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Biomass Waste Processing into Artificial Humic Substances

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***Abstract* – To address the biomass waste processing problem and transform it into refractory forms of carbon, recently suggested hydrothermal carbonization (HTC) of biomass in alkaline environment can be considered as a prospective and climate neutral approach. Biomass HTC provides possibilities to obtain form of biochar (hydrochar) and artificial humic substances, however the conditions of the HTC have not been much studied. Optimization by Response Surface Methodology of the HTC using microwave treatment with three experimental variables (treatment temperature, length of microwave treatment, amount of biomass per volume) provides the possibility to significantly increase the yield of humic substances at the same time reducing the duration of the treatment. However, with an increase in the yield of humic substances, the yield of hydrochar decreases, thus supporting possibilities to obtain most needed biomass waste processing product.**

***Keywords* – Artificial humic acids; biomass waste; humic substances; hydrothermal carbonization**

Nomenclature		
HS	Humic substances	
AHA	Artificial humic acids	
NOM	Natural organic matter	
TOC	Total organic carbon	mg g^{-1}
A_i	Frequency factor for *i*th order	min^{-1}
RSM	Response surface methodology	
CCD	Central composite design	
AC	Ash content	wt. %
HTC	Hydrothermal carbonisation	

1. INTRODUCTION

A major part of soil natural organic matter (NOM) consists of humic substances (HS): high molecular, refractory, acid bearing structures [1]. Soil NOM play a major role in the carbon biogeochemical cycle as soils on the Earth contain more carbon, than the vegetation and atmosphere in the form of CO_2 and CH_4 [2]. Humic substances are formed as a result of complex living organic matter transformation (humification) processes, including three major steps:

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1. Degradation of less stable biomolecules (proteins, carbohydrates, etc.) into low-molecular weight components;
2. Transformation of biopolymers (cellulose, lignin, etc.) into more stable structures;
3. Resynthesis of humic molecules from the remains of natural biopolymers and low molecular substances (including microbial metabolism products) into refractory humic matter molecules [3].

The humification process is slow, and the age of humic substances can reach several thousands of years or more [3].

Humic substances are important for soil fertility as they are able to bind nutrients and trace elements and release them in forms accessible for plants, participate in the soil pH and oxidation-reduction processes and participate in the regulation of soil moisture and temperature regime [4]. Humic substances can act as biostimulants promoting plant growth [5]. However due to climate change and intensive agricultural practices, the organic matter content in soils is decreasing globally [6]. Humic substances nowadays are produced at an industrial scale mostly for agricultural applications as a source material for their production using fossil materials: peat, low rank coal (lignite, leonardite) and others [7]. Biomass waste can be considered as a prospective source material for production of humic substances. Agricultural production, forestry as well as the food industry produce huge amounts of waste with limited application potential, for example, the European Union is producing ~ 88 million tons of food waste yearly [8] and a significant part of it is just discarded, thus causing environmental pollution. The amount of agricultural and forestry waste could be even higher, and the decay or accidental burning of biomass waste is a significant source of greenhouses gases.

A prospective solution that can be considered to address the biomass waste processing problem and produce humic substances for diverse applications is the recently suggested hydrothermal carbonization of biomass in an alkaline environment [9] to produce artificial humic substances (AHS) and hydrochar (HC). This approach supports the accelerated transformation of organic matter (humification), to produce refractory humic substances. Hydrothermal carbonization (HTC) of biomass waste can be considered as a tool for the sequestration of atmospheric CO_2 transforming it into refractory forms of carbon [9]. Artificial humic substances, obtained in such a process express functionalities characteristic of natural humic substances, such as amphiphilicity, ion binding, polyelectrolyte behaviour, phenol-based redox properties [10]. Furthermore, AHS shows the ability to form clay-humin complexes, that are responsible for water and nutrient binding in fertile natural soils [11], [12]. Although the impact of AHS on soil fertility are still not proved, experts state that it is already possible to speak about prospects to create artificial soils on the basis of these materials [10], [13]. Yields of refractory carbon containing compounds and thus the carbon stabilisation efficiency of the HTC process are many times higher than natural humification reactions of organic matter or pyrolysis with the aim to produce biochar and thus HTC process can be considered as climate neutral [9].

