A cascade biorefinery for grape marc: recovery of materials and energy through thermochemical and biochemical processes

Gianluigi Farru [1], Giovanna Cappai [1,2], Alessandra Carucci [1,2], Giorgia De Gioannis [1,2], Fabiano Asunis [1], Stefano Milia [2], Aldo Muntoni [1,2] Matteo Perra [3] and Angela Serpe [1,2]

[1] DICAAR - Department of Civil and Environmental Engineering and Architecture, University of Cagliari, Via Marengo 2, 09123 Cagliari, Italy

[2] IGAG-CNR - Institute of Environmental Geology and Geoengineering, National Research Council, Cagliari, Via Marengo 2, 09123 Italy

[3] Department of Life and Environmental Sciences, University of Cagliari, Via Ospedale 72, Cagliari, 09124, Italy

Corresponding Author: Gianluigi Farru. Email: gianluigi.farru@unica.it.

Abstract:

The agro-industrial sector makes a high contribution to greenhouse gas emissions; therefore, proper waste management is crucial to reduce the carbon footprint of the food chain. Hydrothermal carbonization (HTC) is a promising and flexible thermochemical process for converting organic materials into energy and added-value products that can be used in different applications. In this work, grape marc residues before and after an extraction process for recovering polyphenols were hydrothermally treated at 220 °C for 1 h. The resulting hydrochar and process water were investigated to test an innovative cascade approach aimed at a multiple product and energy recovery based on the integration of HTC with anaerobic digestion. The results show that this biorefinery approach applied to grape marc could allow to diversify and integrate its potential valorisation options. The produced hydrochars possess an increased fixed carbon content compared to the feedstock (up to +70%) and, therefore, can be used in soil, immobilizing carbon in a stable form and partially replacing peat in growing media (up to 5% in case of hydrochar from grape marc after extraction), saving the consumption of this natural substrate. In addition, energy can be recovered from both hydrochar by combustion and from process water through anaerobic digestion to produce biogas. Hydrochars show good properties as solid fuel

similar to lignite, with an energy content of around 27 MJ kg^{-1} (+30% compared to the feedstock). The anaerobic digestion of the process water allowed obtaining up to 137 mL of biomethane per gram of fed COD. Finally, while HTC process waters are suitable for biological treatment, attention must be paid to the presence of inhibiting compounds that induce acute toxic effects in aerobic conditions. The proposed approach is consistent with the principles of circular economy and could increase the overall sustainability and resilience of the agro-industrial sector.

Keywords: Hydrothermal Carbonization, Biofuel, Soil Amendment, Biomethane, Biorefinery.

1. Introduction

The food supply chain is one of the foundations of human society. Its intrinsically positive role, however, is jeopardized by economic, environmental, and social issues, such as: vulnerability to natural calamities; uneven availability of food and fertile land; overuse of land and water resources and related morphological and hydrographic impacts; threats to ecosystems and loss of biodiversity; overuse of pesticides to increase productivity. Over the last few decades, the food supply chain exacerbated the climate emergency. Globally, the food sector account for more than one-third of total global anthropogenic greenhouse gas emissions (3-7 of the 11-19 billion tons of emissions per year) as estimated by the Intergovernmental Panel on Climate change (Crippa et al., 2021). Although land use and production, including fertilizers use, are still the main contributor to agricultural emissions, packaging, transport, "cold chain" activities, processing, and related energy consumption and waste production all play an increasingly large role (Crippa et al., 2021). Reducing the food chain carbon footprint is central to addressing climate change, and proper waste management is of particular importance (Karl & Tubiello, 2021). Improper waste management is pushing the food supply chain to the top of the list of greenhouse gas emitters, as reported by the United Nations Agriculture Agency during the COP26 climate conference in Glasgow in 2021. However, the organic nature of most of the residues produced along the food supply chain is suitable for innovative valorization processes consistent with the principles of the circular bioeconomy.

It is important to develop processes and approaches to flexibly recover both material and energy resources, in accordance with the needs of local and regional market (Muntoni, 2019). In the case of agro-industrial waste, it is ideal if the recovery of resources addresses concomitant problems, such as the need for renewable energy sources to alleviate increasing energy demands of the food chain, and renewal soil amendments to preserve

and restore the carbon balance of the soil (Fryda et al., 2018).

Worldwide, 260 million hectolitres of wine were produced in 2020, which is in line with the yearly average production over the last decade. For every 100 kg of grapes, about 70 litres of wine and 18 kg of grape marc are produced. Accordingly, over 6 million tons of grape marc are produced globally every year (OIV - International Organisation of Vine and Wine Intergovernmental Organisation, 2021).

The management of such a large amount of biowaste, enriched in water (around 60% by weight) and organic matter (up to 900 g $kgTS^{-1}$) and characterized by an acidic pH (3-6), and high lignin and polyphenol content is a task that must be faced consistently, made more challenging by the constraints of current environmental and economic policies. Furthermore, management options must consider the seasonality of production since approximately 75% of solid waste is generated during the harvest period (around 2 months).

Grape marc is sometimes used as a by-product, e.g., for ruminant animal feeding, although some adverse effects may occur due to the presence of substances such as polyphenols (Baumgärtel et al., 2007; Devesa-Rey et al., 2011; Pinelo et al., 2006). Grape marc can be managed using biological treatments such as anaerobic digestion, with reported methane yields spanning 113 - 420 L CH_4 $kgVS^{-1}$, or via composting and subsequent use on soil. The relatively high heating value of grape marc (19 - 22 MJ kg^{-1}) would support its use in direct combustion scenarios (Muhlack et al., 2018); however, the high water content makes pre-drying necessary.

This study proposes an integrated approach for the sustainable management of grape marc and evaluates valorization options that combine the recovery of materials and energy by coupling different processes such as hydrothermal carbonization and anaerobic digestion, investigating multiple by-products valorization pathways. Hydrothermal carbonization (HTC) is a wet thermochemical process that rapidly converts organic substrates into added-value by-products. Under autogenous pressures and temperatures generally ranging from 180 to 250 °C, a coal-like solid material known as hydrochar and process water are produced, whose characteristics suggest a wide range of potential applications (Libra et al., 2011). During the process, water acts as both the reaction medium and catalyst, making HTC suitable for wet biomass valorization.

Hydrothermal carbonization technology is sustainable as it embraces the principles of a circular economy and is a waste management system capable of lowering climate-altering emissions. In its most typical application, biomass-derived hydrochar is used as a fuel for renewable energy production, acting as a neutral combustible and energy-dense carbon source. Being a carbon-enriched solid, hydrochar combustion involves lower CO_2 emissions per unit of energy developed compared to the combustion of the raw material, further offsetting CO_2 emissions. The chemical-physical characteristics of hydrochar make its application interesting

also as a soil amendment, able to improve fertility as well as water retention and carbon sink (Funke & Ziegler, 2010; Libra et al., 2011; Wang et al., 2018).

Besides the hydrochar, the process water may be the bottleneck in view of an industrial up-scaling, since it is still understudied in terms of its sustainable management (Ipiales et al., 2021). Due to the high organic load of process water, it may be a suitable substrate for anaerobic digestion to produce biomethane. Recently, several studies proposed the integration of hydrothermal carbonization with anaerobic digestion as a flexible and circular system to exploit the recovery potential of waste materials (Catenacci et al., 2022; Ipiales et al., 2021).

Few studies are reported in literature focusing on grape marc treated via HTC. Basso et al. (2016) treated grape marc (solid-liquid ratio of 1:10) at 180, 220, and 250 °C for 1, 3, and 8 h in a small-scale reactor (50 mL) reporting increasing carbon content and reduced ash and oxygen content in hydrochar (HC). At the same conditions (Baratieri et al., 2015) developed a thermal dynamic model based on the solid yield obtained after HTC of grape seeds. Same results were stated by Nguyen et al. (2022) and Mäkelä et al. (2017) who worked at similar temperatures (180, 220, and 260 °C) for 0.5 h in a 0.5 L pressurized reactor using an initial solid content of 40 and 25 wt%, respectively. Basso et al. (2018) investigated the behavior of grape marc components (seeds and skins) treated at 180, 220, and 250 °C for 0.5, 1, 3, and 8 h using a 50 mL reactor. Mariuzza et al. (2022) applied HTC to different residues, including grape marc, at 220 °C for 1 h in a 50 mL reactor using a solid liquid ratio of 1:5. Other thermochemical processes have also been applied to grape marc: Lin et al. (2021) studied the valorization of this residue and its mixture with other materials (such as cow manure and HCs) via pyrolysis.

The available studies, all conducted in small-volume reactors (< 0.5 L), demonstrate the possibility of using the hydrochar from grape marc in different applications; however, no information is yet available on the management of the produced process water and, to the best of our knowledge, on the potential integration of HTC and anaerobic digestion for the valorization of this residue.

The specific objective of this work is to assess i) the potential application of the hydrochar produced via HTC from grape marc as a soil improver and solid fuel, and ii) the biological treatability (anaerobic and aerobic) of the process water in view of energetic valorization or disposal. Hydrothermal carbonization was performed on both raw grape marc (GM) and GM after extraction of polyphenols (GM_Ext), compounds that find application in different production sectors.

