CHEMICAL ENGINEERING TRANSACTIONS
VOL. 109, 2024
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Leonardo Tognotti, Rubens Maciel Filho, Viatcheslav Kafarov

ISBN 979-12-81206-09-0; **ISSN** 2283-9216
DOI: 10.3303/CET24109093

Hydrothermal Carbonization of Aquatic Biomass: a Promising Solution for the Invasive Species *Myriophyllum Aquaticum*

Federica Barontini[a,*], Marco Landi[b], Nicola Silvestri[b], Monica Puccini[a]

[a]Dipartimento di Ingegneria Civile e Industriale, Università di Pisa, Largo Lucio Lazzarino 2, 56122 Pisa, Italy.
[b] Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy.
federica.barontini@unipi.it

Myriophyllum aquaticum (Vell.) Verdc., an invasive aquatic plant from South America, is to date worldwide distributed. In Tuscany *M. aquaticum* has colonized much of the surface water network of reclamation channels, forcing the reclamation authority to its removal, with the production of a huge amount of wet biomass to be disposed as a biological waste. In the present study the hydrothermal carbonization (HTC) process was proposed and investigated as a path for the valorisation of this waste biomass. Performed in aqueous conditions at moderate temperatures, HTC does not require energy-intensive pre-drying steps, making HTC applicable to biomass feedstocks with high moisture contents. HTC leads to a carbonaceous solid product, referred to as hydrochar, with different potential applications, such as solid biofuel, or soil-improving material. An experimental investigation was carried out performing HTC tests in a laboratory-scale reactor. The joint effect of operating parameters (temperature, residence time and solid load) on process yields and hydrochar properties was investigated by Design of Experiments – Response Surface Methodology (DoE-RSM), a statistical and mathematical approach for process analysis, prediction, and optimization. The results obtained demonstrated the feasibility of HTC for *M. aquaticum*, suggesting HTC as a promising treatment path. Beyond the use as biofuel, the suitability of the hydrochar produced from *M. aquaticum* as organic growth medium for vegetable seedlings in horticulture and gardening or soil amendment was evaluated. Potential phytotoxic effects of *M. aquaticum* hydrochar were evaluated through bioassays using cress seeds (*Lepidium sativum* L.) as a model species.

1. Introduction

Myriophyllum aquaticum (Vell.) Verdc. (Parrot's Feather) is an amphibious aquatic macrophyte native to South America, which is to date worldwide distributed. It was firstly found in Italy in the early 90s and afterwards the sightings concerned many regions of central and northern Italy. In 2004, *M. aquaticum* was recorded in Tuscany where colonized much of the surface water network of reclamation channels, thereby significantly hindering the normal water outflow. The north-Tuscany reclamation authority has been forced to remove *M. aquaticum* from reclamation channels, producing a huge amount of wet biomass to be disposed as a biological waste. Conversely, it could be usefully allocated to virtuous processes of biomass reuse (according to Agenda 2030 prerogatives, e.g. SDG 12).

In this context, the authors' aim is to propose and study a path for the valorisation of this biomass. In recent years hydrothermal carbonization (HTC) has been receiving increasing attention as a sustainable thermochemical process for the valorisation of waste biomass with high moisture content. Performed in aqueous conditions at moderate temperatures (ranging from 180 to 300 °C) under autogenous pressure, HTC leads to a carbonaceous solid product, referred to as hydrochar (Wang et al., 2018). Various applications of hydrochar have been proposed, such as solid biofuel, electrode material in energy storage devices (capacitors, supercapacitors, and batteries), adsorbent for wastewater treatment, and soil-improving material (Sharma et al., 2020). Unlike other thermochemical processes, HTC does not require energy-intensive pre-drying of the feedstock. This makes HTC applicable to a wide variety of biomass feedstocks with variable but high moisture contents. Several researchers have investigated the possibility of applying HTC as a treatment for different types of substrates, including agriculture residues, agro-industrial wastes, food waste, organic fraction of municipal

Paper Received: 13 January 2024; Revised: 24 February 2024; Accepted: 5 April 2024
Please cite this article as: Barontini F., Landi M., Silvestri N., Puccini M., 2024, Hydrothermal Carbonization of Aquatic Biomass: a Promising Solution for the Invasive Species Myriophyllum Aquaticum, Chemical Engineering Transactions, 109, 553-558 DOI:10.3303/CET24109093

solid waste, and sewage sludge (Cavali et al., 2023). However, to the best of the authors' knowledge, no results are available in the scientific literature concerning the HTC of *M. aquaticum*.