Suggested experimental conditions for hydrothermal carbonization proposes to conduct this process at temperatures starting from 180 °C till 250 °C for 20 to 48 hours [10]–[13]. However, the experimental details for biomass waste hydrothermal transformation have not been studied much. In this respect, a prospective tool to accelerate hydrothermal transformation is considered the treatment with microwaves as cavitation, which are generated in this process and cause the rupture of cell walls, thus releasing their content and this method has been widely applied to assist extraction [14].

The aim of this study is to study typical biomass waste transformation possibilities into humic substances using hydrothermal carbonization and study the possibilities to optimize this process.

2. MATERIALS AND METHODS

2.1. Biomass Hydrothermal Carbonisation

Wheat straw was used for the hydrothermal carbonisation tests of the biomass as the material is useful for processing and represents waste biomass. Wheat straw was ground in a Bosch hand mill and put through a 1mm sieve. Approximately 0.5–3.5 g of straw material were weighed in PTFE capsules (*Milestone*) and 50 mL of 0.65 M KOH (*Sigma-Aldrich*) was added to each capsule. The capsules were placed in a microwave digestion unit (*Milestone Ethos EASY*). Temperature build up was programmed to 15 °C min^{-1} to desired temperature (120 °C; 170 °C; 220 °C) with maximum power output of 1800 W, and were treated for 15 minutes after reaching the specified temperature. After cooling, the samples were filtered through a cellulose filter. The liquid phase was separated for further humic matter concentration analysis as total organic carbon (TOC) analysis. Hydrochar was washed with deionised water (15 MΩ) until humic substances and KOH were removed. Hydrochar material was then dried and weighed on an analytical balance.

The humic substance solution was diluted 100 times with deionised water (15 MΩ) for total organic carbon analysis. Right before analysis, the samples were placed in an ultrasonic bath (Cole-Parmer) for 30 seconds to remove dissolved CO_2. Total organic carbon analysis was carried out with a *Shimadzu TOC-VCSN* total carbon analyser.

2.2. Experimental Design and Statistical Analysis

Variables to be optimised were firstly identified by performing experiments where a single factor (amount of biomass, treatment time, and treatment temperature) was changed at a time. Upon selection of the conditions for preliminary experiments, the conditions as suggested in the literature were considered [10]–[14]. The preliminary range of hydrothermal carbonisation variables were identified – treatment temperature, length of the microwave treatment, amount of biomass per volume. The identified extraction parameters were further optimised using Response Surface Methodology (RSM).

A three-factor and three-level central composite design (CCD) was performed (with three central points), allowing to determine the combined effects of the independent variables on the response. Possible effects of unexplained variability due to extrinsic factors in the two observed responses (yield of humic substances, expressed as total organic carbon (TOC), yield of hydrochar) were minimised by randomising the run (experiment) order. The range and the levels of the variables chosen based on the preliminary results are summarised in Table 1.

The experimental design of CCD consisted of 16 experimental points with 3 central points. The data obtained for RSM was fitted to a second-order polynomial model with a generalised second order (quadratic) model (Eq. (1)):

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i<j} \sum_{j=2}^{k} \beta_{ij} x_i x_j , \quad (1)$$

where

β_0 Regression coefficient for intercept;
β_j Regression coefficient for linear terms;
β_{jj} Regression coefficient for quadratic terms;
β_{ij} Regression coefficient for interaction terms;
x_i, x_j Independent variables.

Experimental data was first fitted to test the obtained model and the resulting prediction values were further used to generate a tri-dimensional regression model. The RSM design and optimal hydrothermal carbonisation conditions were determined by maximising desirability using a response profiler. All the statistical analysis and experimental design were conducted and tested using *SAS JMP*® software. The obtained fit statistics of the generated quadratic regression models were validated based on the determination coefficients by ANOVA and resulting *P*-values, summary of fit statistics can be found in Table 2. ANOVA with post-hoc Tukey's HSD was used to compare means between different treatments.