To achieve the study's objective, cascade processes and treatments were applied; HTC tests were carried out at a temperature of 220 °C for 1 h. After HTC, the products were tested through different valorization

pathways. The feasibility of hydrochar as a solid fuel and as soil improver or peat substitute was investigated, while assessing the suitability of process water as substrate for energy recovery via AD, or for its treatment in a traditional wastewater plant.

2. Materials and methods

2.1 Feedstock

Grape marc (GM) considered in the study consists of skins, stems, and seeds from winemaking with the Cannonau cultivar collected in southern Sardinia (Italy). The GM was dried at 40 °C for about 48 h and stored in the dark at 4 °C in vacuum plastic bags. Considering the significant presence of bioactive molecules in GM, especially polyphenols, their extraction and recovery may be functional to manufacturing nutraceutical and pharmaceutical products for human health (Manca et al., 2020). To this aim, polyphenols separation was carried out through preliminary particle size reduction followed by solid-liquid extraction using a hydroethanolic mixture in a previous study (Perra et al., 2021). Briefly, a part of the dried GM was grinded to obtain a powder with a diameter between 16 and 515 μm, measured with a laser diffraction particle size analyser (Mastersizer 3000, Malvern Panalytical Ltd, Worcestershire, UK), using the wet dispersion method and distilled water as dispersant. 100 g of GM was suspended in 1 L of a mixture of ethanol and water (70:30 v/v, density 0.885 g mL^{-1}), and left under stirring conditions in the dark at 25 °C for 48 h. The dispersion was centrifuged two times (30 min, 8000 rpm) to separate solid and liquid fractions. The exhausted GM (GM_Ext) was recovered as feedstock material for the HTC process.

2.2 Hydrothermal carbonization

Hydrothermal carbonization was carried out in a 1.5 L stainless steel pressurized reactor (Berghof, BR-1000). The reactor was equipped with an electric jacket for heating, a thermocouple for the continuous monitoring of the inside temperature, a stirred shaft and a data logging unit connected to a computer where the main operating parameters (inner temperature and autogenic pressure) were continuously recorded. GM and GM_Ext were both treated at 220 °C for 1 h (with a temperature increase of 2 °C min^{-1}). These conditions were selected to balance the conversion performance and the energy consumption. Cellulose and lignin hydrolysis is significant at above 200 °C (Funke & Ziegler, 2010; Libra et al., 2011) and 1 h treatment may assure a proper conversion (high solid yield (Libra et al., 2011) with minimal energy usage. These hypothesis are also confirmed by Lucian & Fiori, (2017) who defined 220 °C for 1 h as the most favourable conditions. The solid material with its moisture content (6.17 ±0.68 wt% and 7.40 ±0.57 wt% for GM and GM_Ext, respectively) was placed in the

reactor together with distilled water to achieve a solid content of 10 wt% (1 kg of total input mass) filling the reactor at ca. 75% of its volume. After the input material preparation, the reactor was sealed, and the controller was set to the desired temperature and holding time. Once reactor was kept at 220 °C for 1 h, it was turned off and cooled to room temperature overnight (the temperature profile during the cooling phase is reported in Figure S1 in Supplementary Material). Before unsealing it, the final inner pressure was noted and the gas released evaluated through a column gas meter, based on water displacement method, to estimate the volume produced. To assure a mass balance as precise as possible, all the output materials were carefully weighed before and after each step. The carbonized sludge resulting from the HTC process was separated through a press filter obtaining the solid (hydrochar, HC) and the liquid (process water, PW) phases. The hydrochar was stored in vacuum plastic bags at 4 °C while the process water was vacuum filtered at 0.45 μm and stored in plastic bottles at 4 °C. All tests were carried out three times to ensure replicability. According to the feedstocks, the hydrochars were named HC_GM and HC_GM_Ext and the process water samples PW_GM and PW_GM_Ext.

The hydrochars produced were characterized in terms of moisture content, ash content, elemental composition, density, surface chemistry and higher heating value (HHV). The moisture in hydrochar after separation from the liquid phase was estimated by heating three samples (randomly selected from the total mass) to 105 °C. The volatile matter, ash content, and fixed carbon were measured through thermo-gravimetrical analysis (TGA – 710, LECO). Further analyses (i.e., CHN/S, HHV, and FT-IR) were conducted on representative dried and milled samples as detailed in the Supplementary Material. Oxygen was evaluated by difference from C, H, N, S, and ash.

The liquid phase from the HTC runs was characterized in terms of pH and electrical conductivity (EC), total organic carbon (TOC), and chemical oxygen demand (COD). Details on methods are available in Supplementary Material. All the measurements for both solids and liquids were performed 3 times at least and the results were validated through statistical pairwise t tests (significance level $\alpha = 0.05$) performed using the software JMP (v. 15, SAS).

2.3 Germination test

According to the standard test UNI EN 16086-2:2012-01, Sphagnum peat, previously sieved at 10 mm, was mixed with dried GM, HC_GM, and HC_GM_Ext. 5, 25, and 50 wtdb% of feedstock or hydrochars were mixed with peat. The samples were named with the codes formed by the initials of the material mixed with the peat and the used amount; to give an example, the sample with the mixture containing peat and 5% hydrochar from

grape marc was labelled HC_GM_5%. Moisture was checked and adjusted with the fist test, adding water until squeezing the material with the fist did not produce leaching. Around 50 g of each mixture were placed in 3 square Petri dishes and 10 seeds of cress (*Lepidium sativum* L.) were sown at the top of the dish. In addition, 3 more Petri dishes filled with only peat were used as a control. One drop of water was added to each seed. Petri dishes were closed with parafilm and stored in an oven at 25 °C for 72 h for incubation with an inclination of 70 – 80° (seeds at the top). After 72 h of incubation, the Petri dishes were opened, the germinated seeds were counted, and the length of the seedlings was measured. The germination rate was defined as the percentage of germinated seeds on the total number of seeds. The results were validated with statistical tests, such as ANOVA and Tukey's HSD test through the statistical software JMP (v. 15, SAS). Several parameters were calculated: average germination rate (AGR), coefficient of variance for germination rate (CVG), average root length per plant (ARLP), coefficient of variance for root length (CVR), average hypocotyl length per plant (AHLP), coefficient of variance for hypocotyl length (CVH), root length index (RI), and Munoo-Liisa vitality index (MLV).

2.4 Assessment of material stability

Several methods have been proposed to measure biomaterial stability. Among them, respirometric methods based on oxygen consumption are broadly recommended (Sánchez Arias et al., 2012). Electrolytic respirometry is based on the Warburg manometric respirometer and is usually referred to as the Sapromat® system. This system has been extensively applied to estimate O_2 consumption for various purposes/fields: biodegradability of waste and wastewater, toxicity, and inhibition tests, modelling and kinetics of biodegradation, respiration of polluted soils, compost and biologically pretreated waste stability.

10 g of pre-humidified HC were placed in a 500 mL glass reaction bottle, connected to an oxygen generator and a pressure control gauge. The bottle rests in a water bath (20 °C). During aerobic degradation, microorganisms consume oxygen and produce an equivalent amount of carbon dioxide. CO_2 is continuously adsorbed by around 5 g NaOH granules, placed in a small container integrated in the reactor bottle, resulting in the establishment of a negative pressure in the bottle. Oxygen is electrolytically produced until the negative pressure is compensated. The amount of produced oxygen is calculated by the required electric energy (according to Faraday's Law), which is recorded in intervals of 36 s by the control unit (Binner et al., 2012).. The tests were run in triplicate.

The HC stability was assessed in relation to RA_4 (total oxygen consumption after 4 days), which is the recommended parameter for the estimation of compost stability by the European Union (European

214 Commission, 2001).

215

216 ## 2.5 Assessment of Biochemical Methane Potential (BMP)

217 The BMP measurement is a standard test for determining biogas production from an organic substrate using
218 methanogenic bacteria as the inoculum under optimal batch conditions. This test was applied to the PW
219 produced during HTC for assessing the possibility of conversion into biogas, according to the standard
220 UNI1601755EIT, BMP tests were conducted in brown glass bottles, where methanogenic sludge was mixed
221 with HTC PW. The methanogenic inoculum for biochemical methane potential (BMP) tests was collected from
222 a mesophilic anaerobic digestion plant treating cattle slurry and grass silage located in southern Sardinia (Italy).
223 The amount of sludge and sample was set to allow gas to form in the headspace of the bottles and ensure a
224 food-microorganism ratio (F/M) of 0.5 gCOD $gCOD^{-1}$ according to the literature (Boulanger et al., 2012; Pagés-
225 Díaz et al., 2020). Methanogenic sludge with D-glucose was used to assess the specific methanogenic activity
226 (SMA, data not shown). A blank was used with only inoculum. No nutrient solution or pH buffer was added to
227 the bottles. The initial pH value was around 7.0 for all samples. All the tests were performed in triplicate. Before
228 the bottles were sealed with airtight caps, they were purged with nitrogen to ensure anaerobic conditions. The
229 sealed bottles were placed in a thermostated shaker (T = 39 ± 1 °C) and kept for 36 days. An average period
230 of 3 days was adopted, slightly shorter at the beginning of the test and longer at the end, to purge the gas
231 produced and accumulated in the reactors. Partial and cumulative biogas production was measured over time.
232 The volume produced was measured through a water-displacement method using a volumetric column, and
233 the gas samples were analyzed using a GC-FID (7890B, Agilent Technologies) equipped with a thermal
234 conductivity detector (TCD) and two stainless columns packed with HayeSep N (80/100 mesh) and Shincarbon
235 ST (50/80 mesh) connected in series.

