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Potential Use of Plant Biomass from Treatment Wetland Systems for Producing Biofuels through a Biocrude Green-Biorefining Platform

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Abstract: The potential of using the biomass of four wetland plant species (*Iris pseudacorus*, *Juncus effusus*, *Phragmites australis* and *Typha latifolia*) grown in treatment wetland systems and under natural conditions were tested to produce high-value materials using hydro-thermal liquefaction (HTL). The results show that the wetland plants biomass is suitable for biocrude and biochar production regardless of the origin. The hydrothermal liquefaction products' (biocrude, biochar, aqueous and gaseous phase) yields vary according with the specific biomass composition of the species. Furthermore, the results show that the biomass composition can be affected by the growing condition (treatment wetland or natural unpolluted conditions) of the plants. None of the single components seems to have a determinant effect on the biocrude yields, which reached around 30% for all the analyzed plants. On the contrary, the biochar yields seem to be affected by the composition of the biomass, obtaining different yields for the different plant species, with biochar yields values from around 12% to 22%, being that *Phragmites australis* is the one with the highest average yield. The obtained aqueous phase from the different plant species produces homogeneous compounds for each plant species and each growing environment. The study shows that biomass from treatment wetlands is suitable for biocrude production. The environmental value of this biomass lies on the fact that it is considered a residual product with no aggregated value. The treatment wetland biomass is a potential sustainable source for biofuel production since these plants do not need extra land or nutrients for growing, and the biomass does not compete with other uses, offering new sources for enhancing the bioeconomy concepts.

Keywords: treatment wetlands; biocrude; hydrothermal liquefaction; biomass; biorefinery; biofuels; wastewater treatment; biochar; aqueous phase

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1. Introduction

Currently, the world faces diverse and serious environmental challenges. The transport sector, responsible for a significant share of CO_2 emissions, and other sectors such as the agriculture, forestry, or manufacturing ones, demand for sustainable, resilient, affordable, and fair sources of energy that can ease the pressure exerted to nature.

To face those challenges, the bioeconomy model proposes the use of renewable biological resources from the land and sea (e.g., animals, crops, fish, forests and microorganisms) to produce energy, food and materials [1] to reduce the environmental impact of human activity.

In this context, the combined use of green biorefining (GBR) technologies and nature-based solutions (NBS) brings the opportunity of controlling pollution as well as recovering neglected biological resources for use as a sustainable source of biomass for biofuel and later bioenergy production. GBR is a complex and full-integrated technology system that aims to protect the environment and natural resources, making comprehensive use of materials and energy available in green biomasses [2]. NBS are technologies focused in establishing ecosystem services to address societal challenges, such as climate change, food and water security, to tackle natural disasters, and to enhance well-being and biodiversity benefits [3].

Treatment wetlands (TW) are one of the most attractive NBS for wastewater treatment; TW technology mimics processes present in nature, but optimizes them through engineering designs [4]. TW are systems where wastewater, local climate, and energy (mainly coming from the sun) are integrated into an engineered ecosystem formed by plants, bacteria, and depending on the type of TW, filling media to remove pollutants from waters aiming at meeting local discharge standards. The technology is convenient due to the relatively low operative and maintenance cost [5,6], and other ancillary benefits, such as sustaining biodiversity [7], climate change mitigation [8], carbon sequestration [9], hydrological flow regime regulation, public use, education, and habitat conservation [10].

Until now, TW were considered a sustainable technology for wastewater treatment effective for pollution control of domestic wastewater [11–14], rainwater run-off [15–17], slaughterhouses [18,19], industrial [20–22] and urban sewage [8,23], and many other types of polluted waters. However, TW seem to offer a novel and sustainable opportunity to recover neglected resources coming from the produced biomass which can be used to produce plant-based materials and fuels.

To use the TW biomass is attractive because of (1) the high primary productivity reported for plants grown in CW [24], (2) the enhanced capacity for removing pollutants reported from harvested TW [25,26], and (3) the promising results from previous studies regarding the potential for producing high-value products from residual biomass through green biorefining (GBR) processes [2,27,28].

GBR technologies are based on the utilization of plant or other organic materials to produce different high-value materials that can substitute the petroleum-based materials such as plastics, fuels, textiles, and chemicals [29,30]. One of the most promising GBR processes seems to be hydrothermal liquefaction (HTL) technology, which produces biocrude. The HTL process transforms biomass, using high pressure and high temperature under subcritical conditions to obtain a combination of biocrude that can produce biofuel, biochar, gases (mainly CO_2), and an aqueous phase (AqP), rich in organic compounds. The hydrothermal process is essentially similar to how nature has produced fossil fuels over millions of years, heating aqueous organic slurries at elevated pressures to produce an energy carrier with increased energy density [31].

The biomass composition used for HTL processes determines the composition and the yields of the products of the reaction, named biocrude, biochar and AqP. In previous studies, microalgae (*Chlorella*) slurry was processed, obtaining 40 wt% biocrude yields [32]. *Miscanthus*, *Spirulina* and sewage sludge produced biocrudes with average yields of 26 wt%, 33 wt% and 25 wt%, respectively [33]. Maize, oats, and ryegrass had yields of 27.6 wt%, 25.1 wt%, and 22 wt%, respectively [34]. These observations confirm the fact that the predictability of biocrude characteristics is still a topic that needs attention [35].

The HTL process was previously evaluated for the valorization of wastewater-treatment wastes, offering an alternative solution for sludge management. Sludge has been used for biocrude production, reporting yields from 37 wt% to 43 wt% [36]. Other studies have evaluated the biocrude production from willow biomass irrigated with wastewaters at supercritical water conditions (400 °C), reporting a yield of 40 wt% [37].

However, none of the previous studies have evaluated the differences in the capacity of the TW plants for producing biocrude compared with the same plants grown in natural conditions (NC). This study aims to assess the potential of four of the most used plants in

TW for producing biocrude through the HTL technology. The study includes a comparison between the biocrude and biochar yields, as well as the AqP composition, to evaluate how the TW conditions affect the potential of the plant biomass to be used in a HTL platform. The energy balance from the biocrude and biochar is also determined to estimate the potential to produce environmentally friendly renewable biofuels. Additionally, this research evaluates the AqP composition, including a screening of the content of emergent pollutants after the HTL reaction, highlighting the potential environmental problems that can be faced due the complex composition of it. Lastly, a mass balance study is performed to track the fate of carbon (C) and nitrogen (N) in the HTL process, to assess where in the environment those elements could potentially end up. When the C and N of the feed biomass are converted to biocrude, biochar, AqP or a gaseous state, they can later either be emitted to the atmosphere as a gas if the biochar or biocrude are used as fuels, be discharged in the water if the AqP is not used in any other process, or be added to the soil if the biochar is used as a soil amendment acting also as a carbon sequestrator [38–40].

This article is a micro-approach to the development of biofuels from raw materials. The study assesses the potential of TW biomass to be considered as a source of new, renewable biomass to produce biofuels. The biomass from TW is considered sustainable because it reduces the environmental impact, being grown in sustainably way (in the TW), being considered until now a residue, and having not conflicted with land use for agricultural products [1]. TW biomass is considered a by-product with no or low value, but due to the operational characteristics, is an unlimited source of biomass. Up to our knowledge, this approach has not been studied. The combined use of TW for wastewater treatment and biorefinery processes to produce biofuels from the TW biomass can contribute to reaching the UN sustainable goals dealing with the water management and the recovering of resource in a circular bioeconomy. The use of biomass that otherwise would be disposed of can contribute to reaching some of the UN goals as follows: "6, clean water and sanitization", "7, Affordable and clean energy", "11, Sustainable cities and communities", "12, Responsible consumption and production", and "13, climate action." [41].

2. Materials and Methods

2.1. The Plants

During the summer of 2020, four plant species, from two different locations, were selected and harvested to compare their potential for being used in the HTL process. Triplicates of individual samples of *Iris pseudacorus*, *Juncus effusus*, *Phragmites australis*, and *Typha latifolia* were harvested from two different locations. The first place was a local TW system located in the Tilst neighborhood in Aarhus, Denmark, (56°10′20.5″ N, 10°06′21.9″ E). The same plant species were harvested during the same period from Påskehøjgaard growth facilities of Aarhus University (56°13′48.7″ N, 10°07′38.3″ E), where the plants were grown under natural conditions (not exposed to pollutants), referred to herein as NC (natural conditions).