TABLE 1. LEVELS OF VARIABLES EMPLOYED IN THE PRESENT STUDY FOR THE CONSTRUCTION OF CENTRAL COMPOSITE DESIGN (CCD)

Variables	**Coded variables**	**Actual variables**	**Levels**		
			−1	0	1
Temperature, °C	x_1	X_1	120	170	220
Incubation time, min	x_2	X_2	15	127	240
Sample mass (per 50 mL), g	x_3	X_3	0.50	2.00	3.50

TABLE 2. CCD MATRIX OF THE THREE VARIABLES IN CODED UNITS AND RESPONSE VALUES OF THE HYDROTHERMAL CARBONISATION PROCESS

Run	**Temperature, °C**	**Time, min**	**Sample, g 50 mL^{-1}**	**Hydrochar, mg g^{-1}**	**TOC, mg g^{-1}**	**TOC, mg L^{-1}**
1	120.00	15.00	0.50	0.556	302	3051
2	120.00	15.00	3.50	0.536	181	12683
3	120.00	127.50	2.00	0.549	215	8583
4	120.00	240.00	0.50	0.653	302	3036
5	120.00	240.00	3.50	0.488	168	11760
6	170.00	15.00	2.00	0.493	218	8720
7	170.00	127.50	0.50	0.413	295	2981
8	170.00	127.50	2.00	0.414	217	8706
9	170.00	127.50	3.50	0.300	211	14770
10	170.00	240.00	2.00	0.397	197	13800
12	220.00	15.00	0.50	0.283	389	3881
13	220.00	15.00	3.50	0.229	218	15302
14	220.00	127.50	2.00	0.125	292	11680
15	220.00	240.00	0.50	0.179	433	4316
16	220.00	240.00	3.50	0.223	242	16950

TABLE 3. EFFECTS TESTS OF EXPERIMENTAL VARIABLES AND THEIR INTERACTIONS FOR THE PREDICTED QUADRATIC POLYNOMIAL MODELS BASED ON THE TWO INVESTIGATED RESPONSES – TOTAL ORGANIC CARBON AND HYDROCHAR

Variable	*Total organic carbon*			*Hydrochar*		
	Sum of Squares	***F* Ratio**	***P*-value**	**Sum of Squares**	***F* Ratio**	***P*-value**
X_1	27011.25	96.8538	<0.0001*	1.83	112.8077	<0.0001*
X_2	1531.25	5.4906	0.0286*	0.01	0.3205	0.5770
X_3	102 245.00	366.6182	<0.0001*	5.06	312.3591	<0.0001*
X_1X_2	2525.06	9.0541	0.0065*	0.01	0.1216	0.7306
X_1X_3	3108.06	11.1445	0.0030*	0.63	39.2518	<0.0001*
X_2X_3	1350.56	4.8427	0.0386*	0.01	0.9564	0.3387
X_1X_1	3304.55	11.8491	0.0023*	0.17	10.5036	0.0038*
X_2X_2	956.05	3.4281	0.0776	0.38	23.4844	<0.0001*
X_3X_3	10 812.70	38.7709	<0.0001*	0.34	21.1733	0.0001*

3. RESULTS AND DISCUSSION

3.1. Preliminary Single-Factor Experiments

As a tool to accelerate reactions including processes of importance for biomass transformation, treatment with microwaves can be considered as an efficient method [15]. Identification of the most important variables that have the most influence on the yield of either hydrochar or humic substances (as indicated by total organic carbon – TOC) was done by altering a single factor during the hydrothermal treatment using microwaves. Wheat straw was selected as model material of biomass waste, the pH during the hydrothermal treatment was chosen as suggested in the literature (pH > 9) for conventional HTC [10]–[13], influence of the biomass weight to volume ratio, HTC temperature, and duration were tested with low and high variable values. Performed experiments revealed effects of various parameters on the TOC and hydrochar yields and the identified parameters were chosen for further optimisation using RSM. The value range of the variables can be found in Table 1.

3.2. Optimisation of Extraction by RSM

In order to examine the combined effects of the previously chosen HTC variables using RSM, a CCD of 16 runs with 3 centre points was performed randomly. A summary of ANOVA of the used design can be found in Table 4 – analysis of variance was performed in order to determine if the obtained quadratic model was significant. The obtained *F*- and *P*-values for the model were significant. The obtained *F*- and *P*- values for the lack-of-fit show significant fit which indicates that the model prediction equation is adequate for predicting the total hydrochar and humic matter yields under any combination of variable values (Table 4). The adjusted R^2 and total correlation R^2, 0.9019, 0.9304 and 0.9272, 0.9483 for the models of hydrochar and humic matter, respectively, indicate adequate accuracy and general availability of the designed polynomial models (Table 4).

