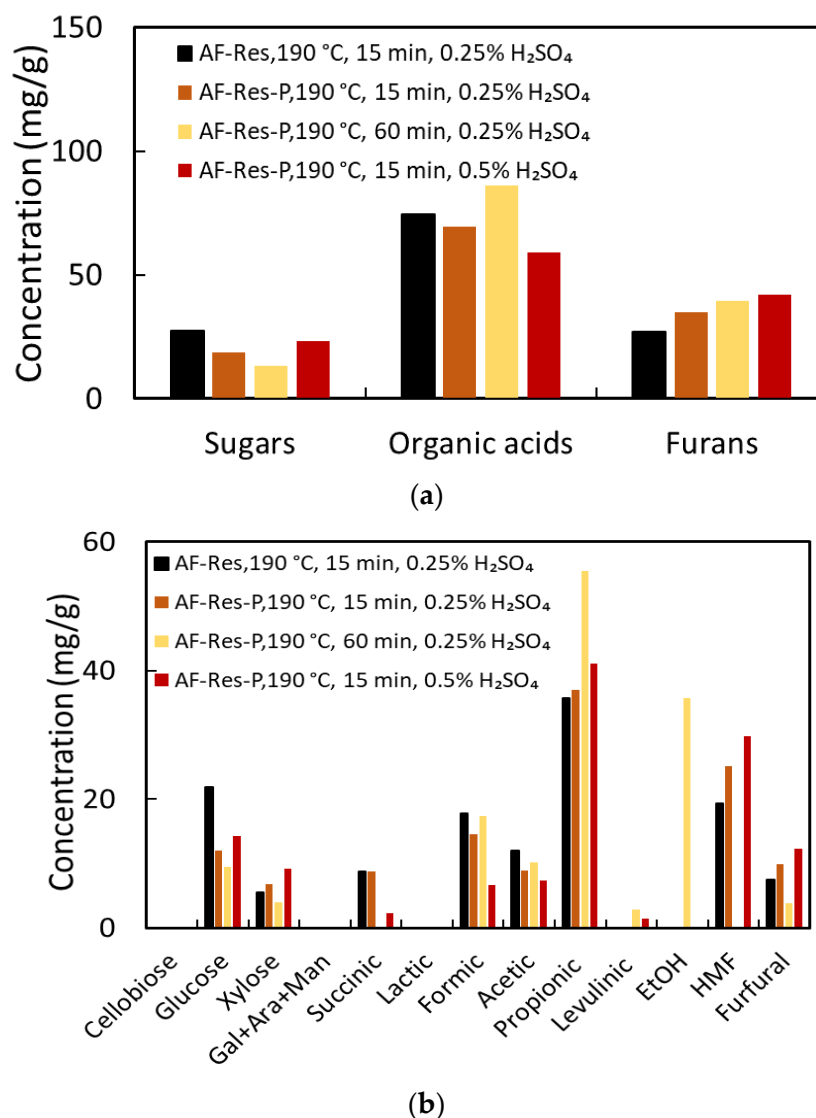
Figure 5. (**a**) Categories of the compounds and (**b**) composition of the AF-Res and AF-Res-P *Chlorella vulgaris* hydrolysates.

Based on the above results, it is shown that the careful selection of hydrolysis conditions (temperature, time and sulfuric acid concentration), which may also induce further/in situ conversion of the initially formed sugars to furans and organic acids, can finetune the composition of the final liquid hydrolysates. Usually, the boundaries between the initial hydrolysis of carbohydrates to monomer sugars, and their consequent conversion to furans and/or acids, are not distinct according to the different types of homogeneous or heterogeneous acid catalysts. As a result, it may be preferable to adjust and increase slightly the severity of the hydrolysis conditions towards the direct/in situ production of furans and/or organic acids. An additional benefit of doing this is that the tedious separation of sugars from the above mixture is avoided. As a general rule, relatively mild hydrolysis/reaction conditions select for sugars and some organic acids, while more severe conditions can shift the selectivity towards furanic compounds and other organic acids (from HMF hydrolysis). Humin formation under more intense conditions should also be taken into consideration. Another important outcome of the present results is the versatile character of the approach with respect to the type of feedstock used, i.e., lipid-extracted microalgae biomass or lipid-protein-extracted biomass. In addition, it is shown that similar results in terms of hydrolysis reactivity and product composition can be obtained from experimental/lab- and pilot-scale biomass cultivation and processing.

Another important point relates to the yields of the above-discussed targeted products (sugars, furans, acids) that are in the range of 7–20 wt.% of biomass. These yields are relatively low, mainly due to the relatively mild reaction conditions applied, which, however, restrict the formation of humins to low levels. Still, the initial sugar content of the biomass feedstocks (shown in Table S1) in the present work are not far from these product yields, i.e., they range from 5 to 40 wt.%, thus showing a relatively moderate/high yield of products when estimated on the basis of initial sugar content rather than the whole residual biomass. This further indicates that the residual biomass samples contain leftover lipids, proteins and ash.

