

Towards a Standard Protocol to Measure Carbon Stability in Hydrochar under Abiotic Storage Conditions

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1. Scope and Rationale

This protocol proposes a standardized methodology to assess the carbon stability of hydrochar (HC) under strictly abiotic and confined storage conditions, without contact with liquid water.

The objective is to:

- Quantify temporal changes in solid-phase properties
- Monitor the evolution of the gaseous phase
- Detect carbon redistribution within a closed system

The system is designed to isolate intrinsic physicochemical ageing processes from biological degradation, aqueous leaching and transport, and mineral–matrix interactions.

2. Experimental Approach

The methodology is based on a dual monitoring strategy under confined, dry conditions, combining destructive and non-destructive analyses.

2.1 Solid-Phase and Gas-Phase Monitoring (Sacrificial Bottles)

A series of sealed bottles containing hydrochar or the control char are sacrificed at predefined time points to analyse gas and hydrochar, determine mass recovery, and analyze structural, chemical and thermal stability evolution. Before opening the bottles, the headspace gas is sampled and analyzed to quantify gaseous carbon species and estimate partial carbon mass balance.

2.2 Gas-Phase Monitoring (Closed Bottles) - optional

A parallel set of bottles remains sealed throughout the experiment. At predefined intervals, headspace gas is sampled and analyzed to quantify gaseous carbon species.

3. Experimental System

Experimental configuration for the sealed airtight bottles filled with hydrochar or control char:

- Crimp-top serum bottles
- Total volume: 100 mL (scalable)
- Material: Borosilicate glass
- Aluminum crimp seal with rubber septum
- Target headspace: approximately one-third (1/3) of total bottle volume

All empty bottles (with caps) shall be weighed prior to loading.

4. Hydrochar Preparation and Mass Definition

Hydrochar samples shall be:

- Oven-dried at 105 °C until constant weight
- Stored in a desiccator
- Sieved to particle size < 2 mm
- Oversized fraction manually reduced below 2 mm.

Preliminary Determination of Hydrochar Mass

The final hydrochar mass per 100 mL bottle will be defined based on preliminary screening tests to define the mass quantity able to provide approximately 1/3 headspace (30–35 mL), allow detectable gas evolution, and ensure enough for characterization. The final mass must be the same in each bottle.

Preliminary Hydrochar and feedstock Characterization

The hydrochar and the feedstock must be characterized according to the Table 1.

5. Control Material

Experiments will be conducted in parallel using a stable reference char material, such as a high-temperature pyrolysis biochar (> 650 °C) considered stable (e.g., [European Biochar Certificate-compliant](#)).

Control samples will be subjected to identical experimental set up, conditions and [sampling intervals](#) to validate the system and allow benchmarking.

6. Experimental Design

Incubation temperatures: Target = 20 °C, plus two extra among 30 °C, 50 °C, 70 °C (?).

Each condition performed in triplicate (n = 3) for both hydrochar and control material.

Sampling times: 1, 5, 10, 20, 30, 50, 70, 90, 150, 200, 365 days.

Total number of bottles (example): 11(times)x3(replicates)x3(Temperatures)x2(HC+control)=198.

7. Monitoring Plan

7.1 Solid-Phase and Gas-Phase Monitoring (Sacrificial Bottles)

At each sampling time point, gas is sampled from sacrificial bottles before opening and analyzed via gas chromatography. Target compounds include CO₂ (mandatory), CH₄, CO, and optional hydrocarbons and VFAs. Then the bottles are opened, let naturally degas in desiccator till room temperature is reached and weighed. Hydrochar is analysed according to section 8.

7.2 Gas-Phase Monitoring (optional Closed Bottles)

At each sampling time point, 1 mL headspace gas is withdrawn and analyzed via gas chromatography. Target compounds include CO₂ (mandatory), CH₄, CO, and optional hydrocarbons and VFAs.

8. Analytical Plan

Details on the analyses are provided in Table 1.

The analytical plan is aimed at assessing any modification over time of hydrochar quality in terms of carbon quantity and speciation. A tentative list of parameters is the following:

- Mass
- Elemental analysis (C, H, N, O) and atomic ratios (H/C, O/C)
- Proximate analysis (Volatile matter, fixed carbon, ash)
- Optical properties (Reflectance and Colorimetry)
- Surface functionalities (FTIR spectroscopy)
- Morphology
- Density
- pH (PZC), EC
- Leachable elements
- Calorimetry (HHV), LHV

9. Open Points for Harmonization

- Time before analyses (and storage conditions)
- Which are the target temperatures for minimum experiments?
- Mandatory analyses
- Minimum duration
- Frequency of analyses
- Gas sampling correction strategy (depending on temperature at sampling, etc.)
- Definition of reference control material
- Others?

10. Expected Outcomes

The protocol provides a reproducible method to assess hydrochar stability under abiotic confinement, enabling cross-laboratory comparability and contributing toward standardized testing procedures.

11. Notes on Ring Test

The motivation to develop this protocol is the desire to carry out a community-wide action to establish the stability of hydrochar, i.e., carry out a ring test. The goal is to collect and publish comparable data on a wide number of hydrochars from a range of feedstocks. This protocol will ensure the experiments in the individual laboratories are comparable. A discussion point to be agreed upon is the type of hydrochars to be compared in this ring test, in terms of age, HTC process conditions, feedstocks, etc. For example, we can define targets for age (e.g., freshly produced (within X days), or stored X months under what conditions), process conditions (T, t, %So, etc.), feedstock types (lignocellulosic, manures, etc.).

12. Test Reporting

- Carbon yield (from HTC production process)
- Carbon loss during storage
- Net CO₂e Removal Calculation

- Statistics and reliability:
 - Number of replicates (for sample bottles, analyses, ...)
 - Variability of climate chamber
 - minimum detection limits:

Others?

Table 1 – list of analyses (in progress)

Analysis	Mandatory	Frequency	Technique	Method/ Equipment	Observations	References
Mass	yes	0, 1, 5, 10, 20, 30, 50, 70, 90, 150, 200 and 365	Weighting	Precision balance (minimum 4 decimals)	Room temperature, open in dessicator	
Elemental analysis	yes	0, 1, 5, 10, 20, 30, 50, 70, 90, 150, 200 and 365	CHN			
Proximate analysis	yes	0, 1, 5, 10, 20, 30, 50, 70, 90, 150, 200 and 365	TGA			
Optical properties	?	?	Diffuse Reflectance spectrophotometry	Spectrophotometer (equipped with an integrating sphere)	calibration using a certified white reference standard is needed	
Surface functionalities	yes	?	FTIR spectroscopy	Spectroscope	3 analysis using 3 samples of the same bottle	
Color	?	?	Colorimetry Value: CIELAB color coordinates (lightness)	Digital Colorimeter		
Morphology	?		Scanning Electron Microscopy + EDX	Microscope	3 analysis using 3 samples of the same bottle	

Density	?		<ul style="list-style-type: none"> · Mass to volume determination · Stereopycnometry · Porosimetry 	<ul style="list-style-type: none"> · Balance (ISO 23499) · (He??)Stereopycnometer · Hg porosimeter 	Measure the HC mass for a known volume
pH (PZC), EC	yes				
Leachable elements	?	0, 365	leaching test followed by chemical analysis of the extract	ICP-OES for solution analysis (DIN 38414-S4)	
Energy content	?	0	HHV Analysis		

Graphical abstract