Within this framework, the specific aims of the present study are multiple and interconnected: i) prove the feasibility of HTC for *M. aquaticum*; ii) investigate the joint effect of operating parameters on the process yields and hydrochar properties; iii) evaluate a potential application of the hydrochar produced from *M. aquaticum*.

HTC experimental tests were carried out in a laboratory-scale reactor. The joint effect of process parameters was investigated by Design of Experiments – Response Surface Methodology (DoE-RSM), a statistical and mathematical approach for analysing, predicting, and optimising a wide range of systems. DoE-RSM is particularly useful for the study and the optimization of the HTC process due to the complexity of HTC reactions which limits scope of process simulation. Among the potential applications of hydrochar, there is a growing interest on its use as organic growth medium for vegetable seedlings in horticulture and gardening (as peat substitute in potting mix) or soil amendment (Islam et al., 2021). The suitability of the hydrochar produced from *M. aquaticum* was evaluated in terms of potential phytotoxicity throughout germination and growth tests in microcosms.

2. Materials and Methods

2.1 Feedstock

M. aquaticum biomass was collected from Barra channel in the catchment of Lake Massaciuccoli (Tuscany, Italy) in March 2023. Harvested biomass was washed with tap water to remove adhering matter. For HTC experiments, the biomass was dried at 105 °C and ground to size < 1 mm. Table 1 summarizes the results of feedstock characterization, i.e., Volatile Matter (VM), Fixed Carbon (FC) and Ash content, Carbon (C), Hydrogen (H), Nitrogen (N) and Oxygen (O) content, and Higher Heating Value (HHV).

Table 1: Results of feedstock characterization (on a dry basis)

VM [wt %]	FC [wt %]	Ash [wt %]	C [wt %]	H [wt %]	N [wt %]	O [wt %]	HHV [MJ/kg]
54.56	12.37	33.07	32.09	4.08	2.14	28.62	12.20

2.2 Design of Experiments – Response Surface Methodology

HTC tests were planned according to the DoE-RSM approach. Temperature (A), reaction time (B), and solid content (C), were selected as independent variables (factors). Table 2 shows the levels selected for the process variables. A face centered central composite design was applied to investigate the possible non-linear interactions between the factors. The experimental design matrix was composed of 20 runs, with 8 runs at factorial points, 6 runs at axial points, and 6 replicates at the central point. The experimental sequence was randomized with the aim to reduce the effects of uncontrolled factors. The responses selected to assess the process yields and the properties of the obtained hydrochars were the following: hydrochar yield (Y); carbon yield (C yield); hydrochar H/C atomic ratio; hydrochar O/C atomic ratio; hydrochar ash content; hydrochar higher heating value (HHV); energy densification (ED); energy yield (EY). Hydrochar yield, carbon yield, energy densification, and energy yield were defined as reported in Eqs (1)–(4).

$$Y = \frac{mass\ of\ dry\ hydrochar}{mass\ of\ dry\ feedstock} \cdot 100 \tag{1}$$

$$C\ yield = Y \cdot \frac{C\ content\ of\ dry\ hydrochar}{C\ content\ of\ dry\ feedstock} \cdot 100 \tag{2}$$

$$ED = \frac{HHV_{dry\ hydrochar}}{HHV_{dry\ feedstock}} \tag{3}$$

$$EY = Y \cdot \frac{HHV_{dry\ hydrochar}}{HHV_{dry\ feedstock}} \tag{4}$$

The analysis of variance (ANOVA) was used to assess suitable models for each response. The Design Expert® 13 (Stat-Ease) software was employed to carry out DoE/RSM trials.

Table 2: Independent variables in HTC experiments: name, units and levels

Factor	Low level (-1)	High level (+1)
A: Temperature [°C]	200	260
B: Time [min]	30	210
C: Solid content [wt %]	5	25

2.3 Hydrothermal Carbonization experiments

HTC experiments were carried out in a 300 mL stainless-steel PARR reactor. Further details on the experimental apparatus are reported elsewhere (Barontini et al., 2023). HTC tests were performed at different reaction temperatures, reaction times, and solid content, according to the randomized design matrix obtained by DoE-RSM. In each test, the reactor was loaded with *M. aquaticum* and demineralized water in order to reach the required solid content value. The slurry obtained at the end of the HTC test was recovered from the reaction vessel and the solid fraction was separated by vacuum filtration. The solid product (hydrochar) was washed with water, dried at 105 °C for 12 hours, weighed and stored at 4 °C for characterization and phytotoxicity tests.