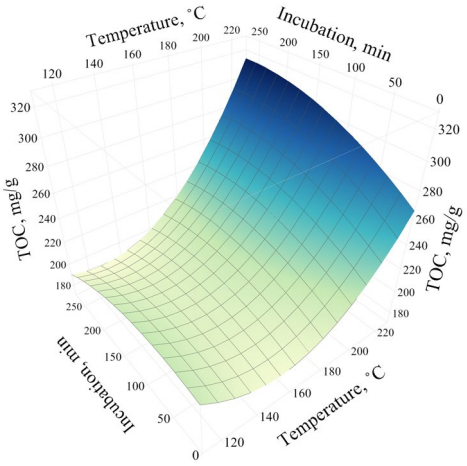
TABLE 4. ANALYSIS OF VARIANCE (ANOVA) FOR THE RESPONSE SURFACE MODEL ON THE TOC AND HYDROCHAR YIELD

Sources	Degrees of freedom	Sum of squares	Mean square	Coefficient	*F*-value	*P*-value
			Response: TOC			
Model	9	131 119	14 568		44.8661	<0.0001
Lack of fit	5	3400	680		3.0878	0.0366
Pure error	17	3743	220			
Error	22	7143	324.7			
Cor. total	31	138 263				
Cor. total R^2	32			0.9483		
Adj – R^2				0.9272		
			Response: Hydrochar			
Model	9	603 512	67 056		32.6882	<0.0001
Lack of fit	5	19 726	3945		2.6400	<0.0001
Pure error	17	25 405	1494			
Error	22	45 131				
Cor. total	31	648 643				
Cor. total R^2	32			0.9304		
Adj – R^2				0.9019		

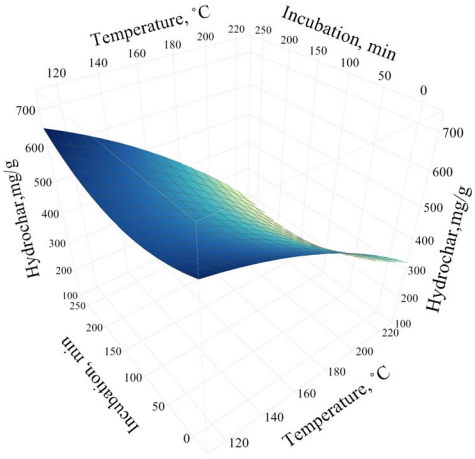
Variables and their interactions were investigated for their significance on the hydrochar and humic matter yields (Table 4). The linear coefficients X_1, X_2, X_3 were significant ($P < 0.05$) for the TOC model, however, the X_2 was insignificant in the hydrochar model (Table 3). Significance of the variable interaction pairs is summarised in Table 3.

Response surface methodology was used to examine the effects of the used variables of the HTC on the yield of humic substances. Humic substances under suggested conditions are formed as a result of hydrolysis of biomass components with following chemical condensation reactions as suggested previously [12], however treatment with microwaves significantly accelerates process in comparison with treatment conditions as suggested in literature (24–48 hrs, at temperature 180–240 °C) (Fig. 1).

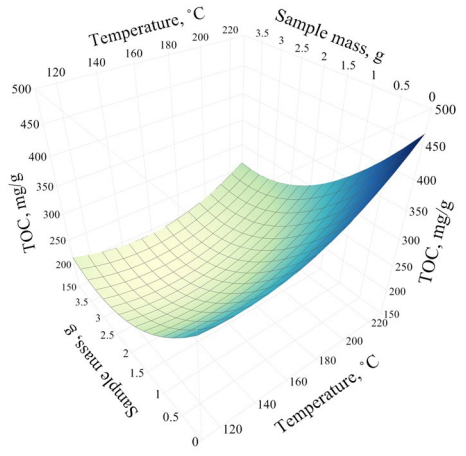
HTC variables and their combinations (Table 4) were optimised to maximise the yield of humic substances, at the same time following the yield of hydrochar. The obtained optimal conditions provide 96.5 % desirability. The minimal obtained humic substance values provided 180 mg TOC g^{-1}, while the conditions of maximised desirability provided 433 mg TOC g^{-1} straw biomass, however concentration of humic substances changed from 2.9 g TOC L^{-1} to 16.9 g TOC L^{-1} under optimal conditions, which is an increase of nearly 5 times, proving the high efficiency of the RSM approach in comparison with experimental conditions, given in literature [13], [16]. The duration of hydrothermal treatment increased the yield of humic substances, most probably due to alkaline hydrolysis of straw, supporting release of low molecular weight biomass components. Similar trends have been found in other studies, however in our case, the duration of the HTC process was hours, not tens of hours as in literature [17].