2.2. Ash Content and Fixed Carbon

The plants were dried at 65 °C for 72 h to drive off water. Samples of the residue were cooled, weighed, and combusted at 550 °C for 3 h to drive off volatile solids. The total solids, volatile solids, and ash content were determined by comparing the mass of the sample before and after each step. The method followed was that of Ref. [42].

A TGA Mettler Toledo SDTA851 was used to analyze the raw materials and biochar samples. The TGA was operated using a constant heating rate of 10 K min^{-1} from 50 °C to 900 °C under nitrogen followed by 10 min under air at constant temperature. A minimum of 5 mg of the mass sample was placed in the TGA ceramic crucibles. The fixed carbon was obtained by calculating the mass difference in the sample, between the weight at the air injection and the final weight in the test.

2.3. Elemental Analysis

For the CHNS content, the solid and biocrude samples were determined using an Elementar vario Macro Cube elemental analyzer (Langenselbold, Germany). The protein content was estimated using a nitrogen Jone's factor of 6.25 [43].

The higher heating value (HHV) was calculated according to the the Channiwala–Parikh [44] correlation

$$\mathrm{HHV}\left[\frac{\mathrm{MJ}}{\mathrm{kg}}\right] = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151N - 0.0211A \quad (1)$$

The equation used to calculate the energy recovery of the biocrude, and biochar was:

$$\text{Energy yield } (\eta_{th}) = \frac{\mathrm{HHV_{oil}}\left[\frac{\mathrm{MJ}}{\mathrm{kg_{oil}}}\right]\cdot \mathrm{yield_{oil}}\left[\frac{\mathrm{kg_{oil}}}{\mathrm{kg_{feed}}}\right]}{\mathrm{HHV_{feed}}\left[\frac{\mathrm{MJ}}{\mathrm{kg_{feed}}}\right]} \times 100 \quad (2)$$

$$\text{Energy yield } (\eta_{th}) = \frac{\mathrm{HHV_{char}}\left[\frac{\mathrm{MJ}}{\mathrm{kg_{char}}}\right]\cdot \mathrm{yield_{char}}\left[\frac{\mathrm{kg_{char}}}{\mathrm{kg_{feed}}}\right]}{\mathrm{HHV_{feed}}\left[\frac{\mathrm{MJ}}{\mathrm{kg_{feed}}}\right]} \times 100 \quad (3)$$

2.4. Compositional Analysis

The structural carbohydrates and lignin content were determined according to [45]. The value of the hemicellulose is from the addition of the obtained values of xylan, arabinan, and galactan. The values of lignin were obtained by the addition of the Klason lignin and the soluble lignin content.

2.5. HTL Reaction

To perform hydrothermal liquefaction of the biomass, and determine the product yields of bio-crude, gas, solid residue (biochar), and water-soluble products (aqueous phase), the reactions were carried out at 340 °C for 15 min residence time in small bomb-type 20 mL batch reactors, and no catalyst was used. Biomass slurries were prepared by mixing 20 wt% and 80 wt% demineralized water. The feedstock consisted of the 8 biomasses described previously. Reactors were sealed and lowered into a preheated fluidized sand bath at 340 °C, then 20 min reaction time was applied. Subsequently, the reactor were cooled quickly to ambient temperature in a water bath. The reactors were vented, and the aqueous phase (AqP) was decanted into a centrifuge tube and centrifuged for 5 min before the AqP was transferred with a glass pipette to a preparative glass. The AqP was then stored at 5 °C for further analysis. The centrifuge tube was washed with 2 mL of dichloromethane and the reactor was extracted with around 4 mL of dichloromethane, which were combined. The dichloromethane phase was vacuum filtered, and the residue washed with dichloromethane until the filtrate appeared clear. Dichloromethane was evaporated under a stream of nitrogen until a constant weight. Each experiment was performed in triplicate and the average values are reported.

2.6. COD, TOC, and TN in Aqueous Phase

AqP samples were analyzed for chemical oxygen demand (COD) content, using Merck Spectroquant cell. The total organic carbon (TOC) and total nitrogen (TN) of the AqP samples were analyzed using a scalar FORMACS HT-I TOC/TN analyzer.

2.7. High-Resolution Mass Spectrometry (HRMS) Analysis of the Aqueous Phase

For a broader screening of the chemical composition of the AqP, a liquid chromatograph (LC) coupled to a 6600 quadrupole-time-of-flight (QTOF) instrument (SCIEX) was used. An Acquity UPLC BEH Shield RP18 1.7 µm column (2.1 mm × 30 mm) (Waters) was used for chromatographic separation. Eluents A and B were water and methanol, both with 0.1% formic acid, respectively. The chromatographic conditions, as well as detailed parameters of the instrument, were similar to those used before [46]. The samples were injected directly (10 µL) to the LC-QTOF and measured in a data-dependent acquisition mode (DDA), with a TOF mass range 100–1000 Da, 250 ms accumulation time, and successive fragmentation and recording of product ion spectra of the 10 highest peaks for 40 ms each. The samples were measured in positive and negative ionization modes with electrospray ionization (with ±5300 V ion spray voltage).

The high-resolution MS data were processed with Marker View (SCIEX) for the molecular feature extraction (feature is defined by exact m/z and retention time), according to [46]. Briefly, one feature was only considered if present in 3 out of the 3 injected replicate samples with a mass tolerance of 20 ppm and a retention time tolerance of 0.1 min. All features present in control blank samples were filtered.

2.8. Statistics

The differences of the analyzed parameter between the two different selected sites (TW and NC) were analyzed using an ANOVA test. Prior to statistical analysis, all data were tested for homogeneity of variance by Levene's test. For clarity, all data are presented as untransformed values. Post hoc Tukey HSD tests were applied to identify significant differences between samples. All statistical analyses were conducted in R studio at a significance level of 0.05 and all figures were prepared in GraphPad Prism 7.00.

For the AqP analysis, a principal component analysis was performed with no weighting and Pareto scaling to analyze the triplicate samples from the 4 plant species, both TW and NC.

3. Results

3.1. Characterization of Harvested Biomass

The characterization of the biomass is relevant since the composition and yield of HTL products depend strongly on the type and composition of the biomass used as feed [39]. A first characterization of the biomass, shown in Table 1, reports the content of cellulose, hemicellulose, lignin, protein, and ash found in the analyzed biomass. A second characterization of the biomass, shown in Table 2 shows the results of the elemental analysis of the feed biomass, reported in C, H, O, N, and S.

From Table 1, it is possible to observe that the cellulose content in the plants did not differ between the two growing environments, having no significative difference for the *Iris pseudacorus*, *Phragmites australis* and *Typha latifolia*, and being lower for around 4.5% for the *Juncus effusus*, grown in TW. The hemicellulose content had a lower concentration in the *Phragmites australis* and *Typha latifolia*, being lower for around 6.6% and 5% respectively. Regarding lignin content, the plants showed significative differences between both growing environments, having a lower value in around 0.3% for *Iris pseudacorus* and *Juncus effusus*, grown in TW, and higher concentration in around 0.3% for *Phragmites australis* and *Typha latifolia* grown in TW. The protein content was significantly higher for all the plant species grown in TW, with differences between 0.5% and 1.4%. Lastly, the ash content was also higher for all the plant species grown in TW conditions, reporting differences of 1.6, 1.3, 0.3, and 2.7% for the *Iris pseudacorus*, *Juncus effusus*, *Phragmites australis* and *Typha latifolia*, respectively.

Regarding the elemental analysis of the biomass, only the N content was higher for all the TW plants. The rest of the elements in the plants did not present significant differences or showed lower concentration in the plants grown in TW. For *Juncus effusus*, *Phragmites australis*, and *Typha latifolia*, the C content was lower in the TW plants. For *Phragmites australis* and *Typha latifolia*, the H content was lower in the TW, and the O was lower in the TW for *Iris pseudacorus*, being that the S in the *Iris pseudacorus* was the only element and plant that showed a higher content for the TW.

This confirms that the composition of the plants grown in TW is not negatively affected due to the presence of wastewater, comparable to other plants grown as a biological source for the production of biofuels grown under natural conditions.

Table 1. Cellulose, hemicellulose, lignin, and protein content in the selected plants for the different growing environments.