2.3. Catalytic Hydrogenation/Hydrogenolysis

The catalytic hydrogenation/hydrogenolysis of microalgae residual biomass was performed using ethanol–water mixtures as solvents under increased hydrogen pressure, a sulfuric acid catalyst to induce the hydrolysis of carbohydrates towards sugar monomers, and ruthenium supported on activated carbon as the hydrogenation catalyst. The physicochemical characteristics of the 5%Ru/C catalyst are shown in Table 2 and Figure S4. The ruthenium was in its metallic phase; its crystallite size was calculated via Scherrer equation and found to be 8 nm. Regarding its porous properties, the catalyst exhibits mainly microporous characteristics, owing to the activated carbon support, alongside significant mesoporosity. The specific surface area, determined via the BET equation, was 1047 m^2/g, while the surface area corresponding to the micropores was 690 m^2/g, the rest being attributed to meso/macropores and external area. Taking into consideration the total pore volume, almost 37% corresponds to the micropore volume and 63% to the meso/macropore volume.

Table 2. Physicochemical characteristics of metallic catalysts supported on activated carbon (AC).

Catalyst	D_{XRD} (nm)	S_{BET} (m^2/g)	S_{micro} (m^2/g)	V_{total} (cm^3/g)	V_{micro} (cm^3/g)	$V_{meso/macro}$ (cm^3/g)
5%Ru/AC	8	1047	690	0.806	0.306	0.500

The biomass used as feed for the hydrogenation/hydrogenolysis experiments was the AF-Res-P, i.e., that derived from the pilot-scale microalgae AF after sequential extraction of lipids and proteins. The liquid products obtained via hydrogenation/hydrogenolysis exhibited a dark brown color, as can be observed in Figure 6.
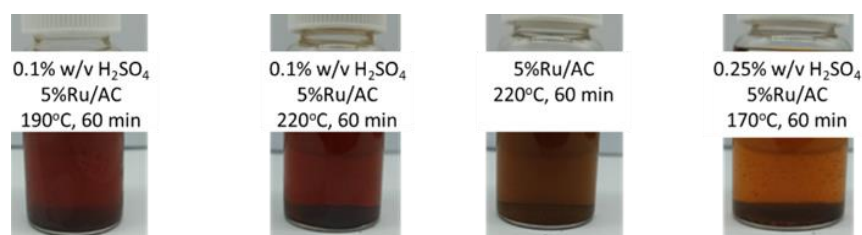

Figure 6. Liquid products obtained via the hydrogenation/hydrogenolysis of AF-Res-P *Chlorella vulgaris* under different reaction conditions.

At 190 °C for 60 min, the solubilization of the AF-Res-P biomass was 80.9 wt.%, and by increasing the reaction temperature to 220 °C, the solubilization was raised to 87.6 wt.%, as shown in Figure S5. For purposes of comparison, the utilization of only the 5%Ru/AC as the catalyst, without the addition of H_2SO_4, resulted in almost the same solubilization, ca. 86.4 wt.%. Furthermore, a higher concentration of sulfuric acid, 0.25% *w/v* instead of 0.10% *w/v*, but under milder reaction conditions (170 °C), resulted in slightly lower solubilization, 74.3 wt.%, possibly due to humin formation.

The composition of the liquid products is shown in Figure 7a,b. Using 0.1% w/v H_2SO_4 + 5%Ru/AC at 190 °C, the concentration of sugars was 20.4 mg/g, organic acids 23.9 mg/g and furanic compounds 6.1 mg/g. Furthermore, under the hydrogenation/hydrogenolysis conditions applied, the in-situ production of sugar alcohols was achieved (21.9 mg/g), with sorbitol (13.6 mg/g) and glycerol (8.3 mg/g) being the main products. Via a generalized mechanism, the hydrogenation of glucose leads to the formation of sorbitol, which can then be converted to glycerol via hydrogenolysis [48,49]. An increase in the reaction temperature from 190 °C to 220 °C resulted in lower sugar concentration (11.1 mg/g), lower furans (5.4 mg/g) and significantly lower sugar alcohols (2.2 mg/g). On the other hand, the more intense conditions induced the conversion of sugars to organic acids, whose concentration increased to 32.2 mg/g, with the most abundant acids being propionic acid (21.5 mg/g), formic acid (3.6 mg/g) and levulinic acid (1.9 mg/g). This shift towards organic acid formation maybe attributed to the increased dehydration of sugars to furans (and their subsequent rehydration to organic acids) and/or the direct conversion of sugars to organic acids catalyzed by H_2SO_4 at higher temperatures, being faster than the hydrogenation reactivity of sugars towards sugar alcohols. Apart from the combination of sulfuric acid with 5%Ru/AC, the activity of neat 5%Ru/AC was examined at 220 °C for 60 min. As can be observed in Figure 7a, 5%Ru/AC enhanced the depolymerization of carbohydrates to monomeric sugars (13.8 mg/g), their dehydration to furanic compounds (4.7 mg/g), their conversion to organic acids (35.7 mg/g) and their hydrogenation/hydrogenolysis to sugar alcohols (18.9 mg/g). Regarding the sugars' composition, the presence of 5%Ru/AC enhanced the formation of glucose (5.6 mg/g) and galactose/arabinose/mannose (6.5 mg/g), while less xylose was produced (0.8 mg/g). Also, a narrower distribution of organic acids was observed with the formation of acetic acid (6.9 mg/g) and propionic acid (28.8 mg/g). The ruthenium-based catalyst enhanced the hydrogenolysis of the formed sorbitol to glycerol, whose concentration increased to 18.9 mg/g. Finally, aiming to achieve milder reaction conditions (mainly a lower temperature), a slightly higher concentration of sulfuric acid was used (0.25% w/v) at a lower reaction temperature (170 °C). Under these conditions, the selectivity towards sugars increased and their concentration amounted to 57.8 mg/g, along with increased concentrations of furanic compounds (11.8 mg/g) and sugar alcohols (40.6 mg/g). The higher concentration of sugars is attributed to the higher concentration of xylose (11.6 mg/g) and galactose/arabinose/mannose (39.1 mg/g). Interestingly, the only organic acids formed were succinic acid (8.1 mg/g) and acetic acid (5.1 mg/g). Regarding the sugar alcohols, sorbitol and glycerol were formed at concentrations of 13.5 mg/g and 27.1 mg/g, respectively.