Temperature, °C
Incubation, min
TOC, mg/g
Incubation, min
Temperature, °C

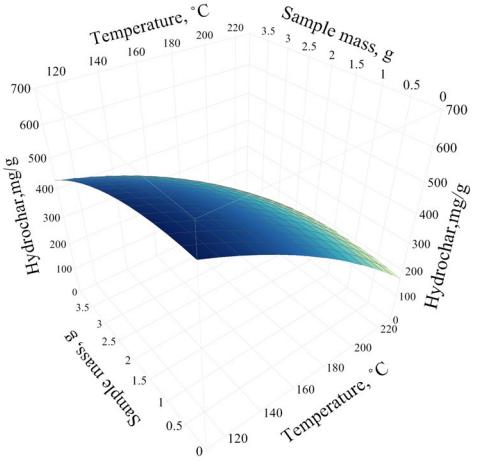
(a)


Temperature, °C
Incubation, min
Hydrochar,mg/g
Incubation, min
Temperature, °C

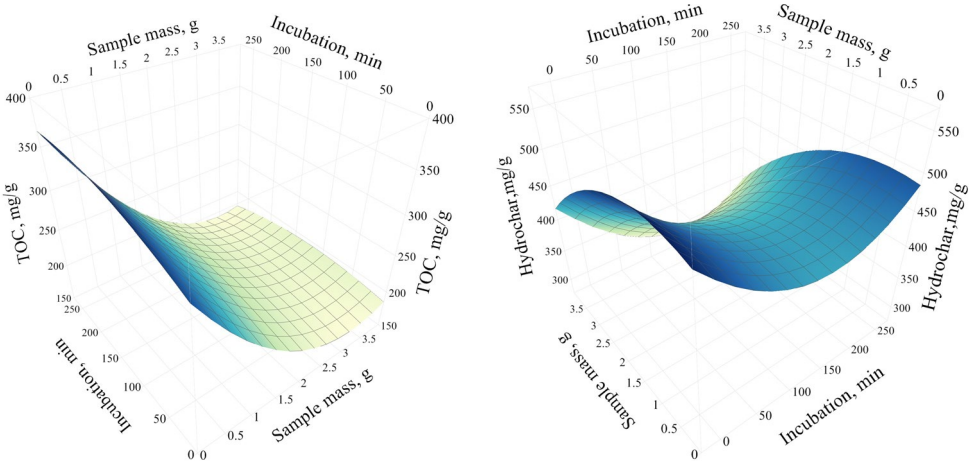
(b)


Temperature, °C
Sample mass, g
TOC, mg/g
Sample mass, g
Temperature, °C

(c)


Temperature, °C
Sample mass, g
Hydrochar,mg/g
Sample mass, g
Temperature, °C

(d)



(e) (f)

Fig. 1. Impact of the conditions (amount of biomass, treatment time, treatment temperature) of the hydrothermal carbonisation process of the wheat straw on the yield of hydrochar (b, d, f) and artificial humic substances (a, c, e) (expressed as Total Organic Carbon - TOC).

Also, temperature have significant impact on the transformation process of straw biomass, however this impact is not so significant, as well as above 220–260 ºC, the mechanism of the hydrothermal process is changing and a release of gaseous substances can occur, thus significantly increasing pressure in the reaction vessel. Thus, in respect of possibilities to raise the temperature there is a strict limit – 260 ºC [18]. The duration of the HTC have an impact on the yield of humic substances, however impact of this factor is regulated, considering economic aspects and the suggested optimal conditions also provides acceptable conditions from the perspective of application at the pilot or industrial scale [19], [20]. Results of the optimisation at the same time demonstrates possibilities to design the biomass waste HTC process accordingly to the aims and interests in the relevant product (hydrochar, considering possibilities to design also properties of this product [21] or AHS), as well as modify the properties of obtained AHS, to adjust their properties for most prospective application areas [22], [23].

4. CONCLUSION

Hydrothermal carbonization of biomass waste (on example of wheat straw) using microwave treatment significantly accelerates the transformation of waste organic matter into artificial humic substances and hydrochar thus supporting practical application potential of this technology. The optimization of the HTC process using the Response Surface Methodology with three experimental variables provide the possibility to reduce process temperature and significantly increase the yield of humic substances, however with an increase of the yield of humic substances, the yield of hydrochar is decreasing.

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