	Plant Description	Cellulose (%)			Hemicellulose (%)			Lignin (%)			Protein (%)			Ash (%)		
		TW		NC	TW		NC	TW		NC	TW		NC	TW		NC
1	*Iris pseudacorus*	37.6 ± 2.6	=	38.9 ± 0.4	17.8 ± 4.5	=	14.6 ± 1.7	5.6 ± 0.4	↓	5.8 ± 0.6	4.8 ± 0.5	↑	3.5 ± 0.3	8.6 ± 0.0	↑	7.0 ± 0.0
2	*Juncus effusus*	36.9 ± 2.6	↓	41.5 ± 4.9	32.8 ± 2.1	=	32.3 ± 1.0	5.8 ± 0.6	↓	6.2 ± 0.7	6.7 ± 0.7	↑	4.5 ± 0.3	5.0 ± 0.2	↑	3.7 ± 0.0
3	*Phragmites australis*	35.0 ± 4.3	=	37.2 ± 1.1	21.0 ± 4.6	↓	27.6 ± 2.9	5.9 ± 0.5	↑	5.6 ± 1.0	7.2 ± 0.2	↑	6.4 ± 0.4	5.0 ± 0.0	↑	4.7 ± 0.0
4	*Typha latifolia*	41.4 ± 4.0	=	37.9 ± 3.8	17.2 ± 4.7	↓	22.2 ± 5.3	7.1 ± 2.4	↑	5.8 ± 1.9	5.8 ± 0.6	↑	4.7 ± 0.2	8.5 ± 0.1	↑	5.8 ± 0.1

↑ Indicates a positive effect of the TW in the concentration of the reported element. ↓ Indicates a negative effect of the TW in the concentration of the reported element. = Indicates a not significative effect of the TW in the concentration of the reported element.

Table 2. C, H, O, N, S content in the selected plants for the different growing environments.

	Plant Description	C [%]			H [%]			O [%]			N [%]			S [%]		
		TW		NC	TW		NC	TW		NC	TW		NC	TW		NC
1	*Iris pseudacorus*	42.4 ± 0.2	=	41.4 ± 0.1	6.4 ± 0.1	=	6.5 ± 0.0	41.8 ± 0.2	↓	44.6 ± 0.2	0.7 ± 0.0	↑	0.5 ± 0.0	0.2 ± 0.1	↑	0.0 ± 0.0
2	*Juncus effusus*	43.9 ± 0.1	↓	45.5 ± 0.2	6.5 ± 0.1	=	6.7 ± 0.1	43.4 ± 0.3	=	43.4 ± 0.2	1.1 ± 0.1	↑	0.7 ± 0.0	0.1 ± 0.0	=	0.1 ± 0.0
3	*Phragmites australis*	44.9 ± 0.0	↓	46.1 ± 0.1	6.4 ± 0.1	↓	6.7 ± 0.0	42.4 ± 0.0	=	41.2 ± 0.1	1.2 ± 0.0	↑	1.1 ± 0.0	0.2 ± 0.1	=	0.2 ± 0.0
4	*Typha latifolia*	41.9 ± 0.2	↓	45.4 ± 3.5	6.1 ± 0.1	↓	6.6 ± 0.5	42.6 ± 0.2	=	41.4 ± 4.2	0.9 ± 0.0	↑	0.8 ± 0.0	0.1 ± 0.0	=	0.0 ± 0.0

↑ Indicates a positive effect of the TW in the concentration of the reported element. ↓ Indicates a negative effect of the TW in the concentration of the reported element. = Indicates a not significative effect of the TW in the concentration of the reported element.

3.2. HTL Yields

Table 3 presents the biocrude yields obtained from the different reactions and biomass. Biocrude yields presented a significative difference only for the *Typha latifolia*, which reported a lower biocrude yield (−6%) in the TW plants. For the biochar, the differences were significant for almost all the plant species, decreasing the yields in the case of *Iris pseudacorus* and *Juncus effusus*, and increasing it for the *Phragmites australis*, grown in TW, without any difference for *Typha latifolia*. The results suggest that the yields of biochar are more sensible than the yields of biocrude to the plant species and composition.

Table 3. Fraction yields for all the selected plant for the different growing environments after HTL reaction.

Plant Description		Biocrude (%)			Biochar (%)			AqP (%)		Gas (%)	
		TW		NC	TW		NC	TW	NC	TW	NC
1	*Iris pseudacorus*	25.6 ±1.2	=	29.6 ± 6.5	12.4 ± 2.4	↓	22.0 ± 3.3	16.5 ± 8.4	16.7 ± 3.5	26.6 ± 3.7	32.0 ± 5.8
2	*Juncus effusus*	31.7 ± 2.6	=	33.9 ± 3.1	14.5 ± 0.7	↓	17.4 ± 1.4	31.2 ± 18.7	22.7 ± 18.8	22.6 ± 18.4	27.1 ± 13.7
3	*Phragmites australis*	26.3 ± 6.2	=	28.6 ± 0.5	23.3 ± 1.7	↑	19.3 ± 3.0	21.6 ± 6.4	20.8 ± 6.2	26.8 ± 3.1	31.4 ± 5.4
4	*Typha latifolia*	31.6 ± 8.9	↓	37.6 ± 7.9	18.7 ± 0.2	=	19.3 ± 6.2	20.1 ± 6.5	15.5 ± 6.9	30.3 ± 5.1	47.9 ± 7.7

↑ Indicates a positive effect of the TW in the concentration of the reported element. ↓ Indicates a negative effect of the TW in the concentration of the reported element. = Indicates a not significative effect of the TW in the concentration of the reported element.

In general, it is expected that the biocrude formation follows the trend lipids/fats > proteins > carbohydrates [40]; nonetheless, it is possible to observe from the results of this study that this trend does not fit with the results obtained from the lignocellulosic analyzed biomass. From Figure 1, it is possible to observe that the biomass with the highest content of protein is not the ones with the highest biocrude or biochar yields, e.g., meanwhile, the plant 3A (*Phragmites australis* grown in TW) is the one with the highest content of protein, the biocrude yield is one of the lowest, and the biochar yield one of the highest. It is important to highlight that the differences in protein content in the biomass, do not represent an increase larger than 3.5% between the protein content of the plant with the highest content and the lowest. Additionally, the highest protein content of all species is 7%, meaning that protein has a relatively small contribution to the overall bio-crude yield; hence, the slight change in protein contents amongst samples does not follow the general trend of biocrude lipids/fats > proteins > carbohydrates.

It was described that the C5 and C6 carbohydrates (from cellulose and hemicellulose) tend to produce mainly biochar in the HTL reactions [31], but according with the obtained results, the plants with the highest content of cellulose (plant 2B and 4A) did not report the highest yield of biochar. In other case, the plant with the highest biochar yield (3A) reported the highest content of protein but the lowest content of cellulose.

From the particular plant analysis, it is not possible to observe any pattern, e.g., in the case of *Phragmites australis*, which reported a significative lower concentration of hemicellulose in the TW plants, the biochar yield was significantly higher, and for *Typha latifolia*, even the lower concentration of hemicellulose in the TW plants, the biochar yield did not show significative differences.

Nevertheless, the results do not contradict the results from other authors since the feed biomass is not a uniform mass with a completely known content. On the contrary, lignocellulosic biomasses are complex systems, with some unknown components, and even though is possible to know the generality of them, when the biomass is forced to react under HTL conditions, all the present elements have a role in the reaction and interfere with the final biocrude and biochar yields and composition.

The interaction in the HTL between the protein, the saccharide and the lignin were studied previously. A study mixed soya with cellulose, xylan, and alkaline lignin to model the interactions of the protein with the other elements, reaching models with an accuracy of around 94% [47]. Some others have studied synergistic effects between the lignocellulosic biomass compounds, reporting that mixtures of protein and cellulose, protein and xylose, cellulose and lignin, and xylose and lignin seem to have synergistic effects on biocrude yield, and mixtures of soybean oil and lignin showed an antagonistic effect [48]. The results seem to confirm the synergistic effect between the protein and cellulose, lignin, and hemicellulose. Figure 1 shows that plants with the higher lignin and protein and low ashes, such as 2A and 2B, report biocrude yields over 30%.