2.4 Feedstock and hydrochar characterization

The physicochemical characterization of *M. aquaticum* and hydrochar samples was performed following the methodologies briefly described herein. Proximate analysis was performed by thermogravimetric analysis employing a TA Instruments Q-500 analyzer. Ultimate analysis was carried out with a LECO TruSpec CHN Elemental Analyzer, the oxygen content was evaluated by difference. A LECO AC-500 Calorimeter was used for heating value determination.

2.5 Phytotoxicity tests

Hydrochar phytotoxicity was assessed through bioassays using cress seeds (*Lepidium sativum* L.), as a model species, according to the Italian regulation (D.Lgs. n. 75/2010) and the methodology reported by APAT (APAT 20/2003).

Hydrochar water extract

For the germination test, hydrochar water extracts from hydrochar with elemental composition similar to peat (HC peat) and lignite (HC lignite) were obtained as follows. Hydrochar samples (20 g) were watered to 85 % humidity with Milli-Q water and shaken for two hours (MR-12 Rocker-Shaker, Biosan) at maximum speed. Subsequently, samples were centrifuged for 15 min at 5000 rpm (MPW med. instruments, Warsaw, Poland), and the obtained supernatants were filtered using a vacuum pump (Delchimica Scientific Glassware Srl, Naples, Italy). Due to the high water-retention ability of HC peat, no water extract was obtained from this treatment. The water extract of HC lignite was diluted to 30% (v/v) with Milli-Q water.

Germination test

For the germination test, 1.5 mL of 30 % (v/v) diluted aqueous hydrochar extract was added to 9 cm diameter Petri dishes (n = 3) containing filter paper and 10 *L. sativum* seeds previously soaked in distilled water for an hour. As a control, 1.5 mL Milli-Q water was used instead of the aqueous hydrochar extract. Finally, the Petri dishes were incubated in the dark for 48 hours at 25 °C.

After incubation, the germination index (I_g) was determined using the following formula:

$$I_g\ (\%) = \frac{Gt \times Lt}{Gc \times Lc} \times 100 \qquad (5)$$

where *Gt* and *Gc* were the average number of germinated seeds in the treated and the control Petri dishes, respectively, while *Lt* and *Lc* were the average root lengths of the treated and control samples.

Growth test

A growth test was performed in 0.55 L pots (n = 5) filled with a layer of expanded clay at the bottom, followed by a substrate consisting of sand and peat 1:1 (v/v) with the addition of 75 g L^{-1} of hydrochar, and finally by a layer of sand on the top. Control pots were made up to volume with the sand and peat substrate. In each pot, 20 seeds were sown and covered with a perlite layer.

Aboveground biomasses were collected after 21 days from sowing to determine fresh weights. To determine dry weights, samples were dried in a ventilated oven (Memmert GmbH Co., KG Universal Oven UN30, Schwabach, Germany) at 105 °C until a constant weight was reached.

The growth index (G_m) was calculated according to the following formula:

$$G_m\ (\%) = \frac{Gt}{Gc} \times 100 \qquad (6)$$

where *Gt* and *Gc* represents the average plant dry weight in treated and in the control pots, respectively.

Statistical analysis

After checking the normality of distribution (Shapiro-Wilk test, 95% confidence interval), the difference between the treatment and the control in the germination test was analyzed by unpaired t-test. On the growth test results, a non-parametric Kruskal-Wallis test was performed and significant differences among treatments were determined by Dunn's multiple comparisons *post-hoc* test ($p \leq 0.05$). GraphPad software (GraphPad, La Jolla, CA, USA) was used for the statistical analysis of the bioassays.