236 The methane yield at 36 days was calculated as follows (Eq. (1)):

237

$$\mathrm{BMP} = (\mathrm{V_{CH_4,s}} - \mathrm{V_{CH_4,blank}})/\mathrm{COD_s} \quad (1)$$

238

239 $V_{CH4,s}$ is the volume of methane produced from the PW measured at the end of the test, $V_{CH4,blank}$ is the
240 volume of methane produced from the inoculum measured at the end of the test and COD_s is the mass of
241 soluble COD contained in the volume of added PW.

The modified Gompertz equation, based on a sigmoid function, was used to predict the methane production and calculate the kinetic parameters, according to Eq. (2) (Pagés-Díaz et al., 2020). This model was originally developed to describe the slowdown of the batch reactor culture of any microorganism and was modified by Zwietering et al. (1990) by incorporating the classic parameters of maximum concentration, latency time and maximum growth rate. The modified Gompertz model assumes that the methane production is proportional to microbial activity (Nielfa et al., 2015).

$$Y(t)CH_4 = Y_{max}CH_4 \exp\left\{-\exp\left[\frac{R_{max} \cdot e}{Y_{max}CH_4}(\lambda - t) + 1\right]\right\} \quad (2)$$

$Y(t)CH_4$ is the cumulative production yield of CH_4 at time t, $Y_{max}CH_4$ is the maximum theoretical production yield, R_{max} is the maximum production rate, λ is the duration of the lag phase, t is the time and 'e' is the Neperian number. The experimental data were fitted through Eq. (2) using the TableCurve 2D® software (v. 5.01, Systat Software Inc.) through least-squares non-linear regression. The coefficient of determination R^2 was used to preliminary evaluate fitting accuracy of Gompertz model for each experimental dataset.

2.6 Assessment of acute toxicity

A pH-stat titration unit (ANITA, Ammonium NITrification Analyser) was used to assess the acute inhibiting effect of HTC process water on the ammonium-oxidizing bacterial community in activated sludge, as described in Ficara & Rozzi (2001). The tests were carried out in a glass vessel containing 1 L of unacclimated activated sludge (total suspended solids, TSS, 4 g L^{-1}) drawn from the Oristano wastewater treatment plant (Italy). The maximum ammonium-oxidizing capacity of nitrifying biomass was measured in the presence of ammonium as the only substrate, considering the stoichiometric relationship between ammonium oxidation and acidity production, and used as reference. Known volumes of PW_GM and PW_GM_Ext were added to the activated sludge every 20 minutes. The resulting nitrifying activity was compared with the reference and the corresponding inhibition was calculated for each dosage. At the end of the test, nitrification was fully inhibited by allylthiourea to detect the presence of interferences. A dose-response curve was plotted for both PW_GM and PW_GM_Ext according to Eq. (3), and the IC_{50} (that is the amount of PW that causes 50% acute inhibition of sludge activity) was determined.

$$I(\%) = \left(1 - \frac{1}{1 + \frac{d^a}{b}}\right) 100 \quad (3)$$

I is the inhibition expressed as percentage, d is the dose in mL L^{-1} and a and b are curve parameters which are obtained by fitting the experimental data.

3. Results and discussion

3.1 Characterization of feedstock and products

The main properties of the two materials used as feedstock for the HTC runs and the characterization of the hydrochars are shown in Table 1. All parameters were evaluated on a dry basis by weight, apart from the moisture content.

After the HTC tests, a solid yield (Y) of 62.28 wtdb% (±0.69) was found for GM_Ext, while a slightly lower value of 59.59 wtdb% (±0.52) was obtained in the tests on GM. This is consistent with the extraction process previously applied to GM_Ext, which may have involved the removal of soluble compounds from the solid matrix. Literature reports solid yield ranging from around 50 and 75 wtdb% under different operating conditions (Basso et al., 2016; Mäkelä et al., 2017). Mariuzza et al. (2022) obtained a solid yield of 69.2 wtdb% (±1.3) at the same temperature and duration used in our experiments. However, it must be highlighted that the experimental conditions differed for the size reactor (30 times smaller than ours) and for the cooling phase, that was quicker compared to the one applied in our study. The cooling conditions could affect the final yield and properties of the produced materials due to the formation of a secondary char on the surface of the primary char as suggested by different authors (Benavente et al., 2022; Lucian et al., 2018). However, due to the reactor characteristics, we studied the HTC process considering the material deriving from the natural cooling phase without accelerating it. While considering the given limitations related to the cooling phase, our solid yields are placed within the range reported in literature.

In comparison to the feedstock, the ash content and the volatile matter in the produced HCs was lower and fixed carbon higher, aspect of interest in view of the use of HC as a fuel and as a carbon replenishment in soil. Regarding the elemental composition, carbon relative content increased, while nitrogen and oxygen ones decreased under the carbonization process. . No significant differences were found between the feedstocks ($p > 0.05$), apart for volatile matter ($p = 0.023$) and fixed carbon ($p = 0.012$). All parameters analysed in HC

reported significant differences compared to the feedstocks ($p < 0.001$), except for hydrogen ($p > 0.05$). Hydrochars reported significant differences in the solid yield only ($p < 0.001$).

Table 1. Main properties of grape marc, extracted grape marc, and hydrochars. STD is reported in brackets.

Material	Code	Solid yield [%]	Moisture [wt%]	Volatile matter [wt%]	Ash [wt%]	Fixed carbon [wt%]	C [wt%]	H [wt%]	N [wt%]	O [wt%]	kg
Grape marc	GM		6.17 (0.07)	71.19 (0.08)	7.64 (0.18)	21.17 (0.10)	49.00 (0.15)	6.09 (0.08)	2.08 (0.13)	35.19 (0.13)	1 (0
Grape marc (extracted)	GM_Ext		7.40 (0.56)	73.24 (0.17)	8.13 (0.46)	18.64 (0.29)	49.63 (0.29)	5.94 (0.08)	2.09 (0.13)	34.22 (0.23)	1 (0
Hydrochar from GM	HC_GM	59.59 (0.52)		63.53 (0.48)	3.96 (0.04)	32.51 (0.52)	61.60 (0.60)	6.14 (0.15)	1.85 (0.11)	26.45 (0.76)	2 (0
Hydrochar from GM_Ext	HC_GM_Ext	62.28 (0.69)		64.95 (1.02)	3.33 (0.01)	31.72 (1.00)	62.46 (0.29)	6.28 (0.12)	1.68 (0.07)	26.25 (0.32)	2 (0

Results are reported on dry basis; moisture on wet basis.

To assess potential modifications induced by HTC on the chemical functions of the materials, ATR-FTIR spectra were recorded on samples before and after the hydrothermal treatment. Figure S2 reports a comparison between the spectra of the GM powder and of selected seeds, stems, and skin, for starting material characterization.

The spectrum of GM consists of the typical peaks reported for polyphenolic lignocellulosic and flavonoid compounds found in grape marc (Casazza et al., 2016), together with a contribution of amines as the result of biogenic decarboxylation processes of amino acids (Grossmann et al., 2007). Details on the peaks attribution are reported on the spectra (Figure S2). In summary, the typical peaks related to lignin and polyphenols - OH (υOH 3300s-br when secondary H-bonding are present, δOH 1378m, υPhO 1263s), C-H (aliphatic CH: υ 2980-2850mw, δ/ρ 1400-1300m; aromatic CH: δ 650-720m), C=C (aliphatic C=C: υ 1650m; aromatic C=C: υ 1540m) and C-O (υC=O 1750m; υC-O 1026s; υC-O-C 1130m), as well as those typical for amines (υNH 3313m and 3271m; δNH 1600m-br; υCN 1063s), are present (Fan et al., 2012). A comparison of the GM and GM_Ext IR spectra is reported in Figure 1a, showing no significant modification in the spectrum profile after solvent extraction. A significant modification of the chemical composition and structure of both feedstock seems occurring during the HTC process as highlighted by the comparison of the spectra reported in Figure 1b, for GM and HC_GM, and Figure S3 for GM_Ext and HC_GM. In agreement with the loss of oxygen and nitrogen

found in the samples after HTC, the peaks related to the phenolic moieties of polyphenols and their intermolecular secondary interactions, as well as those of possible carboxylic groups and NH_2 stretching vibrations (υNH 3271 and 3313 cm^{-1}) are mainly reduced. Correspondingly, peaks typically related to unsaturated aromatic and aliphatic C=C bonds (1650-1598 cm^{-1}) and aromatic CH in bonds (719 cm^{-1}) increased.