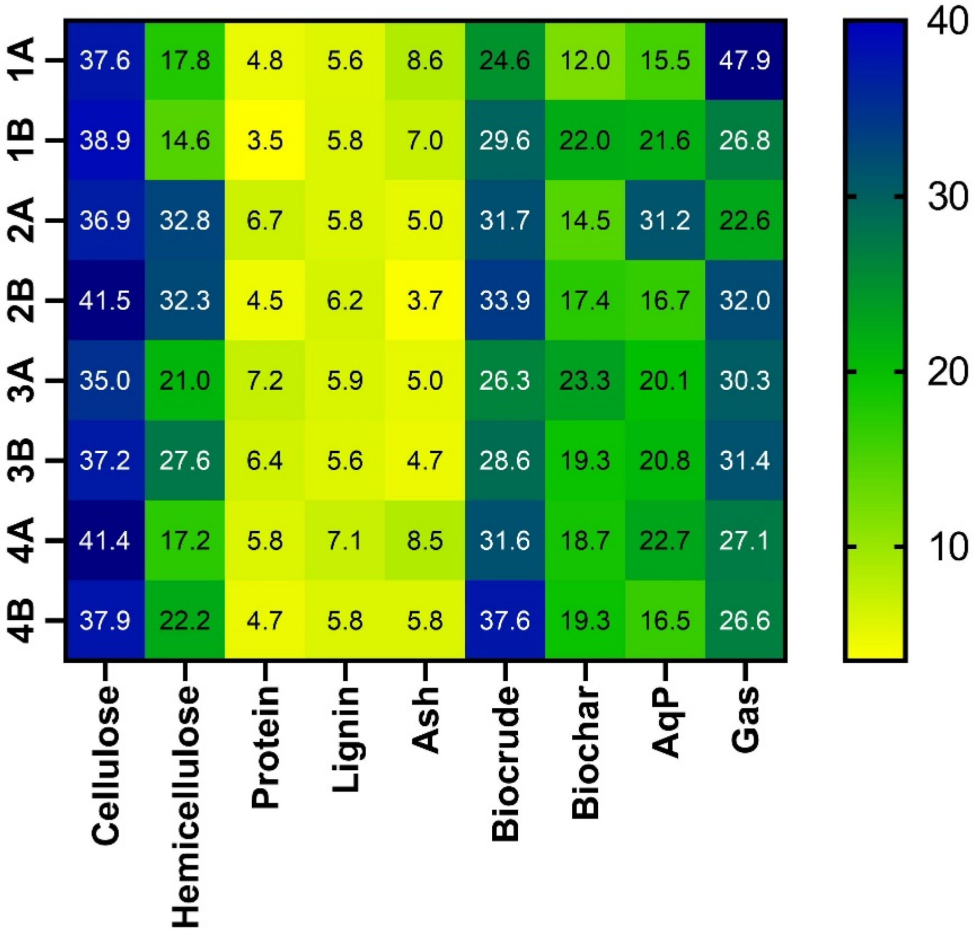

Figure 1. Heat map comparing feed composition and HTL fractions yields. The values in each square are the mean content of each parameter named in the columns. The content is reported in mass fraction (%). In the rows, the numbers refer to each plant species named: 1, *Iris pseudacorus*; 2, *Juncus effusus*; 3, *Phragmites australis*; and 4, *Typha latifolia*. The letters A and B refer to the growing environments: A = TW, and B = NC environment.

The predictably of the HTL fraction yields still needs to be studied further. This study can elucidate how the lignocellulosic biomass, even with no significative differences regarding its composition, can produce different yields. Nonetheless, and despite the differences, the results are consistent with previous studies regarding biocrude, and biochar yields produced from lignocellulosic biomass. The yields, in all the cases, range from 25.6 $\pm$ 1.2% to 37.6 $\pm$ 7.9% for biocrude, and from 12.4 $\pm$ 2.4% to 23.3 $\pm$ 1.7% for the biochar, which correspond with previous studies about cellulosic biomass used in the HTL reaction, such as biocrude yields of 26% obtained from *Miscanthus* [33], 22% reported for ryegrass [34], or the yield of 39.7% reported for willow biomass at supercritical water conditions (400 °C) [37].

These results are important because they provide new information from the simultaneous evaluation of different plant species of different composition for producing biocrude and the different HTL subproducts (biochar, AqP, and gases), showing that even with plants with different composition, the biocrude yields are consistent and comparable. However, the results also indicate that the predictions about biocrude yields can be more effective if they are made based on the plant species more than the specific composition. There is no significative difference between the biocrude yield from plants of the same species grown in TW or NC, but a difference between plant species was found, being that *Juncus effusus* and *Typha latifolia* are the most attractive plants for the production of biocrude.

3.3. Characterization of the HTL Products

3.3.1. Biocrude and Biochar

From Tables 4 and 5 is observed that the N, C, and S content in the plants is densified in the biocrude and biochar, the H is densified only in the biocrude, and O decreases its density in both, biocrude and biochar, this pattern being consistent with previous studies. The N content in the produced biocrude from TW plants seems to have a lower concentration than biocrudes previously studied, e.g., biocrude obtained from *Miscanthus* [33] reported N content from 1.0% to 1.6%, which is a higher concentration than that obtained in this study, from 0.5% to 1.2%. Additionally, the O reported for the biocrude in this study is also higher than the 17.7% reported for the biocrude obtained from *Miscanthus* in the same reference. These differences in the elemental analysis influence the HHV of the biocrudes, and together with the product yield can define the global value or potential of the studied biocrudes.

Table 4. C, H, O, N, and S content in the biocrude produced by the selected plants for the different growing environments.

	Plant Description	C [%]		H [%]		O [%]		N [%]		S [%]	
		TW	NC	TW	NC	TW	NC	TW	NC	TW	NC
1	*Iris pseudacorus*	66.9 ± 11.4	58.3 ± 24.0	7.37 ± 0.6	9.4 ± 3.1	22.9 ± 12.0	30.8 ± 27.8	2.6 ± 0.0	1.5 ± 0.6	0.3 ± 0.0	0.1 ± 0.1
2	*Juncus effusus*	61.4 ± 3.8	49.1 ± 27.4	8.61 ± 0.5	5.1 ± 2.8	27.6 ± 3.9	44.0 ± 31.2	1.6 ± 0.1	1.6 ± 0.9	0.8 ± 0.4	0.2 ± 0.2
3	*Phragmites australis*	69.4 ± 4.3	63.2 ± 6.2	8.47 ± 0.9	7.6 ± 0.7	18.7 ± 4.2	26.9 ± 6.7	3.2 ± 0.1	2.1 ± 0.4	0.3 ± 0.2	0.2 ± 0.0
4	*Typha latifolia*	64.9 ± 5.3	63.5 ± 10.9	8.38 ± 0.4	8.4 ± 0.7	24.0 ± 6.2	26.3 ± 12.1	2.5 ± 0.9	1.6 ± 0.5	0.2 ± 0.1	0.2 ± 0.1

The results offer new information regarding the distribution of elements in the different plant species used in TW. The higher amount of N from the TW plants results in higher concentration of N in the resultant biocrude and the biochar. This is relevant for future studies as well as for the refining of the biocrude since excess N can affect the quality and can potentially generate N emissions.

3.3.2. Aqueous Phase

The elements that constitute this HTL fraction are multiple and with an undefined number of chemicals. In this study, a first approach to the chemical content of the AqP was done using referential parameters as TN, TOC, IC, and COD to determine total nitrogen, the organic, and inorganic carbon content, and the COD as a reference of the polluting potential of the AqP. Then, a second evaluation was performed, using LC-HRMS analysis to compare how different or similar the different samples were, regarding the chemical content, using a full in-depth analysis of the full dataset and characterization of the features considered outside the scope of the present work.

Table 6 shows the obtained values for the COD, TOC, TN, and IC in the AqP obtained from each plant and site. It is possible to observe that IC was not present in any of the cases, being that organic carbon was the only kind of detected carbon. The highest value for COD was reported for *Iris pseudacorus* grown in NC (69 g/L) and the lowest was obtained for *Iris pseudacorus* grown in TW (47 g/L). The highest TOC was obtained for *Phragmites australis* grown in TW (21 g/L), and the lowest was reported for *Juncus effusus* grown in TW (17.4 g/L). The highest TN concentration was obtained for *Iris pseudacorus* grown in TW (1.1 g/L), and the lowest was reported for *Phragmites australis* grown in NC (0.7 g/L).

Table 5. C, H, O, N, S and ash content in the biochar produced by the selected plants for the different growing environments.