3. Results and Discussion

3.1 Results of HTC tests

The HTC treatment converted the *M. aquaticum* sample into a carbonaceous solid product enriched in total and fixed carbon and characterized by lower atomic ratios of hydrogen to carbon (H/C) and oxygen to carbon (O/C) compared to the initial feedstock (Figure 1). During the HTC process, biomass undergoes a series of reactions including hydrolysis, dehydration, decarboxylation, condensation, polymerization, and aromatization. The extent of these reactions mostly depends on the type of feedstock and on reaction conditions like temperature, residence time and solid content. This complex network of reactions lowers atomic H/C and O/C ratios. The extent of carbonization (or coalification) can be visualized using the Van Krevelen diagram (Figure 1). The Van Krevelen diagram helps to assess the extent of the carbonization process, while comparing it with other fossil fuels like peat, lignite and coal. The results obtained for hydrochars produced in different process conditions are reported in Figure 1. To characterize HTC operating conditions and compare hydrochars obtained with different temperature and residence time, the severity factor *f* was used in Figure 1. The severity factor *f* is defined as:

$$f = 50 \cdot t^{0.2} \cdot e^{-\frac{3500}{T}} \qquad (7)$$

where *t* is the duration of the treatment (s) and *T* is the temperature (K). An enhanced process severity is related to a higher temperature and/or major residence time. The results shown in Figure 1 evidence that the HTC process applied to *M. aquaticum* is effective for the production of hydrochars with elemental composition similar to peat and lignite. As expected, the higher the process severity, the higher the carbonization degree.

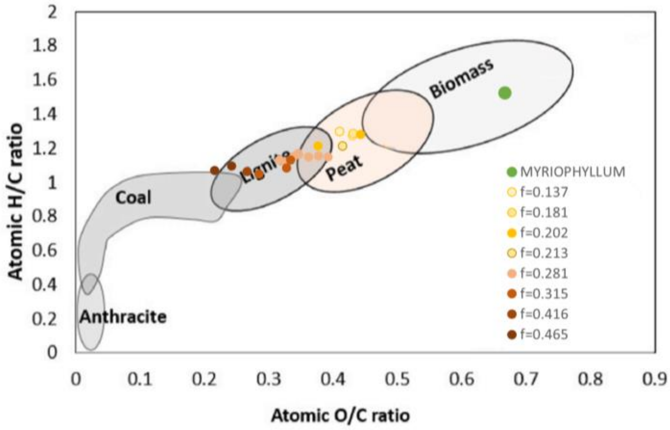

Figure 1: Atomic H/C versus O/C ratio (Van Krevelen diagram) of Myriophyllum aquaticum and hydrochars obtained in different process severity (f factor) conditions.

The influence of temperature, time and solid content on the process yields and properties of the hydrochar produced was analyzed by the RSM approach. The effect of the key operative parameters on the target responses, as well as the influence of their interaction, was investigated, with the aim to provide insight into the choice of the operating conditions required to fit the desired application. Regression calculations were performed to fit polynomial models to the selected responses. The effects (main and interactions) were calculated for all model terms together with statistics values, such as p-values, lack of fit (which determines how well the model fits the experimental data) and adjusted and predicted R^2. The analysis of variance (ANOVA) was applied to verify the statistical significance of all the terms of the obtained regression. For each response, the software suggested a model that is statistically significant with high values for adjusted and predicted R^2. Some of the results obtained are reported and discussed next.

A reduced cubic model was obtained for hydrochar yield. The parametric model in terms of coded values is reported in Eq (8):

$$Y = 58.13 - 4.99A - 3.28B + 1.69C + 0.0399AB + 0.1438AC + 0.7284A^2 - 1.45C^2 + 2.34A^2B + 0.7064A^2C \qquad (8)$$

The relationship between process parameters and responses may be graphically represented by three-dimensional response surface plots. Figure 2 shows the response surfaces of the hydrochar yield as a function of temperature and time for different values of the solid content. It can be observed the hydrochar yield increases with increasing solid content and decreasing temperature and residence time. As expected, temperature greatly influences solid yield. Temperature enhances the decomposition reactions of the components of the lignocellulosic biomass. A decrease in the solid yield with increasing temperature is mainly expected from the solubilisation of reaction intermediates into the aqueous phase. The present results are in agreement with the findings of previous studies on HTC of various feedstocks (Nizamuddin et al., 2017). The observed increase in hydrochar yield at increasing solid load may be related to secondary char formation, suggesting that increasing the solid content causes polymerisation of chemical species from the liquid phase.