The aim of this study was to provide a first proof of concept of the possible valorization of microalgae residual carbohydrates to sugar alcohols via hydrogenation/hydrogenolysis. The leaching of Ru was not detected in the liquid products, as the use of sulfuric acid was kept to very low levels, just to initiate the hydrolysis of carbohydrates to monomeric sugars that could then be hydrogenated using Ru. Certainly, an in-depth characterization of the spent catalysts and reuse/regeneration studies are needed to further investigate the exploitation potential of the proposed approach.
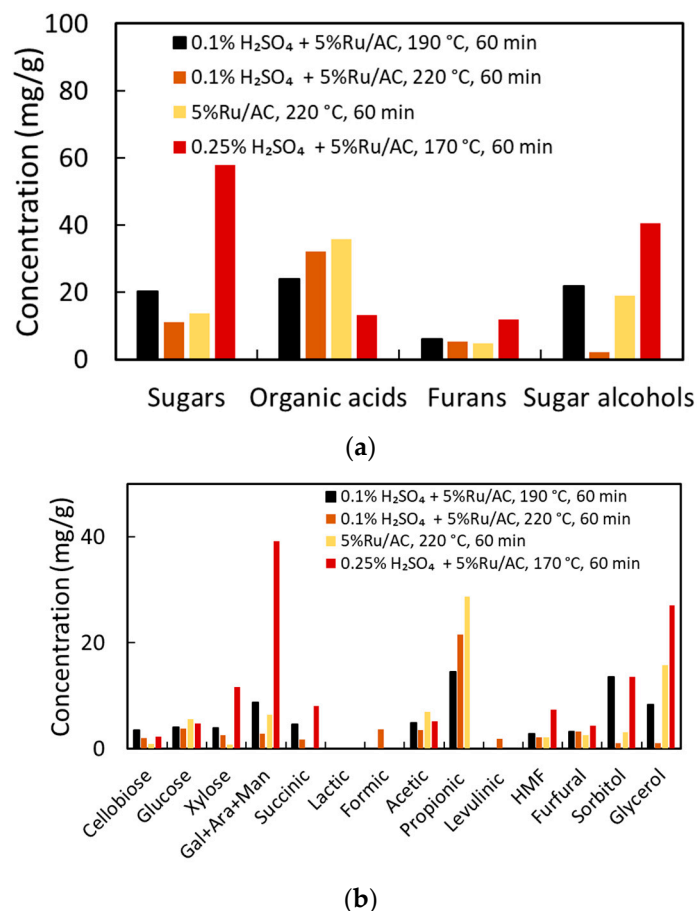
(a)

(b)

Figure 7. (**a**) Categories of the compounds and (**b**) composition of the AF-Res-P *Chlorella vulgaris* hydrolysates obtained via the hydrogenation/hydrogenolysis experiments.

3. Materials and Methods

3.1. Feedstocks

The microalgae samples used in this study are shown in Table 3. Two lab-scale cultures of *Chlorella vulgaris* were produced under different cultivation conditions, mainly nitrogen abundance, according to previously published procedures [33]. The high nitrogen concentration in the cultivation medium led to the *Chlorella vulgaris* LL (i.e., low lipid) sample, while the lower nitrogen concentration led to the production of the ML (i.e., moderate lipid) sample. Larger-scale amounts of *Chlorella vulgaris* biomass were produced by the Faculty of Agriculture, Aristotle University of Thessaloniki (photobioreactor, 2000L); this sample was named AF. For comparison purposes, a commercially available *Chlorella vulgaris* was also used (MF).