	Plant Description	C [%]		H [%]		O [%]		N [%]		S [%]		Ash [%]	
		TW	NC	TW	NC	TW	NC	TW	NC	TW	NC	TW	NC
1	*Iris pseudacorus*	61.9 ± 1.6	63.8 ± 1.4	4.4 ± 0.2	4.7 ± 0.2	11.0 ± 5.5	21.3 ± 0.6	2.7 ± 0.2	1.8 ± 0.1	1.6 ± 0.2	0.2 ± 0.1	17.8 ± 3.5	11.9 ± 2.4
2	*Juncus effusus*	70.4 ± 0.1	70.5 ± 0.1	4.6 ± 0.2	4.5 ± 0.1	13.0 ± 0.4	13.3 ± 0.6	2.2 ± 0.1	2.7 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	8.2 ± 1.0	11.1 ± 6.2
3	*Phragmites australis*	67.7 ± 1.2	70.5 ± 1.2	4.6 ± 0.1	4.7 ± 0.4	15.6 ± 1.4	21.6 ± 1.3	3.6 ± 0.1	2.8 ± 0.4	0.2 ± 0.0	0.4 ± 0.1	9.5 ± 0.9	7.5 ± 0.1
4	*Typha latifolia*	69.0 ± 1.1	69.0 ± 1.5	4.6 ± 0.2	4.9 ± 0.3	23.8 ± 1.6	24.0 ± 1.4	2.3 ± 0.5	1.9 ± 0.6	0.2 ± 0.1	0.2 ± 0.1	8.3 ± 0.4	7.7 ± 1.8

Table 6. Pollutant analysis in the AqP produced after the HTL by the selected plants for the different growing environments.

	Plant Description	COD (g/L)			TOC (g/L)			TN (g/L)			IC (g/L)	
		TW		NC	TW		NC	TW		NC	TW	NC
1	*Iris pseudacorus*	47.0 ± 4	↓	69.0 ± 3	18.8 ± 0	↑	17.6 ± 1	1.1 ± 0.1	=	1.0 ± 0.1	BLD	BLD
2	*Juncus effusus*	57.0 ± 2	↑	49.5 ± 2	17.4 ± 1	↓	19.4 ± 1	0.9 ± 0.1	↓	1.1 ± 0.4	BLD	BLD
3	*Phragmites australis*	54,5 ± 2	↑	48.7 ± 4	21.0 ± 2	↑	18.1 ± 0	1.3 ± 0.8	↑	0.7 ± 0.0	BLD	BLD
4	*Typha latifolia*	57.7 ± 2	=	55.2 ± 7	18.3 ± 1	↑	17.5 ± 1	1.3 ± 0.2	↑	0.9 ± 0.4	BLD	BLD

↑ indicates a positive effect of the TW in the concentration of the reported element. ↓ Indicates a negative effect of the TW in the concentration of the reported element. = Indicates a not significative effect of the TW in the concentration of the reported element. BLD: below limit of detection.

It is possible to observe from all the comparisons a high heterogeneity between all the cases. The COD concentration for *Typha latifolia* did not show a significative difference between the TW and NC environments. On the contrary, the COD concentration for *Juncus effusus* and *Phragmites australis* was higher in the TW with a corresponding negative effect on the water quality and eventually on the environment. Lastly, it was observed for *Iris pseudacorus*, and *Salix viminalis* a lower COD concentration in the TW AqP compared with the concentration of the NC one.

The statistical analysis shows significative differences between all the cases regarding TOC concentration. Only two comparisons, 1B–4B and 2A–4B, did not show a significative difference, observing no tendency in the TOC concentrations.

The high values of TOC, TN, and COD in the AqP are important and demand special attention since the reported values are high when compared with regular wastewater. For example, while the AqP of the present experiment reports COD values between 47 g/L and 69 g/L, the municipal wastewater COD is in the range of 0.3 g/L to 1 g/L, and after biological treatment, the COD drops to 0.02–1.0 g/L [49]. It means that the concentration of pollutants in the AqP is up to 70 times higher than regular wastewater. The AqP results with transformation products of aromatic and other organic compounds. The study presents an initial screening of these compounds to the composition of this AqP, showing the relation between the plants used, and the compounds that are present in the liquid. The AqP should receive special attention since is the phase that is the least known regarding the compounds that constitute it, which can be evaluated for further use.

3.3.3. LC-HRMS Analysis of the Aqueous Phase

The complexity of the LC-QTOF-MS dataset was reduced by the application of a PCA analysis shown in Figure 2 and Supplementary Materials. The PCA results showed that the three replicates of each sample type are in general grouped, stressing the reproducibility of the different processed reactions in the HTL. The major differences between samples are due to plant species (e.g., 1 *Iris pseudacorus* vs. 3 *Phragmites australis*), meaning that the type of biomass fed in the HTL reactors produced an aqueous phase of different chemical composition. The *Iris pseudacorus* (1) samples seemed more different than all others, while those from *Typha latifolia* (4), as well as *Juncus effusus* (2) and *Phragmites australis* (3), tended to be more similar. Moreover, it is also clear that for the same plant type, plants sourced in treatment wetlands generated a different aqueous phase than that from natural systems (e.g., 1A vs. 1B or 3A vs. 3B). These difference between TW and NC is especially marked for *Iris pseudacorus* (1), *Juncus effusus* (2) and *Phragmites australis* (3).

The high heterogeneity in the AqP points to the fact that each plant species, depending on the growing environment, have the potential to produce certain components in the AqP. This is confirmed by the LC-HRMS analysis, which shows that not only the type of plant, but also the growing environment conditions the composition of the AqP.

This characterization is useful also because HTL could have the potential to treat biomass polluted with a specific kind of persistent components. It would be interesting for further studies to evaluate how the HTL process deals with biomass that have already uptaken persistent pollutants. Previous studies have evaluated the potential of HTL to degrade pesticides and pharmaceutical compounds, due to the reactions at supercritical conditions [50] and this kind of component being present in treatment sludges [44] or in wetland biomass [45], with promising results.

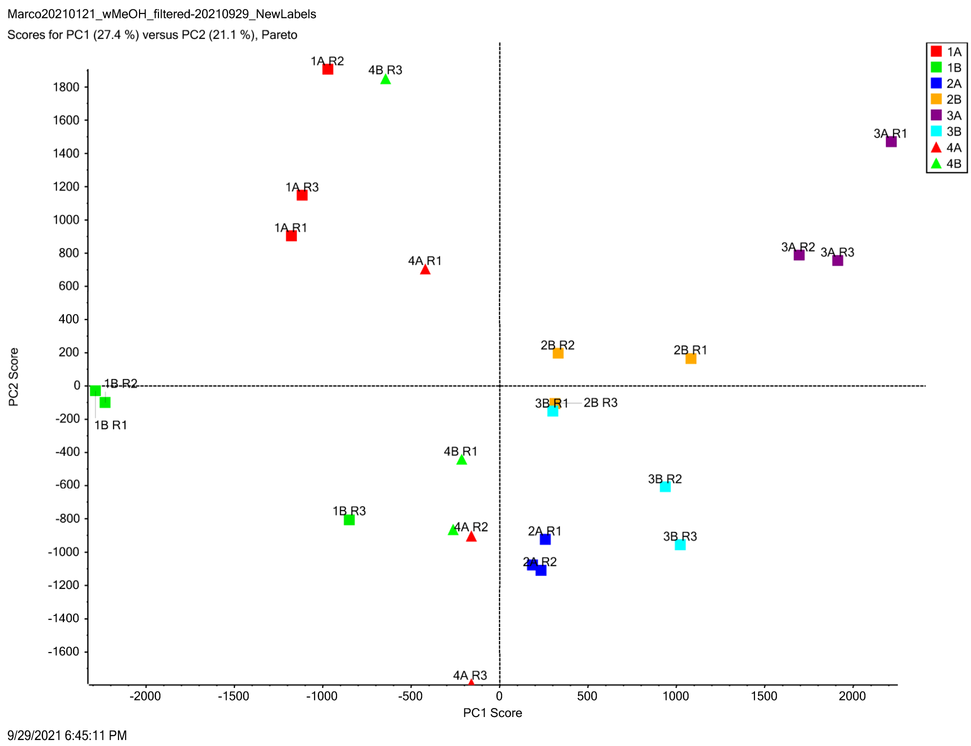

Figure 2. PCA plot from the LC-QTOF-MS dataset.