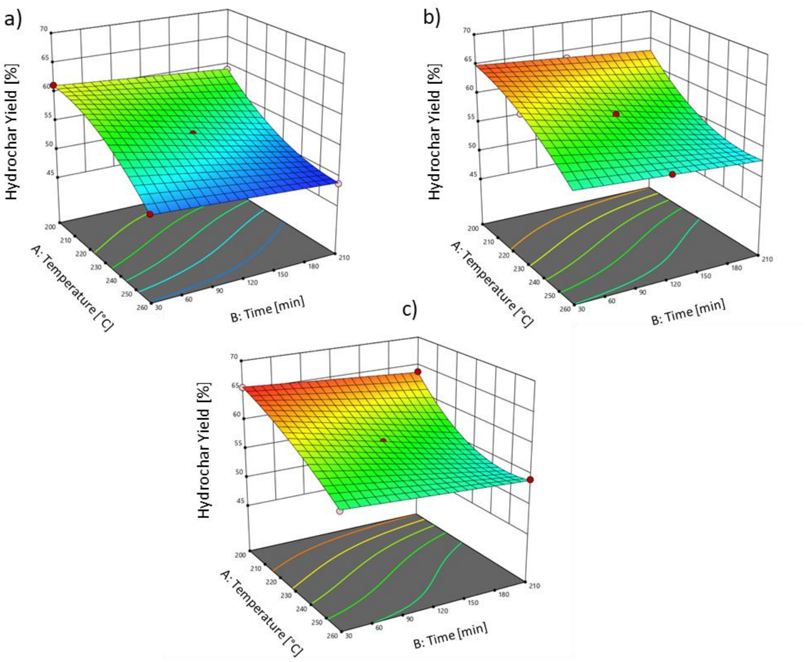

Figure 2: 3D response surface graphs obtained by RSM for hydrochar yield as a function of temperature and time; (a) 5 wt % solid content, (b) 15 wt % solid content, (c) 25 wt % solid content.

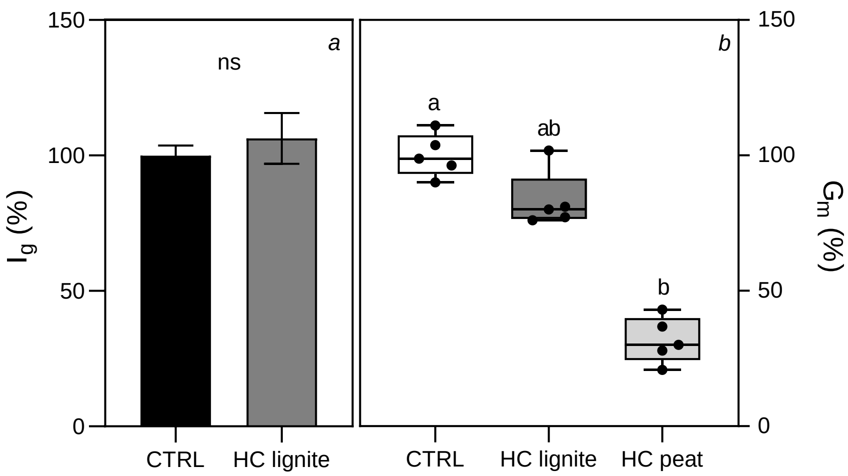

Figure 3: Gemination index (I_g; a) of Lepidium sativum *in control (CTRL) or treated with water extract of hydrochar with an elemental composition similar to peat (HC peat). Means (n =3 ± SD) were subjected to an* unpaired t-test. ns*, non-significative difference.* G*rowth index (G_m;* b*) of* Lepidium sativum *in control (CTRL) condition and after treatment with hydrochars with elemental composition similar to lignite (HC lignite) or peat (HC peat). Means (n =5 ± SD) were subjected to a non-parametric Kruskal-Wallis test and significant differences among treatments were determined by Dunn's multiple comparisons* post-hoc *test (*p ≤ *0.05) and indicated with different letters.*

3.2 Results of phytotoxicity tests

Hydrochars obtained from a range of raw material might contain some potentially phytotoxic compounds which usually are water soluble and are part of the dissolved organic carbon fraction, thus posing a threat for seed germination and plant growth (Bargmann et al., 2013; Lang et al., 2023). In the present experiment, the use of hydrochar with an elemental composition similar to lignite (HC lignite) did not reveal any significant phytotoxicity effect on germination efficiency within 48 h after sowing nor in germination index after 21 days (Figure 3a,b), thus suggesting that HC lignite may have low content in phytotoxic compounds. Moreover, Luutu et al. (2023) reported that high HTC (260 °C) has no effect on seedlings emergence. Despite data on germination index on HC peat were not available, the growth index revealed a possible phytotoxicity after chronic seedlings exposure (Figure 3b). These results contrast with previous experiment which evidenced that higher HTC temperatures are most likely to increase its phytotoxic compound content (Celletti et al., 2021; Luutu et al., 2023). Anyway, these tests represent only a preliminary screening to disentangle possible phytotoxicity of hydrochar obtained by *M. aquaticum* and further agronomic studies must be performed.