Table 3. *Chlorella vulgaris* residual biomass used in the hydrolysis/dehydration and hydrogenation/hydrogenolysis experiments.

Sample	Pretreatment
LL-Res	Lab-scale cultivation sample after lipid extraction of low lipid content strain
ML-Res	Lab-scale cultivation sample after lipid extraction of medium lipid content strain
MF-Res	Commercially available biomass after lipid extraction
AF-Res	Upscale cultivation sample after lipid extraction
LL-Res-P	Sample after protein extraction of LL-Res
ML-Res-P	Sample after protein extraction of ML-Res
MF-Res-P	Sample after protein extraction of MF-Res
AF-Res-P	Sample after protein extraction of AF-Res

3.2. Fractionation of Chlorella vulgaris Microalgae

The fractionation of the parent microalgae biomass was based on the extraction of lipids in the first step, followed by protein extraction in the second step. Thus, in the first step, 5 g of dry biomass was placed in a cellulose thimble. The extraction was performed in a Soxhlet apparatus, using a mixture of chloroform and methanol (2:1 *v/v*) as solvents, at 80 °C for 18 h. After the extraction, the solvent was recovered in a rotary evaporator (HB10, IKA) and the remaining lipid fraction was dried at 40 °C for 6 h. The remaining solid fraction, after lipid extraction, containing proteins and carbohydrates, was dried at room temperature overnight and at 40 °C for 6 h. The obtained samples were denoted as LL-Res, ML-Res, MF-Res and AF-Res.

In the second step, proteins were isolated according to the procedure described by Safi et al. [50]. Briefly, 0.5 g of dry biomass (after lipid extraction) was added to 25 mL 2 N NaOH solution (pH = 12) and left under stirring for 2 h at 40 °C. The supernatant was separated from the solid via centrifugation at $10{,}000\times g$, 40 °C, 10 min. The solid residue enriched in carbohydrates was washed several times with deionized water and centrifuged until pH = 6, dried at room temperature and at 40 °C for 6 h. Proteins were isolated after precipitation at pH = 3, with 0.1 M HCl and centrifugation at $10.000\times g$, 20 °C, 10 min. The obtained samples after protein extraction were denoted as LL-Res-P, ML-Res-P, MF-Res-P and AF-Res-P.

3.3. Characterization of Biomass Feedstocks

The characterization of the microalgae biomass feedstocks was performed according to NREL protocols. Moisture content and total solids were based on NREL/TP-5100-60956 protocol, according to which 100 mg of pulverized biomass was placed at 40 °C under vacuum for at least 18 h [51]. Afterwards, the ash content was determined after ignition at 575 °C for 3 h, according to the same protocol. The elemental composition (C/H/N/S) was determined in an elemental analyzer EA 3100 (EuroVector, Pavia, Italy). The samples were heated at 980 °C, under constant helium flow. Oxygen was determined via the equation O (wt.%) = 100 − C (wt.%) − H (wt.%) − N (wt.%) − S (wt.%) − Ash (wt.%). The carbohydrate content was determined via two-step sulfuric acid hydrolysis, according to NREL/TP-5100-60957 protocol [52].

3.4. Catalytic Hydrolysis/Dehydration

The hydrothermal hydrolysis/dehydration experiments were carried out in a batch stirred autoclave reactor. In each experiment, microalgal biomass was mixed with distilled water at liquid-to-solid ratio L/S = 32 and placed in the reactor, along with 0.25 or 0.50% *w/v* H_2SO_4 as an acid catalyst. After closing the reactor, 10 bar of nitrogen gas was purged into the reactor. The experiments were performed in the temperature range of 175–250 °C for 15–60 min, with a 400 rpm stirring rate to eliminate any mass transfer phenomena. The average heating rate to the targeted temperature was ~14 °C/min, thus limiting the conversion of biomass taking place during the heat-up of the experiment. After the reaction, the reactor was cooled to room temperature and vacuum filtration was applied to separate the liquid fraction from the biomass. The solid fraction, corresponding to the non-solubilized biomass, was dried at 80 °C for 6 h under vacuum, while the liquid fraction containing the solubilized biomass was analyzed by high-performance liquid chromatography (HPLC). Analysis was performed on an HPLC (LC-20AD, HPLC, Shimadzu, Tokyo, Japan) equipped with a refractive index detector (RID-10A, Shimadzu) and an oven (CTO-20A, Shimadzu). The identification and quantification of sugars, organic acids and furanic compounds was carried out in an SH-1011 column, at 45 °C and 0.01 N H_2SO_4 as the mobile phase with a flow of 0.7 mL/min. The recovered solids were characterized via thermogravimetric analysis (TGA). The measurements were performed in the Netzsch (Selb, Germany) STA 449 F5 Jupiter, at a temperature range of 25–950 °C, under N_2 atmosphere and a 10 °C/min heating rate. The experiments were performed in triplicate and the standard error was <5%.