3.4. Energy Balance

The relation between the different components in the biomass, named C, H, N, S, O, and ash, determine the HHV of the analyzed biomass, and the relation between these compounds and the obtained yields of the different HTL fractions determine their energy yields (η_{th}). The energy yield, then, is a reference parameter about the potential of a feedstock to be used for producing biocrude and biochar through HTL reactions. From the obtained results (shown in Table 7), it is possible to observe that there is no significant difference between the biocrude energy yields for the same species grown in the two different environments, TW and NC. Conversely, the biochar energy yield seems to be more sensible to the biomass composition, it being possible to observe that two of the TW plants, *Iris pseudacorus* and *Phragmites australis*, produced biochar with a significative difference of energy yield compared with the plants grown in NC. However, these differences point to two different directions: while the biochar obtained from *Iris pseudacorus* in the TW had a lower energy yield, the one obtained from *Phragmites australis* produced a higher one.

Table 7. Calculated HHV for the feed biomass, biocrude and biochar for the selected plants and the different growing environments.

Plant Description		Feed Biomass		Biocrude		Biochar		Biocrude Energy Yield			Biochar Energy Yield		
		HHV (MJ/kg)		HHV (MJ/kg)		HHV (MJ/kg)		η_{th}			η_{th}		
		TW	TW	TW	TW	TW	NC	TW		NC	TW		NC
1	*Iris pseudacorus*	17.8 ± 0.1	17.3 ± 0.1	29.7 ± 5.9	28.2 ± 14.9	25.4 ± 1.2	25.3 ± 0.6	42.8 ± 10.0	=	33.4 ± 7.4	17.9 ± 4.4	↓	32.2 ± 5.3
2	*Juncus effusus*	18.3 ± 0.2	19.2 ± 0.1	28.8 ± 2.2	27.8 ± 2.9	28.5 ± 0.5	28.4 ± 0.2	49.7 ± 3.4	=	49.1 ± 4.5	22.4 ± 1.5	=	25.8 ± 2.1
3	*Phragmites australis*	18.8 ± 0.1	19.7 ± 0.1	32.3 ± 1.7	28.3 ± 3.0	27.2 ± 0.6	27.5 ± 1.3	44.9 ± 8.6	=	41.1 ± 4.8	33.8 ± 3.0	↑	27.0 ± 5.1
4	*Typha latifolia*	17.2 ± 0.2	19.2 ± 2.3	30.1 ± 3.0	29.4 ± 5.8	26.9 ± 0.7	27.1 ± 1.0	42.8 ± 10.0	=	33.4 ± 7.4	29.2 ± 1.0	=	27.9 ± 11.5

↑ indicates a positive effect of the TW in the concentration of the reported element. ↓ indicates a negative effect of the TW in the concentration of the reported element. = indicates a not significative effect of the TW in the concentration of the reported element.

Figure 3 shows that since the HHV is more homogeneous for all the studied plants, the element which generates the difference in the energy yields is the biocrude or biochar yield. It points to the fact that the combination between all the big chemical structures in the biomass, named structural sugars, lignin, and protein, is more relevant for the global potential of a feedstock than the molecular composition (amount of C, H, O, N, and S). It can be a direct consequence of the chemical reactions that take place in the HTL process because the elements do not interact directly but are associated with different molecules, which dictate the chemical pathway. Additionally, the high molecular weight of the organic compounds contains a lot of energy because of the many chemical bonds between the elements, particularly carbon, in the molecules, where other elements, such as N and S, are more loosely bound, which may even occur in inorganic forms.

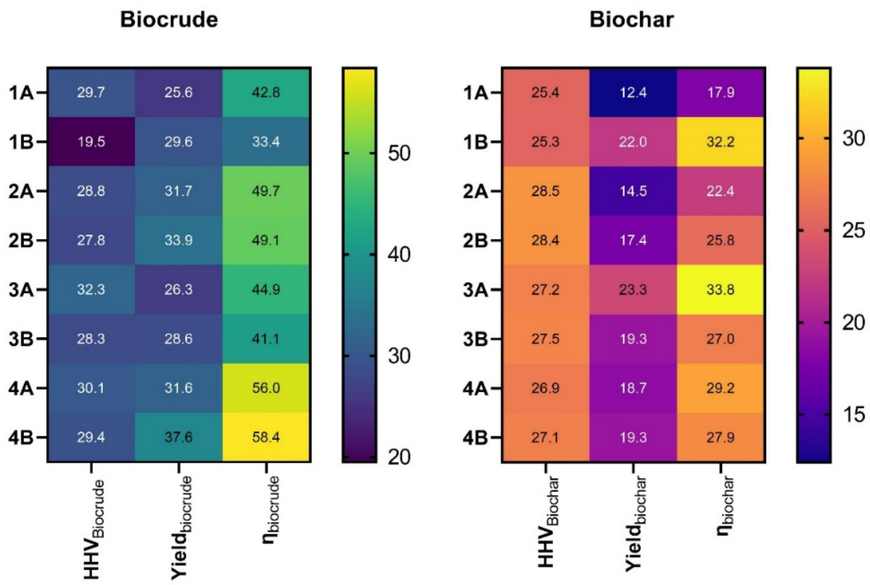

Figure 3. Heat map comparing the energy yields of the obtained biocrude and biochar. The HHV is reported in MJ/kg and the yields in %. In the rows, the numbers refer to each plant species: 1, *Iris pseudacorus*; 2, *Juncus effusus*; 3, *Phragmites australis*; and 4, *Typha latifolia*. The letters A and B refer to the growing environments: A = TW, and B = NC environment.

TW conditions do not seem to affect significantly the biocrude energy yield. For the same plant species in different environments, the biocrude energy yield does not present significative differences. However, Figure 2 shows significative differences between the energy yield reported by different plant species. The results suggest that for the biocrude energy yield, it is more important to select a species with higher biocrude yield (associated with the content of specific molecules) than the influence of the TW environment and its effect on the presence of the different elements. On the other hand, it seems that some plant species tend to produce different energy yields for the biochar, being relevant, in some cases, even if grown in the same place, to the conditions where they grow.

From the biofuel and bioeconomy perspective, it is relevant to make an assertive selection of the plants used in the TW in order to obtain higher energy yields in possible later biocrude production from these biomasses. This study presents for the first time an energy balance to approach which plant species, commonly used in TW, are more attractive for biocrude production, with *Typha latifolia* and *Juncus effusus* being the most attractive for this purpose.

3.5. Mass Balance

Carbon Balance

Table 8 shows how C is distributed in the different HTL products, biocrude being the fraction accumulating the highest portion, reporting values from 35% for the *Juncus* *effusus* grown in NC, to values of around 59% for *Typha latifolia* grown in NC, with a global average of 43.4%. The biochar reported values from around 18% to around 35% for *Iris pseudacorus* in TW and *Phragmites australis* in TW, respectively, with a global average of 28.5%. The AqP is the third phase that accumulates the most C, reporting values from around 17% for *Juncus effusus* in TW to around 20% for *Iris pseudacorus* in TW, having a global average of 18.5%. Lastly, the gas phase reported values from around 3.5% to 21.2%, and an average of 10.1%.

Table 8. Carbon balance [1] for the different HTL fractions obtained from the selected plants and the different growing environment.

Plant Description	Biocrude			Biochar			AqP [3]			Gas [2]	
	TW		NC	TW		NC	TW		NC	TW	NC
Iris pseudacorus	40.5 ± 8.5	=	44.1 ± 28.5	18.2 ± 4.0	↓	33.9 ± 5.7	20.1 ± 0.4	↑	18.4 ± 0.7	21.2 ± 4.7	3.5 ± 24.0
Volatile C				10.2 ± 1.7		20.2 ± 3.7					
Fixed C				7.6 ± 2.3	↓	13.0 ± 2.0					
Juncus effusus	44.3 ± 2.4	=	35.4 ± 18.2	23.2 ± 1.3	=	27.1 ± 2.2	16.7 ± 1.4	↓	18.3 ± 0.5	15.8 ± 4.0	19.2 ± 17.6
Volatile C				12.2 ± 1.2		13.2 ± 1.3					
Fixed C				10.7 ± 2.5	↓	13.6 ± 0.9					
Phragmites australis	40.2 ± 7.0	=	39.1 ± 4.4	35.2 ± 3.0	↑	29.5 ± 5.0	19.5 ± 1.7	↑	16.8 ± 1.3	5.1 ± 6.3	14.6 ± 0.9
Volatile C				18.3 ± 2.0		15.2 ± 2.1					
Fixed C				16.5 ± 0.9	↑	13.8 ± 2.8					
Typha latifolia	49.6 ± 17.4	=	53.4 ± 16.6	30.8 ± 0.6	=	29.7 ± 11.4	18.5 ± 0.2	↓	19.5 ± 5.9	1.1 ± 17.1	0.6 ± 21.8
Volatile C				15.9 ± 0.5		15.1 ± 4.9					
Fixed C				14.4 ± 0.2	=	14.1 ± 6.1					
Mean		43.4			28.5			18.5		10.1	
SD		13.9			6.9			2.3		14.7	