The main characteristics of the process water are reported in Table 2. The HTC treatment led to an acidic pH and an electrical conductivity (EC) value of around 12 mS cm^{-1} for both the PW, typical features of the liquid phase from HTC (Funke & Ziegler, 2010; Libra et al., 2011). The total organic carbon (TOC) accounted for 9.69 g L^{-1} for PW_GM and 7.68 g L^{-1} for PW_GM_Ext. Similarly, the soluble COD was higher in GM than GM_Ext, with values higher than 30 gO_2 L^{-1}. The combined analysis of the characteristics of HC and process water suggests that the preliminary extraction treatment slightly enhances the fixation of carbon in the HC, limiting its release into the PW.

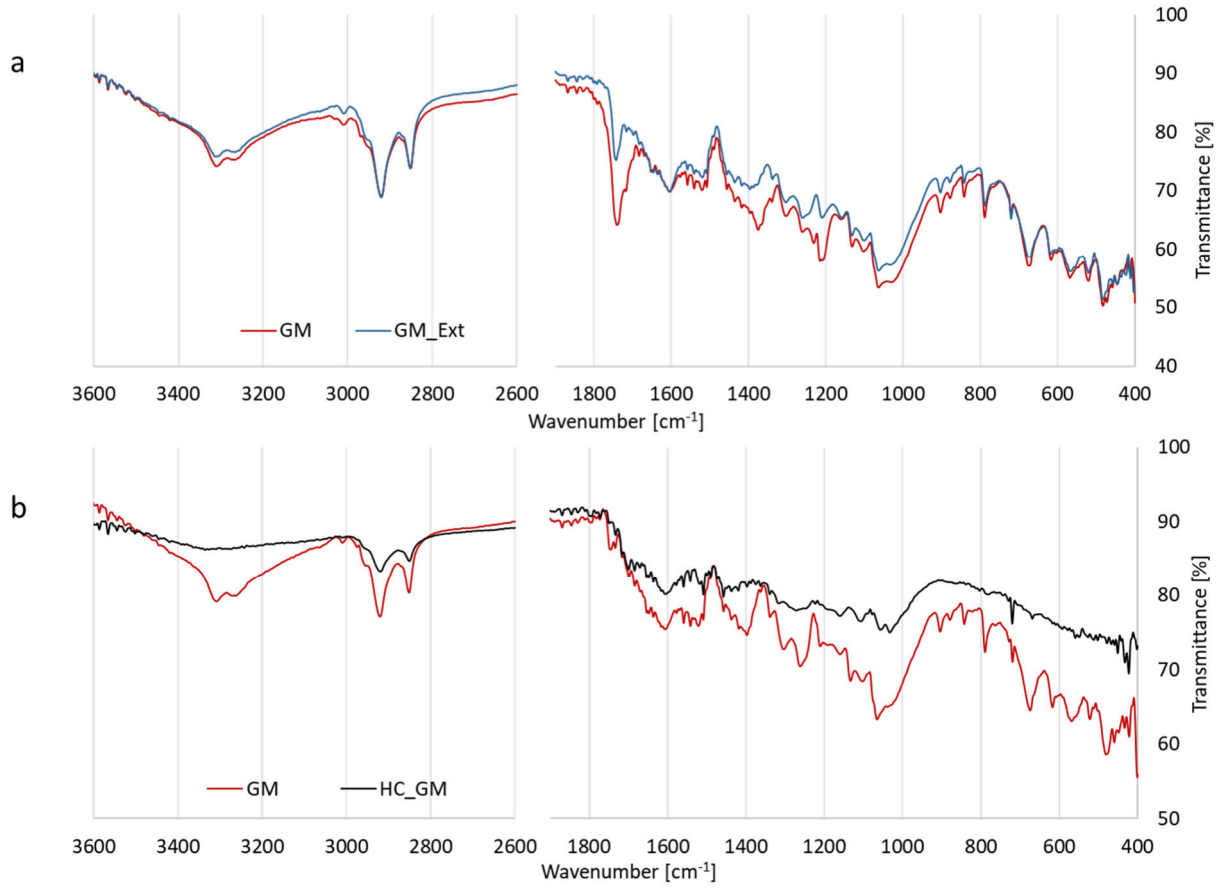


Fig. 1. FTIR spectra of a) GM and GM_Ext and b) GM and HC_GM.

Table 2. Process water characterization (average of triplicates). STD is reported in brackets.

Sample	pH [-]	EC [mS cm^{-1}]	TSS [g L^{-1}]	NVSS [g L^{-1}]	TOC [g L^{-1}]	COD [gO_2 L^{-1}]
PW_GM	4.40 (0.02)	12.52 (0.88)	24.73 (0.01)	9.84 (0.99)	9.69 (0.33)	33.28 (0.42)
PW_GM_Ext	4.37 (0.08)	11.37 (0.16)	21.59 (0.02)	8.90 (0.36)	7.68 (0.48)	31.08 (0.97)

EC = electrical conductivity; TSS = total suspended solids; NVSS = non-volatile suspended solids; TOC = total organic carbon; COD = chemical oxygen demand.

The product mass distribution in the solid, liquid and gas phases observed after the HTC treatment is reported in Table 3. The response to the conditions of the HTC process was similar for the two types of grape marc, regardless of the preliminary extraction. As described in 2.2, the solid content at the beginning of the HTC test was 10%. The treatment resulted in a dramatic reduction of the solid phase with a modest gasification. The solid reduction was likely due to both to dehydration reactions and solid mass solubilisation, the latter resulting in an increased density of the liquid solution. As for the fate of the inorganics, as much as 69.11 and 74.49 wtdb% of the initial ash content of the GM and GM_Ext, respectively, was solubilised in the process water.

Table 3 Solid, liquid and gas mass fractions after HTC treatment. STD is reported in brackets.

Phase	GM [wt%]	GM_Ext [wt%]
Solid (Hydrochar)	6.05 (0.07)	6.32 (0.08)
Liquid (Process water)	92.85 (0.13)	92.78 (0.05)
Gas	1.10 (0.06)	0.90 (0.03)

The results of the characterization analyses of the phases that result from the HTC treatment were used to perform mass balances for the elements carbon and nitrogen according to equation 4.

$$M_{IN} = M_{HC\,OUT} + M_{PW\,OUT} + M_{GAS\,OUT} = M_{OUT} \quad (4)$$

The carbon and nitrogen distributions in the HTC products are presented in Figure 2, where the gas phase was evaluated considering the measured volume and the density of CO_2 (1.87 kg m^{-3}) . Most of the carbon is

present in the solid phase, to a higher extent in the sample derived from GM_Ext while the carbon dissolved in the liquid phase is higher for the samples derived from GM. In contrast, nitrogen is almost uniformly distributed among the solid and liquid phases, as far as the latter is counted as a complement to 100 and assuming zero nitrogen in the gaseous phase, since the HTC gas is mainly composed by CO_2 (> 95%), CO, and H_2 (Libra et al., 2011).

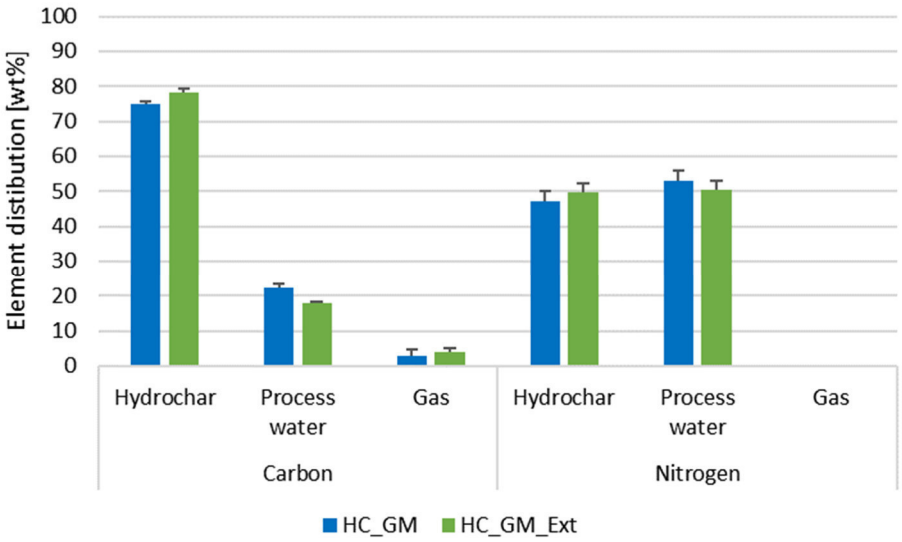


Fig. 2. Carbon and nitrogen distribution in the HTC products from GM and GM_Ext

3.2 Potential valorization of hydrochars

3.2.1 Assessment of the energetic valorization of hydrochars

In Figure 3, a Van Krevelen diagram, The feedstocks GM and GM_Ext fall within the typical area of biomass, while the obtained HC, as expected, approach the lignite region as a consequence of the higher grade of carbonization. Similar results were reported by Mariuzza et al. (2022) with O/C values around 0.3 and H/C approximately 1.1.