3.5. Catalytic Hydrogenolysis/Hydrogenation

The hydrogenation/hydrogenolysis experiments were carried out in a batch autoclave reactor, using 0.4 g of biomass and a solvent mixture of 20 mL ethanol–water at a ratio of 90–10%. Dilute H_2SO_4 0.1 or 0.25% *w/v* was used as an acid catalyst, combined with the solid hydrogenation catalyst 5%Ru/AC. All experiments were performed with 30 bar hydrogen gas pressure (measured at room temperature), in the temperature range of 190–220 °C for 60 min and with a 400 rpm stirring rate to eliminate any mass transfer phenomena. The average heating rate to the targeted temperature was ~14 °C/min. Afterwards, the reactor was cooled to room temperature and vacuum filtration was applied to separate the liquid fraction from the biomass. The solid fraction was dried at 80 °C for 6 h under vacuum, while the liquid fraction was analyzed by high-performance liquid chromatography (HPLC), similarly to Section 3.4. The experiments were performed in triplicate and the standard error was <5%.

The catalyst 5%Ru/AC was synthesized via a wet impregnation method. Norit SX-Plus carbon was used as support. Prior to the impregnation, the support was thermally treated at 500 °C, 3 h under N_2 flow. Briefly, an appropriate amount of $RuCl_3$ was dissolved in 20 mL H_2O and then added dropwise and under stirring to the suspension of 10 g support which was suspended in 100 mL H_2O. Stirring continued for 1 h before the water was removed using a rotary evaporator. The final solid was dried overnight at 100 °C, calcined at 500 °C, 3 h under He (50 mL/min) and reduced under H_2 flow 50 mL/min. The catalyst was characterized via X-ray powder diffraction. The pattern was recorded using CuKa X-ray radiation, at 2θ = 5–85°, with 0.02°/step and 2 s/step. The porous properties were determined via nitrogen physisorption at −196 °C using an automatic volumetric sorption analyzer (Autosorb-1 MP, Quantachrome, Boynton Beac, FL, USA). Prior to taking the measurements, the sample was outgassed at 250 °C, 19 h under 5×10^{-9} Torr vacuum. Surface areas were determined via the multipoint BET method; the total pore volume was determined at P/Po = 0.99 while the microporous surface areas and volumes were determined via t-plot method.

4. Conclusions

This work focused on the potential valorization of various types of microalgal residual biomass towards the production of platform chemicals. The residual carbohydrate biomass that remained after the extraction of lipids and proteins can be converted to monomeric sugars, which can serve as precursors of a wide range of chemicals. The intensity of the mild acid-hydrothermal treatment of this residual biomass determined the extent of hydrolysis–dehydration–rehydration reactions that led to sugar monomers being produced at concentrations of 4.6–32.5 mg/g, furans at up to 42 mg/g and organic acids between 30 and 144 mg/g, respectively. Under more intense conditions, i.e., higher acid concentration and/or temperature, all the above products were suppressed due to further condensation reactions that lead to humin formation.

Each one of the above group of products, e.g., monomeric sugars, organic acids and furanic compounds, has its value in an integrated biorefinery scheme. Monomeric sugars can be used to produce bioethanol/butanol, a wide range of acids (succinic, lactic, etc.) as well as furans (furfural, HMF), all of them being high-added-value platform chemicals towards the production of fuels or biobased polymers. With regard to the furan/acid mixtures, these may be more easily separated, compared to when monomer sugars are also present, and utilized; for example, furfural can make furfuryl alcohol resins or HMF to produce furan dicarboxylic acid (FDCA) and then polyethylene furanoate (PEF), a potential replacement for PET. On the other hand, organic acids such as lactic and succinic are being used to produce valuable green plastics based on polylactic acid (PLA) or polybutylene succinates (PBS). Alternatively, the whole mixture of organic acids may find application in the production of phenol–formaldehyde resins (P–F) where a commercial/petroleum-derived, usually acetic acid-based, acidification medium is used to control/regulate the pH of the process.

A promising alternative pathway is the catalytic hydrogenation/hydrogenolysis of this residual carbohydrate biomass, with the assistance of homogeneous mild acid catalysts, towards the in-situ production of sugar alcohols, such as sorbitol and glycerol, whose concentrations add up to 2–41 mg/g. These products are also of high value and can be used as food ingredients, in polymer synthesis, in pharmaceuticals, etc., thus increasing the exploitation potential of microalgae residual biomass.