[1] The values are reported in % in reference to the amount of carbon present in the biomass feed. [2] The value of gas is the difference between 100% and the addition of the biocrude, biochar and AqP fractions; it means gas = 100 − (biocrude + biochar + AqP). [3] The value is obtained from the TOC concentration in the AqP and the amount of water in the reaction. ↑ indicates a positive effect of the TW in the concentration of the reported element. ↓ indicates a negative effect of the TW in the concentration of the reported element. = indicates a not significative effect of the TW in the concentration of the reported element

Regarding the fixed carbon, it is possible to observe that the highest value of fixed carbon was found for *Phragmites australis* grown in TW (16.5%) and the lowest was reported for *Iris pseudacorus* in the NC (7.6%). The statistical analysis for the comparison of fixed carbon in the different biochar shows for *Iris pseudacorus*, *Juncus effusus*, and *Phragmites australis* a significative difference in the fixed carbon content between the plants grown in TW and the ones grown in NC. The TW showed a positive effect in the fixed carbon content for the *Phragmites australis*, and a negative effect on the *Iris pseudacorus* and *Juncus effusus*. Typha latifolia did not show significative differences between the two growing environments.

4. Conclusions

The results of this study show that biomass from TW is suitable for biocrude production. In general, the TW conditions did not affect the biocrude yields of the HTL reaction; however, the biochar yield seems to be more sensitive to the biomass composition, showing yield differences between both environments, TW and NC, and those differences are more evident between plant species. The amount of fixed carbon in the biochar also seems to depend on the plant species, showing also differences between the growing environments. Lastly, the AqP composition showed to be different according with the growing environment and the plant species. This was confirmed by LC-HRMS analysis performed.

The biomass produced in TW can produce biochar and biocrude in similar amounts to the plants grown in NC, being a suitable source of sustainable biomass to be used in biorefinery processes, which demand, based on the bioeconomy concepts, sustainable and sources of biomass. The environmental value of the TW plants lies in the fact that the biomass is considered a residual product and up to now, no aggregated value has been found from this wastewater treatment; the plants capture the CO_2 that later can be emitted by the combustion of the biofuels, and to produce these TW plants, no extra land or nutrients are needed for growing the plants.

However, further studies dealing with the primary production of the plants, HTL pathways, uses of the AqP, and alternatives to treat the AqP are needed. Additionally, and to increase the global impact of the study, it would be relevant to select other species used in TW, especially for systems running in warm and tropical countries where TW are becoming widely used. The approach from this study can transform the wastewater treatment process into a productive system, simultaneously improving water quality and generating resources.

Lastly, the use of the HTL technology represents new environmental concerns, such as the management of the AqP, which has up to 70 times the pollutant concentration found in typical municipal wastewater. In this context, it seems that it is necessary to explore more and new biorefining process to produce from the AqP new products that take advantage of the high concentration of N and C.

This study is evidence of the possibilities of the circular economy concepts, where the biomass that already has been used for treating wastewater in a previous and sustainable way can be used and re-used not only once, but many more times. Moreover, it shows that the use of sustainable sources of biomass for producing biofuel and later bioenergy allows to bring to the present the future externalities caused by fossil fuels, which are even cheaper in present value and have a high cost because of the future environmental externalities.

Lastly, the study supports the bioeconomy values, where environmental externalities are controlled and managed in the present time. This model can create an invisible self-regulated system, compared to and readapted from the concept of the invisible hand of Adam Smith [1], where the development is invisibly controlled by the capacity of the systems to keep and control in the present, using sustainable resources for energy and goods production, the environmental externalities for their activities, avoiding the inheritance of environmental problems by the future generations because of present actions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14238157/s1, Figure S1: Marco20210121_wMeOH_filtered-20210929_NewLabels Loadings for PC1 (27.4%) vs. PC2 (21.1%), Pareto.

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References

1. D'Adamo, I.; Morone, P.; Huisingh, D. Bioenergy: A Sustainable Shift. *Energies* **2021**, *14*, 5661. [CrossRef]
2. Birgit, K.; Michel, K.; Patrick, R.G.; Stefan, K. Biorefinery Systems-An Overview. In *Biorefineries—Industrial Processes and Products. Status Quo and Future Directions*; Elsevier: Amsterdam, The Netherlands, 2006; Volume 1, pp. 3–39. ISBN 3527310274.