The Van Krevelen diagram highlights the transformation induced by the process, underlying that the carbonization decreased both the atomic ratios H/C and O/C as a results of dehydration and decarboxylation reactions (Funke & Ziegler, 2010; Libra et al., 2011). This is also supported by the FTIR analyses, which show a reduction in the number of carboxylic and hydroxyl groups due to HTC. The

diagram is usually used to define the quality of a solid fuel paired with the assessment of the higher heating value (HHV) data. The HHV of the samples is shown in Figure 4 along with the HHV of some common solid fuels. Through HTC, the energy content of the hydrochar (around 27 MJ kg^{-1}) was increased compared to the feedstock (around 19 MJ kg^{-1}), exceeding the values associated with the solid fuels considered. Similar HHVs were found by other authors like Mäkelä et al. (2017) and Mariuzza et al. (2022) who reported values of 26.5 and 26.26 MJ kg^{-1}, respectively.

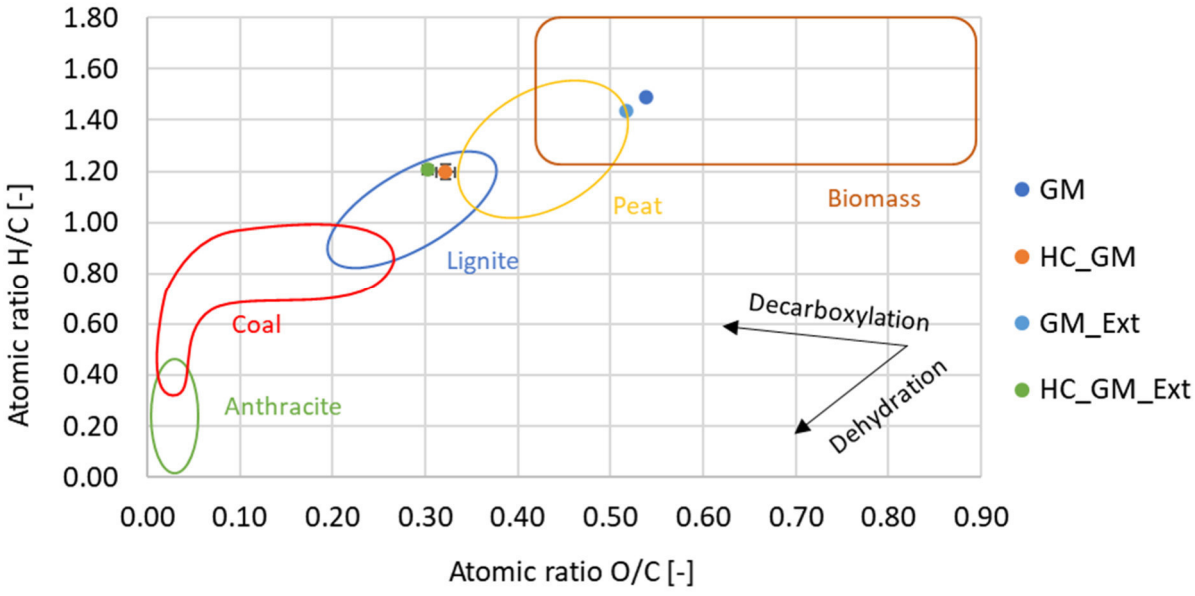


Fig. 3. Van Krevelen diagram of the feedstock (GM and GM_Ext) and hydrochars

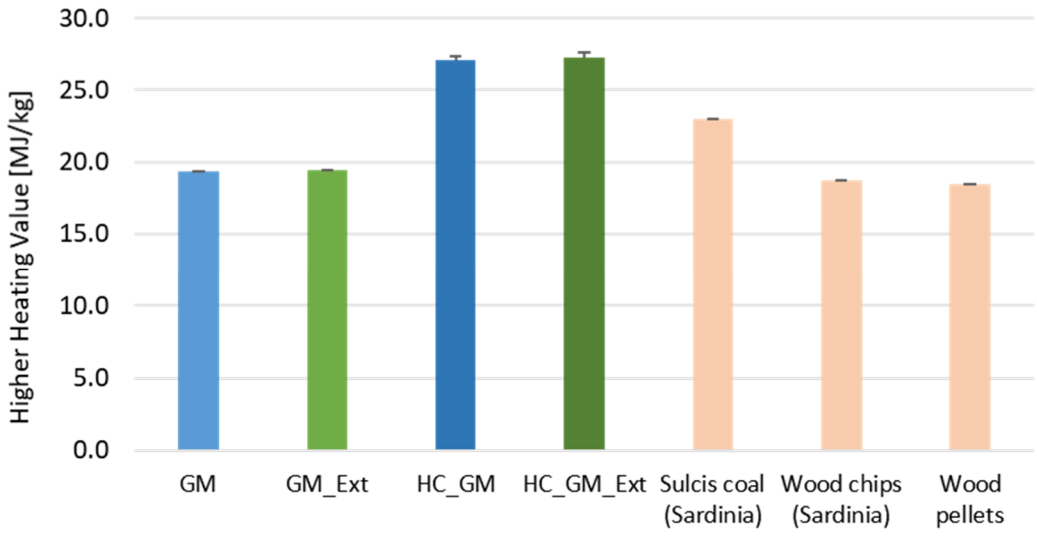


Fig. 4. HHV of feedstocks, hydrochar and some solid fuels of common use

3.2.2 Assessment of the potential use of hydrochar as a soil amendment

The results of the germination tests are shown in Table 4. When GM and HC_GM results are compared on the basis of the RI and Munoo-Liisa vitality index (which takes into account both the germination and growth aspects normalized by the control), hydrothermal carbonization is able to produce a better substrate for the germination and growth of seedlings. Replacing peat with an equivalent quantity of HC slightly lowers the germination rate, yet leads to longer seedlings compared to the use of raw GM. the best results were achieved for HC_GM_Ext. On the other hand, the higher the concentration of hydrochar, the lower the germination rate, with a high inhibition effect in the samples with 50% peat substitution. However, the presence of hydrochar in a percentage below 25% does not affect the germination significantly, but only the growth of the seedlings. the HC_GM_Ext_5% sample achieved a high RI and MLV values. In contrast, all the other samples have shown inhibition of germination and growth resulting in a lower MLV (less than 80%, which is considered the inhibition threshold as reported in Maunuksela et al. (2012). The lack of inhibition associated with HC_GM_Ext might be due to the preliminary ethanol extraction of GM (Wang et al., 2022). the production of aromatic compounds during the HTC process, as evidenced by the FTIR analysis, does not appear to affect the growth of the seedlings. The statistical analysis derived from one-way ANOVA and Tukey's HSD test is shown in Figure 5 where each letter links samples that are found to be statistically similar ($p < 0.05$). The statistical test confirmed that the samples HC_GM_Ext_5% belong to the same group as the control. It may suggest that the substitution of peat with a 5% hydrochar produced by HTC of extracted grape marc does not affect the seed germination and seedling growth. When hydrochar concentration higher than 5% was added, a detrimental effect on germination was evidenced, indicating that the use in growing medium can be carried out only to a limited extent. However, in a previous study (Farru et al., 2022) we found that some post-treatment can be applied to enhance the germination performance even at higher hydrochar concentrations. Also, it is demonstrated by different authors (Fryda et al., 2018; Lanza et al., 2016, 2018) that the addition of char to soil may replenish and store carbon in soil.

Table 4. Main parameters calculated in germination tests for each sample.

Sample	AGR [%]	*CVG [%]*	ARLP [cm]	*CVR [%]*	AHLP [cm]	*CVH [%]*	RI [%]	MLV [%]

Control	100	*0*	3.30	*12.58*	1.67	*4.54*	-	-
HC_GM_5%	96.66	*5.77*	2.19	*15.92*	1.74	*5.47*	66.26	63.67
HC_GM_25%	93.33	*11.55*	0.42	*35.46*	0.47	*40.69*	12.82	12.31
HC_GM_50%	0	*0*	0	*0*	0	*0*	0	0
HC_GM_Ext_5%	96.66	*5.77*	3.22	*6.56*	2.13	*2.58*	97.35	94.35
HC_GM_Ext_25%	96.66	*5.77*	0.89	*49.78*	0.70	*36.30*	27.20	26.74
HC_GM_Ext_50%	63.33	*15.28*	0	*0*	0.01	*2.44*	0	0
GM_5%	100	*0*	0.97	*28.22*	1.54	*13.25*	29.47	29.47
GM_25%	46.66	*40.41*	0.18	*59.16*	0.17	*54.27*	5.30	2.12
GM_50%	90	*10.00*	0	*0*	0.01	*0.00*	0	0