References

1. Lee, J.Y.; Yoo, C.; Jun, S.Y.; Ahn, C.Y.; Oh, H.M. Comparison of several methods for effective lipid extraction from microalgae. *Bioresour. Technol.* **2010**, *101* (Suppl. 1), S75–S77. [CrossRef] [PubMed]
2. Lee, S.Y.; Khoiroh, I.; Vo, D.-V.N.; Senthil Kumar, P.; Show, P.L. Techniques of lipid extraction from microalgae for biofuel production: A review. *Environ. Chem. Lett.* **2020**, *19*, 231–251. [CrossRef]
3. Jeevan Kumar, S.P.; Vijay Kumar, G.; Dash, A.; Scholz, P.; Banerjee, R. Sustainable green solvents and techniques for lipid extraction from microalgae: A review. *Algal Res.* **2017**, *21*, 138–147. [CrossRef]
4. Chew, K.W.; Yap, J.Y.; Show, P.L.; Suan, N.H.; Juan, J.C.; Ling, T.C.; Lee, D.J.; Chang, J.S. Microalgae biorefinery: High value products perspectives. *Bioresour. Technol.* **2017**, *229*, 53–62. [CrossRef] [PubMed]
5. Yen, H.W.; Hu, I.C.; Chen, C.Y.; Ho, S.H.; Lee, D.J.; Chang, J.S. Microalgae-based biorefinery—From biofuels to natural products. *Bioresour. Technol.* **2013**, *135*, 166–174. [CrossRef] [PubMed]
6. Kwak, H.S.; Kim, J.Y.H.; Woo, H.M.; Jin, E.; Min, B.K.; Sim, S.J. Synergistic effect of multiple stress conditions for improving microalgal lipid production. *Algal Res.* **2016**, *19*, 215–224. [CrossRef]
7. Yeletsky, M.P.; Kukushkin, G.R.; Stepanenko, A.S.; Koskin, P.A. Prospects in One-stage Conversion of Lipid-based Feedstocks into Biofuels Enriched with Branched Alkanes. *Curr. Org. Chem.* **2023**, *27*, 1114–1118. [CrossRef]
8. Koskin, A.P.; Zibareva, I.V.; Vedyagin, A.A. Conversion of Rice Husk and Nutshells into Gaseous, Liquid, and Solid Biofuels. In *Biorefinery of Alternative Resources: Targeting Green Fuels and Platform Chemicals*; Nanda, S., Vo, D.-V.N., Sarangi, P.K., Eds.; Springer: Singapore, 2020; pp. 171–194.
9. Levasseur, W.; Perre, P.; Pozzobon, V. A review of high value-added molecules production by microalgae in light of the classification. *Biotechnol. Adv.* **2020**, *41*, 107545. [CrossRef]
10. Karageorgou, D.; Rova, U.; Christakopoulos, P.; Katapodis, P.; Matsakas, L.; Patel, A. Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid. *Trends Food Sci. Technol.* **2023**, *136*, 169–180. [CrossRef]
11. Patel, A.; Rova, U.; Christakopoulos, P.; Matsakas, L. Introduction to Essential Fatty Acids. In *Nutraceutical Fatty Acids from Oleaginous Microalgae*; Wiley: Hoboken, NJ, USA, 2020; pp. 1–22.

12. Brown, M.R. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **1991**, *145*, 79–99. [CrossRef]

13. Moreira, J.B.; Vaz, B.d.S.; Cardias, B.B.; Cruz, C.G.; Almeida, A.C.A.d.; Costa, J.A.V.; Morais, M.G.d. Microalgae Polysaccharides: An Alternative Source for Food Production and Sustainable Agriculture. *Polysaccharides* **2022**, *3*, 441–457. [CrossRef]

14. Delattre, C.; Pierre, G.; Laroche, C.; Michaud, P. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnol. Adv.* **2016**, *34*, 1159–1179. [CrossRef] [PubMed]

15. de Carvalho Silvello, M.A.; Severo Goncalves, I.; Patricia Held Azambuja, S.; Silva Costa, S.; Garcia Pereira Silva, P.; Oliveira Santos, L.; Goldbeck, R. Microalgae-based carbohydrates: A green innovative source of bioenergy. *Bioresour. Technol.* **2022**, *344*, 126304. [CrossRef] [PubMed]

16. Gouda, M.; Tadda, M.A.; Zhao, Y.; Farmanullah, F.; Chu, B.; Li, X.; He, Y. Microalgae Bioactive Carbohydrates as a Novel Sustainable and Eco-Friendly Source of Prebiotics: Emerging Health Functionality and Recent Technologies for Extraction and Detection. *Front. Nutr.* **2022**, *9*, 806692. [CrossRef] [PubMed]

17. Smachetti, M.E.S.; Rizza, L.S.; Coronel, C.D.; Nascimento, M.D.; Curatti, L. Microalgal Biomass as an Alternative Source of Sugars for the Production of Bioethanol. In *Principles and Applications of Fermentation Technology*; Wiley: Hoboken, NJ, USA, 2018; pp. 351–386.