3. Walters, G.; Janzen, C.; Maginnis, S. *Nature-Based Solutions to Address Global Societal Challenges*; Cohen-Shacham, E., Walters, G., Janzen, C., Maginnis, S., Eds.; IUCN International Union for Conservation of Nature: Gland, Switzerland, 2016; ISBN 9782831718125.
4. Rodriguez-Dominguez, M.A.; Konnerup, D.; Brix, H.; Arias, C.A. Constructed Wetlands in Latin America and the Caribbean: A Review of Experiences during the Last Decade. *Water* **2020**, *12*, 1744. [CrossRef]
5. Uggetti, E.; Ferrer, I.; Arias, C.; Brix, H.; García, J. Carbon footprint of sludge treatment reed beds. *Ecol. Eng.* **2012**, *44*, 298–302. [CrossRef]
6. Mannino, I.; Franco, D.; Piccioni, E.; Favero, L.; Mattiuzzo, E.; Zanetto, G. A Cost-Effectiveness Analysis of Seminatural Wetlands and Activated Sludge Wastewater-Treatment Systems. *Environ. Manag.* **2007**, *41*, 118–129. [CrossRef]
7. McKinney, R.A.; Raposa, K.B.; Cournoyer, R.M. Wetlands as habitat in urbanizing landscapes: Patterns of bird abundance and occupancy. *Landsc. Urban Plan.* **2011**, *100*, 144–152. [CrossRef]
8. Stefanakis, A. The Role of Constructed Wetlands as Green Infrastructure for Sustainable Urban Water Management. *Sustainability* **2019**, *11*, 6981. [CrossRef]
9. Brix, H.; Sorrell, B.; Lorenzen, B. Are Phragmites-dominated wetlands a net source or net sink of greenhouse gases? *Aquat. Bot.* **2001**, *69*, 313–324. [CrossRef]
10. Brix, H. How "green" are aquaculture, constructed wetlands and conventional wastewater treatment systems? *Water Sci. Technol.* **1999**, *40*, 45–50. [CrossRef]
11. Konnerup, D.; Koottatep, T.; Brix, H. Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with Canna and Heliconia. *Ecol. Eng.* **2009**, *35*, 248–257. [CrossRef]
12. Vymazal, J. Constructed Wetlands for Wastewater Treatment: Five Decades of Experience. *Environ. Sci. Technol.* **2011**, *45*, 61–69. [CrossRef]
13. Wallace, S. Feasibility, Design Criteria, and O & M Requirements for Small Scale Constructed Wetland Wastewater Treatment Systems. *Water Intell. Online* **2006**, *5*, 304. [CrossRef]
14. Brix, H.; Arias, C.A. The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. *Ecol. Eng.* **2005**, *25*, 491–500. [CrossRef]
15. Malaviya, P.; Singh, A. Constructed Wetlands for Management of Urban Stormwater Runoff. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 2153–2214. [CrossRef]
16. Istenič, D.; Arias, C.A.; Matamoros, V.; Vollertsen, J.; Brix, H. Elimination and accumulation of polycyclic aromatic hydrocarbons in urban stormwater wet detention ponds. *Water Sci. Technol.* **2011**, *64*, 818–825. [CrossRef]
17. Istenič, D.; Arias, C.A.; Vollertsen, J.; Nielsen, A.H.; Wium-Andersen, T.; Hvitved-Jacobsen, T.; Brix, H. Improved urban stormwater treatment and pollutant removal pathways in amended wet detention ponds. *J. Environ. Sci. Health Part A* **2012**, *47*, 1466–1477. [CrossRef]
18. Gutiérrez-Sarabia, A.; Fernandez-Villagómez, G.; Martínez-Pereda, P.; Rinderknecht-Seijas, N.; Poggi-Varaldo, H.M. Slaughter-house wastewater treatment in a full-scale system with constructed wetlands. *Water Environ. Res.* **2004**, *76*, 334–343. [CrossRef]
19. Soroko, M. Treatment of wastewater from small slaughterhouse in hybrid constructed wetlands systems. *Ecohydrol. Hydrobiol.* **2007**, *7*, 339–343. [CrossRef]
20. Vymazal, J. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecol. Eng.* **2014**, *73*, 724–751. [CrossRef]
21. Hadad, H.R.; Mufarrege, M.M.; Pinciroli, M.; Di Luca, G.A.; Maine, M.A. Morphological Response of Typha domingensis to an Industrial Effluent Containing Heavy Metals in a Constructed Wetland. *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 666–675. [CrossRef]
22. Maine, M.; Sanchez, G.; Hadad, H.; Caffaratti, S.; Pedro, M.; Mufarrege, M.; Di Luca, G. Hybrid constructed wetlands for the treatment of wastewater from a fertilizer manufacturing plant: Microcosms and field scale experiments. *Sci. Total. Environ.* **2019**, *650*, 297–302. [CrossRef]
23. Pozo-Morales, L.; Moron, C.; Garvi, D. Ecologically Designed Sanitary Sewer Based on Constructed Wetlands Technology—Case Study in Managua (Nicaragua). *J. Green Eng.* **2017**, *7*, 421–450. [CrossRef]
24. Vymazal, J. Emergent plants used in free water surface constructed wetlands: A review. *Ecol. Eng.* **2013**, *61*, 582–592. [CrossRef]
25. Zhu, N.; An, P.; Krishnakumar, B.; Zhao, L.; Sun, L.; Mizuochi, M.; Inamori, Y. Effect of plant harvest on methane emission from two constructed wetlands designed for the treatment of wastewater. *J. Environ. Manag.* **2007**, *85*, 936–943. [CrossRef]
26. Zheng, Y.; Wang, X.; Ge, Y.; Dzakpasu, M.; Zhao, Y.; Xiong, J. Effects of annual harvesting on plants growth and nutrients removal in surface-flow constructed wetlands in northwestern China. *Ecol. Eng.* **2015**, *83*, 268–275. [CrossRef]
27. Camelo, A.; Genuino, D.A.; Maglinao, A.L.; Capareda, S.C.; Paes, J.L.; Owkusumsirisakul, J. Pyrolysis of Pearl Millet and Napier Grass Hybrid (PMN10TX15): Feasibility, byproducts, and comprehensive characterization. *Int. J. Renew. Energy Res.* **2018**, *8*, 682–691.
28. Karmee, S.K. Liquid biofuels from food waste: Current trends, prospect and limitation. *Renew. Sustain. Energy Rev.* **2016**, *53*, 945–953. [CrossRef]
29. Kamm, B.; Hille, C.; Schönicke, P.; Dautzenberg, G. Green biorefinery demonstration plant in Havelland (Germany). *Biofuels Bioprod. Biorefining* **2010**, *4*, 253–262. [CrossRef]
30. Balan, V.; Kumar, S.; Bals, B.; Chundawat, S.; Jin, M.; Dale, B.E. Biochemical and Thermochemical Conversion of Switchgrass to Biofuels. In *Appraisal: From Theory to Practice*; Springer: Singapore, 2012; pp. 153–185.

31. Biller, P.; Ross, A. Production of biofuels via hydrothermal conversion. In *Handbook of Biofuels Production*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 509–547.
32. Biller, P.; Sharma, B.K.; Kunwar, B.; Ross, A.B. Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel* **2015**, *159*, 197–205. [CrossRef]
33. Anastasakis, K.; Biller, P.; Madsen, R.B.; Glasius, M.; Johannsen, I. Continuous Hydrothermal Liquefaction of Biomass in a Novel Pilot Plant with Heat Recovery and Hydraulic Oscillation. *Energies* **2018**, *11*, 2695. [CrossRef]
34. Biller, P.; Lawson, D.; Madsen, R.B.; Becker, J.; Iversen, B.B.; Glasius, M. Assessment of agricultural crops and natural vegetation in Scotland for energy production by anaerobic digestion and hydrothermal liquefaction. *Biomass Convers. Biorefinery* **2017**, *7*, 467–477. [CrossRef]
35. Pedersen, T.H.; Rosendahl, L. Production of fuel range oxygenates by supercritical hydrothermal liquefaction of lignocellulosic model systems. *Biomass Bioenergy* **2015**, *83*, 206–215. [CrossRef]
36. Biller, P.; Johannsen, I.; dos Passos, J.S.; Ottosen, L.D.M. Primary sewage sludge filtration using biomass filter aids and subsequent hydrothermal co-liquefaction. *Water Res.* **2018**, *130*, 58–68. [CrossRef]
37. Conti, F.; Toor, S.S.; Pedersen, T.H.; Nielsen, A.H.; Rosendahl, L.A. Biocrude production and nutrients recovery through hydrothermal liquefaction of wastewater irrigated willow. *Biomass Bioenergy* **2018**, *118*, 24–31. [CrossRef]
38. Tenenbaum, D.J. Biochar: Carbon Mitigation from the Ground Up. *Environ. Health Perspect.* **2009**, *117*, A70–A73. [CrossRef]
39. Kwapinski, W.; Byrne, C.M.P.; Kryachko, E.; Wolfram, P.; Adley, C.; Leahy, J.J.; Novotny, E.H.; Hayes, M.H.B. Biochar from Biomass and Waste. *Waste Biomass Valorization* **2010**, *1*, 177–189. [CrossRef]
40. Qambrani, N.A.; Rahman, M.; Won, S.; Shim, S.; Ra, C. Biochar properties and eco-friendly applications for climate change mitigation, waste management, and wastewater treatment: A review. *Renew. Sustain. Energy Rev.* **2017**, *79*, 255–273. [CrossRef]
41. United Nations Sustainable Development Goals. Available online: https://sdgs.un.org/goals (accessed on 4 October 2021).
42. Sluiter, A.; Hames, B.; Hyman, D.; Payne, C.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Nrel, J.W. Determination of total solids in biomass and total dissolved solids in liquid process samples. *Natl. Renew. Energy Lab.* **2008**, *9*, 3–5.
43. Jones, D.B. *Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins*; US Department of Agriculture: Washington, DC, USA, 1941; Volume 183, p. 22.
44. Channiwala, S.; Parikh, P. A unified correlation for estimating HHV of solid, liquid and gaseous fuels. *Fuel* **2002**, *81*, 1051–1063. [CrossRef]
45. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass—NREL/TP-510-42618. Laboratory Analytical Procedure (LAP). 2012. Available online: nrel.gov/docs/gen/fy13/42618.pdf (accessed on 10 September 2021).
46. Tisler, S.; Liang, C.; Carvalho, P.N.; Bester, K. Identification of more than 100 new compounds in the wastewater: Fate of polyethylene/polypropylene oxide copolymers and their metabolites in the aquatic environment. *Sci. Total. Environ.* **2021**, *761*, 143228. [CrossRef] [PubMed]
47. Yang, J.; He, Q.; Corscadden, K.; Niu, H.; Lin, J.; Astatkie, T. Advanced models for the prediction of product yield in hydrothermal liquefaction via a mixture design of biomass model components coupled with process variables. *Appl. Energy* **2019**, *233–234*, 906–915. [CrossRef]
48. Lu, J.; Liu, Z.; Zhang, Y.; Savage, P.E. Synergistic and Antagonistic Interactions during Hydrothermal Liquefaction of Soybean Oil, Soy Protein, Cellulose, Xylose, and Lignin. *ACS Sustain. Chem. Eng.* **2018**, *6*, 14501–14509. [CrossRef]
49. Frimmel, F.; Abbt-Braun, G. Sum Parameters: Potential and Limitations. In *Treatise on Water Science*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 3–29.
50. Thomsen, L.B.S.; Carvalho, P.; dos Passos, J.S.; Anastasakis, K.; Bester, K.; Biller, P. Hydrothermal liquefaction of sewage sludge; energy considerations and fate of micropollutants during pilot scale processing. *Water Res.* **2020**, *183*, 116101. [CrossRef]