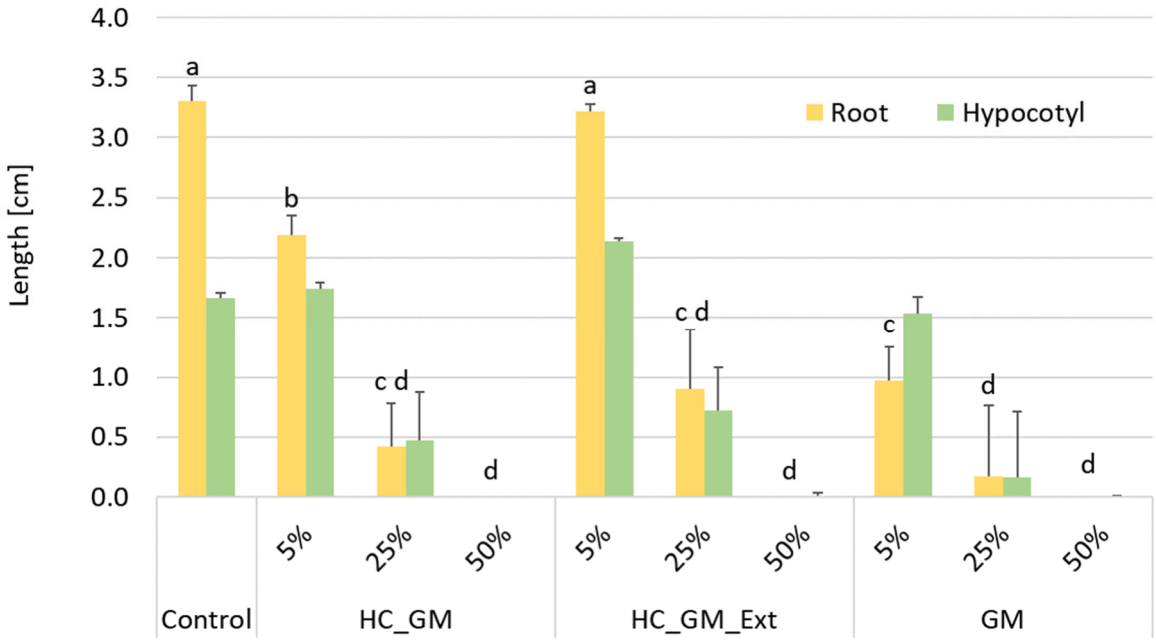


Fig. 5 Root and hypocotyl length of seedlings in germination tests and statistical results derived by Tukey's HSD test.

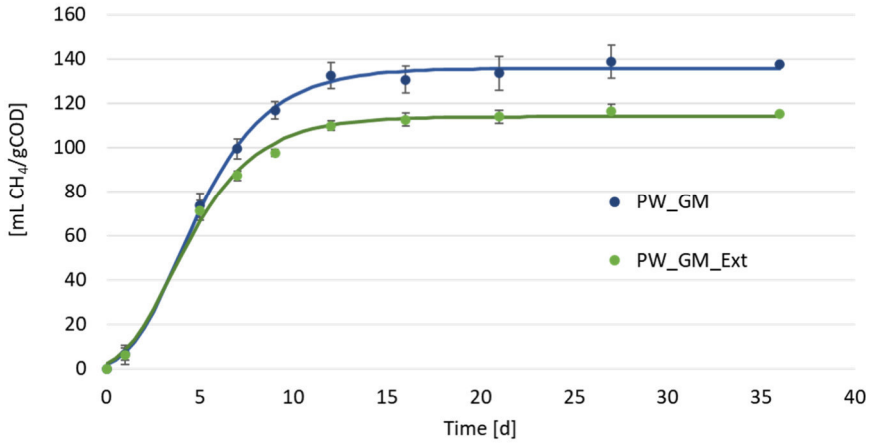
The HC produced from the GM is characterized by a lower respirometric activity, as evidenced by the respirometric index value RA_4, calculated between the end of the lag phase and the end of the test, of 9.95 $mgO_2\ gTS^{-1}$, that is 33% of that associated to HC_GM_Ext (29.9 $mgO_2\ gTS^{-1}$). This feature could be explained by a possible removal of soluble and biodegradable organic compounds during the preliminary phase of extraction. The hypothesis seems to be confirmed by what observed regarding the germination

tests; the greater presence of soluble and biodegradable compounds in HC from non-extracted GM and the consequent higher consumption of oxygen could have determined a competition effect between microorganisms and seeds with a prevalence of the former.

3.3 Potential valorization/management of PW

3.3.1 Biomethanization Potential of PW

The results of the BMP tests performed on the PW samples are summarized in Figure 6 in terms of average cumulative biogas production over time. Methane production started almost immediately in all tests and increased exponentially during the initial phase up to days 12 – 14, albeit at different rates depending on the samples tested. A plateau was reached after about 15 days. The final specific production of methane accounted for 137 and 115 mL CH_4 $gCOD^{-1}$ for PW_GM and PW_GM_Ext, respectively. These results are comparable to those reported by other authors (Ahmed et al., 2021; Parmar & Ross, 2019) for PW generated from HTC applied to sewage digestate or agricultural waste and fall in the range between 22 and 427 mL CH_4 $gCOD^{-1}$ indicated by Pagés-Díaz et al. (2020) for HTC liquid phase of organic wastes. The average volumetric methane content in the produced biogas was always higher than 40% v/v. The modified Gompertz model matches the BMP experimental results very closely ($R^2 > 0.99$); the kinetic parameters are reported in Table 5. The process water obtained from HTC of GM and GM_Ext produced methane with a short lag phase of 0.89 – 1.21 days, probably due to the presence of both highly acclimatized microbial consortia and readily degradable organic compounds (Xu et al., 2020). This is confirmed also by the obtained values of R_{max} (16.57 – 19.07 mL CH_4 $gCOD_i^{-1}$ d^{-1}), similar to those obtained by Gaur et al., (2020) for PW generated from HTC applied to sewage sludge. Pagés-Díaz et al. (2020) reported higher values of λ (0.1 - 7.3 mL CH_4 $gCOD_i^{-1}$ d^{-1}) and lower values of R_{max} obtained in similar experimental condition for PW generated from HTC applied to lignocellulosic wastes (6 - 13 mL CH_4 $gCOD_i^{-1}$ d^{-1}), explained by the formation of some toxic compounds released during the HTC. The lower values of maximum CH_4 production rate and Y_{CH4max} observed for PW_GM_Ext could be linked to the already mentioned possible removal of readily available organic compounds during the preliminary extraction treatment.



453

454 Fig. 6. Cumulative specific biomethane production from the HTC PW versus time and 95% confidence limits (solid
455 line indicates Gompertz-model curve).

456

457 Table 5. Kinetic parameters calculated for the BMP tests and associated 95% confidence limits.

Sample		$Y_{max}CH_4$ [mL CH_4 $gCOD_i^{-1}$]	R_{max} [mL CH_4 $gCOD_i^{-1}$ d^{-1}]	λ [d]	R^2 [-]
PW_GM	Value	135.71	19.07	1.21	0.998
	Lower 95% conf. limit	132.67	16.64	0.64	
	Upper 95% conf. limit	138.75	21.50	1.78	
PW_GM_Ext	Value	113.86	16.58	0.90	0.996
	Lower 95% conf. limit	110.35	13.86	0.19	
	Upper 95% conf. limit	117.38	19.30	1.61	

458

459 3.3.2 Assessment of the potential toxicity of PW in wastewater treatment plants

460 As autotrophic ammonium-oxidizing bacteria are characterized by slow growth rates and high sensitivity
461 to inhibitors compared to heterotrophs, they can be conveniently employed as test organisms to assess
462 the potential toxicity of liquid streams to be treated in wastewater treatment plants. Moreover, their
463 toxicological responses to several chemicals correlate well to that of *Vibrio fischeri* strain NRRL B11177
464 and methanogens (Ficara & Rozzi, 2001). The assessments performed in the present study showed
465 potential acute toxicity of PW_GM and PW_GM_Ext at relatively low dosage (the IC_{50} values were 0.5
466 and 2.0 mL L^{-1}, respectively) (Figure 7).