4. Conclusions

The results of the present study offer the clear evidence that HTC of *M. aquaticum* can be a win-win strategy to a wise and sustainable reuse of this biomass derived from an alien invasive species allowing to produce a product (hydrochar) which can be proficiently used as a substrate for plant cultivation. Further investigation is recommended, as the produced hydrochar offers promising potential for agronomic applications.

Acknowledgments

The study has been funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 1561 of 11.10.2022 of Ministero dell'Università e della Ricerca (MUR); funded by the European Union – NextGenerationEU; Project code PE0000021, Concession Decree No. 1561 of 11.10.2022 adopted by Ministero dell'Università e della Ricerca (MUR), CUP I53C22001450006, Project title "Network 4 Energy Sustainable Transition – NEST".

References

APAT (Italian Environmental Agency), 2003. Metodi microbiologici di analisi del compost. www.isprambiente.gov.it/contentfiles/00003500/3544-manuali-2003-20.pdf/

Bargmann, I., Rillig, M. C., Buss, W., Kruse, A., & Kuecke, M., 2013, Hydrochar and biochar effects on germination of spring barley. Journal of agronomy and crop science, 199(5), 360-373.

Barontini F., Vitolo S., Gori R., Trivelli L., Puccini M., 2023, The Hydrothermal Carbonization Process for Waste Valorisation: a Study on the Effect of Process Conditions on the Yield and Properties of Hydrochars from Municipal Solid Waste, Chemical Engineering Transactions, 99, 181-186.

Cavali M., Libardi J.N., Dutra de Sena J., Woiciechowski A.L., Soccol C.R., Filho P.B., Bayard R., Benbelkacem H., Borges de Castilhos J.A., 2023, A review on hydrothermal carbonization of potential biomass wastes, characterization and environmental applications of hydrochar, and biorefinery perspectives of the process, Science of the Total Environment, 857, 159627.

Celletti, S., Bergamo, A., Benedetti, V., Pecchi, M., Patuzzi, F., Basso, D., ... & Mimmo, T., 2021, Phytotoxicity of hydrochars obtained by hydrothermal carbonization of manure-based digestate. Journal of Environmental Management, 280, 111635.

D.Lgs 29 April 2010 n°75, 2009. Riordino e revisione della disciplina in materia di fertilizzanti, a norma dell'articolo 13 della legge 7 luglio 2009. vol. 88. www.gazzettaufficiale.it/eli/id/2010/05/26/010G0096/sg

Islam Md.A., Limon Md.S.H., Romić M., Islam Md.A., 2021, Hydrochar-based soil amendments for agriculture: a review of recent progress, Arabian Journal of Geosciences, 14, 102.

Lang, Q., Guo, X., Wang, C., Li, L., Li, Y., Xu, J., ... & Zou, G., 2023, Characteristics and phytotoxicity of hydrochar-derived dissolved organic matter: Effects of feedstock type and hydrothermal temperature, Journal of Environmental Sciences. doi.org/10.1016/j.jes.2023.10.007

Luutu, H., Rose, M. T., McIntosh, S., Van Zwieten, L., Weng, H. H., Pocock, M., & Rose, T. J., 2023, Phytotoxicity induced by soil-applied hydrothermally-carbonised waste amendments: effect of reaction temperature, feedstock and soil nutrition. Plant and soil, 1-15.

Nizamuddin S., Baloch H.A., Griffin G.J., Mubarak N.M., Bhutto A.W., Abro R., Mazari S.A., Ali B.S., 2017, An overview of effect of process parameters on hydrothermal carbonization of biomass, Renewable and Sustainable Energy Reviews, 73, 1289-1299.

Sharma H.B., Sarmah A.K., Dubey B., 2020, Hydrothermal carbonization of renewable waste biomass for solid biofuel production: A discussion on process mechanism, the influence of process parameters, environmental performance and fuel properties of hydrochar, Renewable and Sustainable Energy Reviews, 123, 109761.

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 properties, Renewable and Sustainable Energy Reviews, 90, 223-247.