18. Markou, G.; Angelidaki, I.; Georgakakis, D. Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 631–645. [CrossRef] [PubMed]

19. Morales-Jiménez, M.; Gouveia, L.; Yáñez-Fernández, J.; Castro-Muñoz, R.; Barragán-Huerta, B.E. Production, Preparation and Characterization of Microalgae-Based Biopolymer as a Potential Bioactive Film. *Coatings* **2020**, *10*, 120. [CrossRef]

20. Madadi, R.; Maljaee, H.; Serafim, L.S.; Ventura, S.P.M. Microalgae as Contributors to Produce Biopolymers. *Marine Drugs* **2021**, *19*, 466. [CrossRef] [PubMed]

21. Rachidi, F.; Benhima, R.; Sbabou, L.; El Arroussi, H. Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution. *Biotechnol. Rep.* **2020**, *25*, e00426. [CrossRef] [PubMed]

22. Sarma, S.; Sharma, S.; Rudakiya, D.; Upadhyay, J.; Rathod, V.; Patel, A.; Narra, M. Valorization of microalgae biomass into bioproducts promoting circular bioeconomy: A holistic approach of bioremediation and biorefinery. *3 Biotech* **2021**, *11*, 378. [CrossRef] [PubMed]

23. Wild, K.J.; Steingass, H.; Rodehutscord, M. Variability in nutrient composition and in vitro crude protein digestibility of 16 microalgae products. *J. Anim. Physiol. Anim. Nutr.* **2018**, *102*, 1306–1319. [CrossRef]

24. Eilam, Y.; Khattib, H.; Pintel, N.; Avni, D. Microalgae-Sustainable Source for Alternative Proteins and Functional Ingredients Promoting Gut and Liver Health. *Glob. Chall* **2023**, *7*, 2200177. [CrossRef] [PubMed]

25. Lucakova, S.; Branyikova, I.; Hayes, M. Microalgal Proteins and Bioactives for Food, Feed, and Other Applications. *Appl. Sci.* **2022**, *12*, 4402. [CrossRef]

26. Amorim, M.L.; Soares, J.; Coimbra, J.; Leite, M.O.; Albino, L.F.T.; Martins, M.A. Microalgae proteins: Production, separation, isolation, quantification, and application in food and feed. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1976–2002. [CrossRef] [PubMed]

27. Lortou, U.; Panou, M.; Papapanagiotou, G.; Florokapi, G.; Giannakopoulos, C.; Kavoukis, S.; Iakovou, G.; Zalidis, G.; Triantafyllidis, K.; Gkelis, S. Beneath the Aegean Sun: Investigating *Dunaliella* Strains' Diversity from Greek Saltworks. *Water* **2023**, *15*, 1037. [CrossRef]

28. Global Beta Carotene Market—Segmented by Application (Pharmaceuticals, D.S., Food & Beverage, Animal Feed, Others), By Source (Synthetic, Algae, Fungi, Palm Oil, Others), & By Regional Analysis (North America, Europe, Asia Pacific, Latin America, and Middle East & Africa)—Global Industry Analysis, Size, Share, Growth, Trends, and Forecast (2023–2028). Available online: https://www.marketdataforecast.com/market-reports/beta-carotene-market (accessed on 5 January 2024).

29. Marcilla, A.; Catalá, L.; García-Quesada, J.C.; Valdés, F.J.; Hernández, M.R. A review of thermochemical conversion of microalgae. *Renew. Sustain. Energy Rev.* **2013**, *27*, 11–19. [CrossRef]

30. Yang, C.; Li, R.; Zhang, B.; Qiu, Q.; Wang, B.; Yang, H.; Ding, Y.; Wang, C. Pyrolysis of microalgae: A critical review. *Fuel Process. Technol.* **2019**, *186*, 53–72. [CrossRef]

31. Li, F.; Srivatsa, S.C.; Bhattacharya, S. A review on catalytic pyrolysis of microalgae to high-quality bio-oil with low oxygeneous and nitrogenous compounds. *Renew. Sustain. Energy Rev.* **2019**, *108*, 481–497. [CrossRef]

32. Liu, X.; Guo, Y.; Dasgupta, A.; He, H.; Xu, D.; Guan, Q. Algal bio-oil refinery: A review of heterogeneously catalyzed denitrogenation and demetallization reactions for renewable process. *Renew. Energy* **2022**, *183*, 627–650. [CrossRef]

33. Adamakis, I.D.; Lazaridis, P.A.; Terzopoulou, E.; Torofias, S.; Valari, M.; Kalaitzi, P.; Rousonikolos, V.; Gkoutzikostas, D.; Zouboulis, A.; Zalidis, G.; et al. Cultivation, characterization, and properties of Chlorella vulgaris microalgae with different lipid contents and effect on fast pyrolysis oil composition. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 23018–23032. [CrossRef]

34. Sharma, N.; Jaiswal, K.K.; Kumar, V.; Vlaskin, M.S.; Nanda, M.; Rautela, I.; Tomar, M.S.; Ahmad, W. Effect of catalyst and temperature on the quality and productivity of HTL bio-oil from microalgae: A review. *Renew. Energy* **2021**, *174*, 810–822. [CrossRef]