467

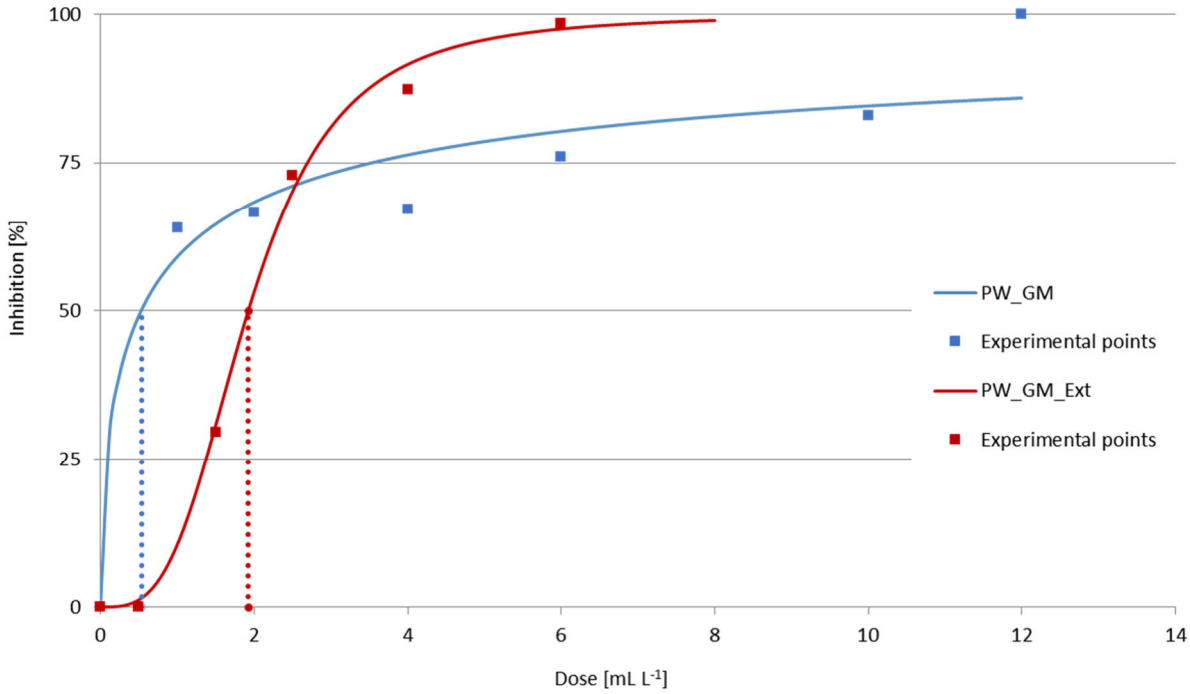


Fig. 7. Dose-Response curves showing the acute inhibiting effects of PW_GM and PW_GM_Ext on unacclimated activated sludge. A sigmoid-function was used to interpolate the experimental data (PW_GM, a=0.58, b= 0.694, R^2= 0.944; PW_GM_Ext, a= 3.28, b= 8.547, R^2= 0.997).

Milia et al., (2016b) reported that phenolic compounds might severely inhibit unacclimated activated sludge even at low concentrations. Accordingly, the higher inhibiting effect of PW_GM is likely due to the expected higher concentration of phenols. As reported in previous studies, liquid streams containing bio-recalcitrant compounds with high acute inhibiting effects may be successfully treated using advanced, low-cost, and efficient biological treatment technologies based on self-aggregated biomass such as aerobic granular sludge (Milia et al., 2016a), or well acclimated floc-shaped biomass (Milia et al., 2016b). In this sense, the high potential inhibiting effect observed in our study does not necessarily imply that aerobic biological treatment of PW_GM and PW_GM_Ext is not feasible, although particular attention should be paid to biomass selection and acclimation strategies.

4. CONCLUSIONS

This work demonstrated the possibility of developing a cascading biorefinery scheme for a multi-purpose valorization of grape marc residues. Hydrothermal carbonization, one of the emerging and most

promising technologies for the conversion of biomass and wet materials into added-value by-products, represents the core of this biorefinery approach, in which three consecutive processes were considered for their potential integration: a) the recovery of polyphenols by ethanol extraction, b) the production of added-value materials and energy through thermochemical conversion, and c) the production of CH_4-rich biogas through anaerobic digestion. The potential for the implementation of HTC in an integrated context of waste biorefinery was addressed by applying the process to both raw grape marc and grape marc previously subjected to polyphenol extraction and recovery treatment. The aerobic treatability of process water was also evaluated in view of its final disposal.

According to the mentioned strategy, the integrated production of bioproducts, soil amendments, peat substitutes, solid fuels, and biogas was demonstrated.

Compared to the feedstock, the produced hydrochars possess an increased content of fixed carbon and a reduced volatile solids and ash content, as well as an increased HHV and improved quality as a solid fuel, suggesting both the feasibility of using HC as a soil amendment for restoring its organic content and exploiting grape marc by producing energy. The outcomes of the germination tests indicated that HC could be used in low concentration to partially replace peat as a substrate for plant growth. This substitution could shorten the supply chain of soil improvers for Mediterranean grape-producing countries that do not have peat deposits.

The biogas production from PW assessed during the BMP test indicates that energetic valorization through anaerobic biological process may be a feasible option. Over 90% of the output mass from HTC is PW, therefore, this additional option allows for more extensive exploitation of grape marc and the minimization of the potential environmental impact of PW. In contrast, PW showed toxic effects on aerobic microorganisms suggesting that the feasibility of a traditional aerobic treatment must be carefully evaluated.

The results obtained and the comparison with what was observed for the process applied to non-extracted GM, although in need of further investigation, seem to suggest that integration can have positive effects in terms of HC quality as soil improver due to the improved biostability and the reduced phytotoxicity.

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REFERENCES

Ahmed, M., Andreottola, G., Elagroudy, S., Negm, M. S., & Fiori, L. (2021). Coupling hydrothermal carbonization and anaerobic digestion for sewage digestate management: Influence of hydrothermal treatment time on dewaterability and bio-methane production. *Journal of Environmental Management*, *281*, 111910. https://doi.org/10.1016/j.jenvman.2020.111910

Baratieri, M., Basso, D., Patuzzi, F., Castello, D., & Fiori, L. (2015). Kinetic and thermal modeling of hydrothermal carbonization applied to grape marc. *Chemical Engineering Transactions*, *43*, 505–510. https://doi.org/10.3303/CET1543085

Basso, D., Patuzzi, F., Castello, D., Baratieri, M., Rada, E. C., Weiss-Hortala, E., & Fiori, L. (2016). Agro-industrial waste to solid biofuel through hydrothermal carbonization. *Waste Management*, *47*, 114–121. https://doi.org/10.1016/j.wasman.2015.05.013

Baumgärtel, T., Kluth, H., Epperlein, K., & Rodehutscord, M. (2007). A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research*, *67*(2–3), 302–306. https://doi.org/10.1016/j.smallrumres.2005.11.002

Benavente, V., Lage, S., Gentili, F. G., & Jansson, S. (2022). Influence of lipid extraction and processing conditions on hydrothermal conversion of microalgae feedstocks – Effect on hydrochar composition, secondary char formation and phytotoxicity. *Chemical Engineering Journal*, *428*, 129559. https://doi.org/10.1016/j.cej.2021.129559

Binner, E., Böhm, K., & Lechner, P. (2012). Large scale study on measurement of respiration activity (AT4) by Sapromat and OxiTop. *Waste Management*, *32*(10), 1752–1759. https://doi.org/10.1016/j.wasman.2012.05.024

Boulanger, A., Pinet, E., Bouix, M., Bouchez, T., & Mansour, A. A. (2012). Effect of inoculum to substrate

ratio (l/S) on municipal solid waste anaerobic degradation kinetics and potential. *Waste Management*, *32*(12), 2258–2265. https://doi.org/10.1016/j.wasman.2012.07.024

Casazza, A. A., Aliakbarian, B., Lagazzo, A., Garbarino, G., Carnasciali, M. M., Perego, P., & Busca, G. (2016). Pyrolysis of grape marc before and after the recovery of polyphenol fraction. *Fuel Processing Technology*, *153*, 121–128. https://doi.org/10.1016/j.fuproc.2016.07.014

Catenacci, A., Boniardi, G., Mainardis, M., Gievers, F., Farru, G., Asunis, F., Malpei, F., Goi, D., Cappai, G., & Canziani, R. (2022). Processes, applications and legislative framework for carbonized anaerobic digestate: Opportunities and bottlenecks. A critical review. *Energy Conversion and Management*, *263*, 115691. https://doi.org/10.1016/j.enconman.2022.115691

Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, *2*(3), 198–209. https://doi.org/10.1038/s43016-021-00225-9

Devesa-Rey, R., Vecino, X., Varela-Alende, J. L., Barral, M. T., Cruz, J. M., & Moldes, A. B. (2011). Valorization of winery waste vs. The costs of not recycling. *Waste Management*, *31*(11), 2327–2335. https://doi.org/10.1016/j.wasman.2011.06.001

European Commission. (2001). *Working Document on the Biological Treatment of Biowaste*. http://www.cre.ie/docs/EU_BiowasteDirective_workingdocument_2nddraft.pdf

Fan, M., Dai, D., & Huang, B. (2012). Fourier Transform Infrared Spectroscopy for Natural Fibres. In S. Salih (Ed.), *Fourier Transform—Materials Analysis*. InTech. https://doi.org/10.5772/35482

Farru, G., Dang, C. H., Schultze, M., Kern, J., Cappai, G., & Libra, J. A. (2022). Benefits and Limitations of Using Hydrochars from Organic Residues as Replacement for Peat on Growing Media. *Horticulturae*, *8*, 325. https://doi.org/10.3390/horticulturae8040325

Ficara, E., & Rozzi, A. (2001). PH-Stat Titration to Assess Nitrification Inhibition. *Journal of Environmental Engineering*, *127*(8), 698–704. https://doi.org/10.1061/(ASCE)0733-9372(2001)127:8(698)

Fryda, L., Visser, R., & Schmidt, J. (2018). Biochar replaces peat in horticulture: Environmental impact assessment of combined biochar & bioenergy production. *Detritus*, *Volume 05-March 2019*(0), 1. https://doi.org/10.31025/2611-4135/2019.13778

Funke, A., & Ziegler, F. (2010). Hydrothermal carbonization of biomass: A summary and discussion of chemical mechanisms for process engineering. *Biofuels, Bioproducts and Biorefining*, *4*(2), 160–177. https://doi.org/10.1002/bbb.198

Gaur, R. Z., Khoury, O., Zohar, M., Poverenov, E., Darzi, R., Laor, Y., & Posmanik, R. (2020). Hydrothermal carbonization of sewage sludge coupled with anaerobic digestion: Integrated approach for sludge management and energy recycling. *Energy Conversion and Management*, *224*, 113353. https://doi.org/10.1016/j.enconman.2020.113353

Grossmann, M., Smit, I., & Loehnertz, O. (2007, November 20). *Biogenic amines and grapes: Effect of microbes and fining agents.* International symposium of microbiology and food safety, Vilafranca, Spain.