35. Arun, J.; Varshini, P.; Prithvinath, P.K.; Priyadarshini, V.; Gopinath, K.P. Enrichment of bio-oil after hydrothermal liquefaction (HTL) of microalgae *C. vulgaris* grown in wastewater: Bio-char and post HTL wastewater utilization studies. *Bioresour. Technol.* **2018**, *261*, 182–187. [CrossRef] [PubMed]

36. Wang, K.; Brown, R.C.; Homsy, S.; Martinez, L.; Sidhu, S.S. Fast pyrolysis of microalgae remnants in a fluidized bed reactor for bio-oil and biochar production. *Bioresour. Technol.* **2013**, *127*, 494–499. [CrossRef] [PubMed]

37. Kim, S.-S.; Ly, H.V.; Kim, J.; Lee, E.Y.; Woo, H.C. Pyrolysis of microalgae residual biomass derived from *Dunaliella tertiolecta* after lipid extraction and carbohydrate saccharification. *Chem. Eng. J.* **2015**, *263*, 194–199. [CrossRef]

38. Obeid, S.; Beaufils, N.; Peydecastaing, J.; Camy, S.; Takache, H.; Ismail, A.; Pontalier, P.-Y. Microalgal fractionation for lipids, pigments and protein recovery. *Process Biochem.* **2022**, *121*, 240–247. [CrossRef]

39. Gerde, J.A.; Wang, T.; Yao, L.; Jung, S.; Johnson, L.A.; Lamsal, B. Optimizing protein isolation from defatted and non-defatted Nannochloropsis microalgae biomass. *Algal Res.* **2013**, *2*, 145–153. [CrossRef]

40. Harun, R.; Danquah, M.K. Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochem.* **2011**, *46*, 304–309. [CrossRef]

41. Yang, Z.; Guo, R.; Xu, X.; Fan, X.; Luo, S. Fermentative hydrogen production from lipid-extracted microalgal biomass residues. *Appl. Energy* **2011**, *88*, 3468–3472. [CrossRef]

42. Naresh Kumar, A.; Min, B.; Venkata Mohan, S. Defatted algal biomass as feedstock for short chain carboxylic acids and biohydrogen production in the biorefinery format. *Bioresour. Technol.* **2018**, *269*, 408–416. [CrossRef] [PubMed]

43. Roy Chong, J.W.; Tan, X.; Khoo, K.S.; Ng, H.S.; Jonglertjunya, W.; Yew, G.Y.; Show, P.L. Microalgae-based bioplastics: Future solution towards mitigation of plastic wastes. *Environ. Res.* **2022**, *206*, 112620. [CrossRef]

44. Arora, Y.; Sharma, S.; Sharma, V. Microalgae in Bioplastic Production: A Comprehensive Review. *Arab. J. Sci. Eng.* **2023**, *48*, 7225–7241. [CrossRef]

45. Klingler, D.; Berg, J.; Vogel, H. Hydrothermal reactions of alanine and glycine in sub- and supercritical water. *J. Supercrit. Fluids* **2007**, *43*, 112–119. [CrossRef]

46. Martins, L.B.; Soares, J.; da Silveira, W.B.; Sousa, R.d.C.S.; Martins, M.A. Dilute sulfuric acid hydrolysis of *Chlorella vulgaris* biomass improves the multistage liquid-liquid extraction of lipids. *Biomass Convers. Biorefinery* **2021**, *11*, 2485–2497. [CrossRef]

47. Miranda, J.R.; Passarinho, P.C.; Gouveia, L. Pre-treatment optimization of Scenedesmus obliquus microalga for bioethanol production. *Bioresour. Technol.* **2012**, *104*, 342–348. [CrossRef] [PubMed]

48. Lazaridis, P.A.; Karakoulia, S.; Delimitis, A.; Coman, S.M.; Parvulescu, V.I.; Triantafyllidis, K.S. d-Glucose hydrogenation/hydrogenolysis reactions on noble metal (Ru, Pt)/activated carbon supported catalysts. *Catal. Today* **2015**, *257*, 281–290. [CrossRef]

49. Lazaridis, P.A.; Karakoulia, S.A.; Teodorescu, C.; Apostol, N.; Macovei, D.; Panteli, A.; Delimitis, A.; Coman, S.M.; Parvulescu, V.I.; Triantafyllidis, K.S. High hexitols selectivity in cellulose hydrolytic hydrogenation over platinum (Pt) vs. ruthenium (Ru) catalysts supported on micro/mesoporous carbon. *Appl. Catal. B Environ.* **2017**, *214*, 1–14. [CrossRef]

50. Safi, C.; Ursu, A.V.; Laroche, C.; Zebib, B.; Merah, O.; Pontalier, P.-Y.; Vaca-Garcia, C. Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods. *Algal Res.* **2014**, *3*, 61–65. [CrossRef]

51. *NREL/TP-5100-60956*; Determination of Total Solids and Ash in Algal Biomass. NREL: Golden, CO, USA, 2015.

52. *NREL/TP-5100-60957*; Determination of Total Carbohydrates in Algal Biomass. NREL: Golden, CO, USA, 2015.