Ipiales, R. P., de la Rubia, M. A., Diaz, E., Mohedano, A. F., & Rodriguez, J. J. (2021). Integration of Hydrothermal Carbonization and Anaerobic Digestion for Energy Recovery of Biomass Waste: An Overview. *Energy & Fuels*, *35*(21), 17032–17050. https://doi.org/10.1021/acs.energyfuels.1c01681

Karl, K., & Tubiello, F. N. (2021). *Methods for estimating greenhouse gas emissions from food systems*. FAO. https://doi.org/10.4060/cb7028en

Lanza, G., Rebensburg, P., Kern, J., Lentzsch, P., & Wirth, S. (2016). Impact of chars and readily available carbon on soil microbial respiration and microbial community composition in a dynamic incubation experiment. *Soil and Tillage Research*, *164*, 18–24. https://doi.org/10.1016/j.still.2016.01.005

Lanza, G., Stang, A., Kern, J., Wirth, S., & Gessler, A. (2018). Degradability of raw and post-processed chars in a two-year field experiment. *Science of The Total Environment*, *628–629*, 1600–1608. https://doi.org/10.1016/j.scitotenv.2018.02.164

Libra, J. A., Ro, K. S., Kammann, C., Funke, A., Berge, N. D., Neubauer, Y., Titirici, M.-M., Fühner, C., Bens, O., Kern, J., & Emmerich, K.-H. (2011). Hydrothermal carbonization of biomass residuals: A comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels*, *2*(1), 71–106. https://doi.org/10.4155/bfs.10.81

Lin, J.-C., Mariuzza, D., Volpe, M., Fiori, L., Ceylan, S., & Goldfarb, J. L. (2021). Integrated thermochemical conversion process for valorizing mixed agricultural and dairy waste to nutrient-enriched biochars and biofuels. *Bioresource Technology*, *328*, 124765. https://doi.org/10.1016/j.biortech.2021.124765

Lucian, M., & Fiori, L. (2017). Hydrothermal Carbonization of Waste Biomass: Process Design, Modeling, Energy Efficiency and Cost Analysis. *Energies*, *10*(2), 211. https://doi.org/10.3390/en10020211

Lucian, M., Volpe, M., Gao, L., Piro, G., Goldfarb, J. L., & Fiori, L. (2018). Impact of hydrothermal carbonization conditions on the formation of hydrochars and secondary chars from the organic fraction of municipal solid waste. *Fuel*, *233*, 257–268. https://doi.org/10.1016/j.fuel.2018.06.060

Mäkelä, M., Kwong, C. W., Broström, M., & Yoshikawa, K. (2017). Hydrothermal treatment of grape marc for solid fuel applications. *Energy Conversion and Management*, *145*, 371–377. https://doi.org/10.1016/j.enconman.2017.05.015

Manca, M. L., Casula, E., Marongiu, F., Bacchetta, G., Sarais, G., Zaru, M., Escribano-Ferrer, E., Peris, J. E., Usach, I., Fais, S., Scano, A., Orrù, G., Maroun, R. G., Fadda, A. M., & Manconi, M. (2020). From waste to health: Sustainable exploitation of grape pomace seed extract to manufacture antioxidant, regenerative and prebiotic nanovesicles within circular economy. *Scientific Reports*, *10*(1), 14184. https://doi.org/10.1038/s41598-020-71191-8

Mariuzza, D., Lin, J.-C., Volpe, M., Fiori, L., Ceylan, S., & Goldfarb, J. L. (2022). Impact of Co-Hydrothermal carbonization of animal and agricultural waste on hydrochars' soil amendment and solid fuel properties. *Biomass and Bioenergy*, *157*, 106329. https://doi.org/10.1016/j.biombioe.2021.106329

Maunuksela, L., Herranen, M., & Torniainen, M. (2012). Quality Assessment of Biogas Plant End Products by Plant Bioassays. *International Journal of Environmental Science and Development*, 305–310. https://doi.org/10.7763/IJESD.2012.V3.236

Milia, S., Malloci, E., & Carucci, A. (2016a). Aerobic granulation with petrochemical wastewater in a sequencing batch reactor under different operating conditions. *Desalination and Water Treatment*, 1–10. https://doi.org/10.1080/19443994.2016.1191778

Milia, S., Porcu, R., Rossetti, S., & Carucci, A. (2016b). Performance and Characteristics of Aerobic Granular Sludge Degrading 2,4,6-Trichlorophenol at Different Volumetric Organic Loading Rates: Water. *CLEAN - Soil, Air, Water*, *44*(6), 615–623. https://doi.org/10.1002/clen.201500127

Muhlack, R. A., Potumarthi, R., & Jeffery, D. W. (2018). Sustainable wineries through waste valorisation: A review of grape marc utilisation for value-added products. *Waste Management*, *72*, 99–118. https://doi.org/10.1016/j.wasman.2017.11.011

Muntoni, A. (2019). Waste biorefineries: Opportunities and perspectives. *Detritus*, *Volume 05-March 2019*(0), 1. https://doi.org/10.31025/2611-4135/2019.13791

Nguyen, D., Zhao, W., Mäkelä, M., Alwahabi, Z. T., & Kwong, C. W. (2022). Effect of hydrothermal

carbonisation temperature on the ignition properties of grape marc hydrochar fuels. *Fuel*, *313*, 122668. https://doi.org/10.1016/j.fuel.2021.122668

Nielfa, A., Cano, R., & Fdz-Polanco, M. (2015). Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports*, *5*, 14–21. https://doi.org/10.1016/j.btre.2014.10.005

OIV - International Organisation of Vine and Wine Intergovernmental Organisation. (2021). *Statistical Report on World Vitiviniculture*. https://www.oiv.int/public/medias/7909/oiv-state-of-the-world-vitivinicultural-sector-in-2020.pdf

Pagés-Díaz, J., Cerda Alvarado, A. O., Montalvo, S., Diaz-Robles, L., & Curio, C. H. (2020). Anaerobic bio-methane potential of the liquors from hydrothermal carbonization of different lignocellulose biomasses. *Renewable Energy*, *157*, 182–189. https://doi.org/10.1016/j.renene.2020.05.025

Parmar, K. R., & Ross, A. B. (2019). Integration of Hydrothermal Carbonisation with Anaerobic Digestion; Opportunities for Valorisation of Digestate. *Energies*, *12*(9), 1586. https://doi.org/10.3390/en12091586

Perra, M., Lozano-Sánchez, J., Leyva-Jiménez, F.-J., Segura-Carretero, A., Pedraz, J. L., Bacchetta, G., Muntoni, A., De Gioannis, G., Manca, M. L., & Manconi, M. (2021). Extraction of the antioxidant phytocomplex from wine-making by-products and sustainable loading in phospholipid vesicles specifically tailored for skin protection. *Biomedicine & Pharmacotherapy*, *142*, 111959. https://doi.org/10.1016/j.biopha.2021.111959

Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology*, *17*(11), 579–590. https://doi.org/10.1016/j.tifs.2006.05.003

Sánchez Arias, V., Fernández, F. J., Rodríguez, L., & Villaseñor, J. (2012). Respiration indices and stability measurements of compost through electrolytic respirometry. *Journal of Environmental Management*, *95*, S134–S138. https://doi.org/10.1016/j.jenvman.2010.10.053

Wang, G., Yang, Y., Kong, Y., Ma, R., Yuan, J., & Li, G. (2022). Key factors affecting seed germination in phytotoxicity tests during sheep manure composting with carbon additives. *Journal of Hazardous Materials*, *421*, 126809. https://doi.org/10.1016/j.jhazmat.2021.126809

Wang, T., Zhai, Y., Zhu, Y., Li, C., & Zeng, G. (2018). A review of the hydrothermal carbonization of biomass waste for hydrochar formation: Process conditions, fundamentals, and physicochemical

661 properties. *Renewable and Sustainable Energy Reviews*, *90*, 223–247.
662 https://doi.org/10.1016/j.rser.2018.03.071
663 Xu, H., Li, Y., Hua, D., Zhao, Y., Mu, H., Chen, H., & Chen, G. (2020). Enhancing the anaerobic digestion
664 of corn stover by chemical pretreatment with the black liquor from the paper industry. *Bioresource*
665 *Technology*, *306*, 123090. https://doi.org/10.1016/j.biortech.2020.123090
666 Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & van 't Riet, K. (1990). Modeling of the Bacterial
667 Growth Curve. *Applied and Environmental Microbiology*, *56*(6), 1875–1881.
668 https://doi.org/10.1128/aem.56.6.1875-1881.1990
669