SAINS TANAH – Journal of Soil Science and Agroclimatology

Journal homepage: http://jurnal.uns.ac.id/tanah

Soil carbon mineralization affected by hot water and ultrasound pretreatment

Toan Nguyen-Sy[1]*, Thi Van Thanh Do[1], Dong Pham Duy[2]

[1] Faculty of Chemical Technology and Environment, University of Technology and Education - The University of Da Nang, 48-Cao Thang, Da Nang, Viet Nam.

[2] Faculty of Environmental Engineering, Hanoi University of Civil Engineering, 55 Giai Phong Street, Hai Ba Trung District, Hanoi 100803, Viet Nam.

ARTICLE INFO

Keywords:
Carbon mineralization
Extracted carbohydrate
Hot water extraction
Soil organic carbon
Ultrasound assist extraction

Article history
Submitted: 2021-12-05
Accepted: 2022-01-18
Available online: 2022-05-26
Published regularly: June 2022

***** *Corresponding Author*
Email address:
nstoan@ute.udn.vn

ABSTRACT

Paddy soil has attracted several studies; however, the effects of pretreatment on soil carbon mineralization remain unclear. This study aimed at validating the effects of soil pretreatment by performing anaerobic incubation of 15 soil samples before treating at room temperature water boiling at 80°C or ultrasound assist at 37Hz and combining (hereafter are control, hot water, ultrasound, mixed hot water, and mixed ultrasound treatments) conducted with three replications. Results showed that initial extracted carbohydrate and incubation extracted carbohydrate (Ini-ECH and Incu-ECH) ranged from 211 to 691 mg kg^{-1} and 229 to 961 mg kg^{-1}, respectively, and reached the highest values with hot water. control, ultrasound, and mixed ultrasound treatments showed the lowest Ini-ECH (211–269 mg kg^{-1}), while the lowest Incu-ECH was linked to both mixed soil treatments with similar amounts (229–264 mg kg^{-1}). Conversely, soil carbon mineralization (generated extracted carbohydrates during anaerobic incubation, Min-ECH) was similar in control, hot water, and ultrasound treatments (ranged from 271 to 393 mg kg^{-1}) but tended to be a negative value in mixed soil treatments. Therefore, we conclude that hot water and ultrasound pretreatments do not increase soil carbohydrate potential but likely promote carbon decomposition.

How to Cite: Nguyen-Sy, T., Do, V.T.T., Duy, D.P. (2022). Soil carbon mineralization affected by hot water and ultrasound pretreatment. Sains Tanah Journal of Soil Science and Agroclimatology, 19(1): 73-79. https://dx.doi.org/10.20961/stjssa.v19i1.57554

1. INTRODUCTION

Carbohydrate is an essential constituent of soil microorganisms and is present as structural constituent and exocellular intracellular component (Chantigny et al., 2014; Fischer et al., 2007; Lowe, 1978; Rakhsh & Golchin, 2018). Behaviors of carbohydrates in the soil are crucial to understanding soil fertility and genesis. Therefore, soil extracted carbohydrate was recommended as a potential indicator for soil quality assessment (Nguyen-Sy, Tan, et al., 2021; Rakhsh & Golchin, 2018). Various methods for carbohydrate extraction have been reported, including chemical-based or nonchemical-based extraction, where the most typical and straightforward extraction method is to dilute the sample with distilled water extraction(Albalasmeh et al., 2013; Gunina & Kuzyakov, 2015; Nguyen-Sy, Tan, et al., 2021; Tanaka et al., 1990; Uzoho & Igbojionu, 2014). Cold water extractable carbohydrate regards to the most labile fraction of soil organic carbon (SOC), while hot water extractions are mainly used to achieve higher extraction yield, including microbial assessable, non-structural plant hydrate, and extracellular microbial carbohydrate (Bongiorno et al., 2019; Chantigny et al., 2014; Ghani et al., 2003). Ultrasound has also been effectively used to assist water extraction. For instance, ultrasound-assisted extraction helps to remove soil microparticles or extracts organic contaminants (Albero et al., 2019; Nguyen-Sy, Nguyen-Thi, et al., 2021). As agriculture is currently moving toward sustainable organic farming (Sardiana & Kusmiyarti, 2021), research on soil physical and chemical properties was considered important (Rendana et al., 2021). Using hot water and ultrasound are the two environment-friendly methods for soil analysis. Therefore, it is important to evaluate the efficiency of using these methods to shed light on the derivatives of soil labile pools like extracted carbohydrates.

To enhance the carbon extraction amount more, incubation for several weeks was recommended. Some organic carbon cannot be extracted immediately but can be decomposed and mineralized via incubation conditions. Hence, the incubation experiment has been considered as a useful method to investigate soil mineralization and is specified as a good soil quality indicator (Cheng et al., 2007; Pansu & Thuriès, 2003; Wang et al., 2016). Several studies showed that soil mineralization potential could be regulated by elevated temperatures (Davidson & Janssens, 2006; Gao et al., 2020; Tang et al., 2016). However, there was no report on soil pretreatment before the regular incubation experiment. As the soil organic matter processes were mainly regulated by the active microorganisms living in the soil, we hypothesized that pretreatment before incubation could affect the soil's mineralized carbohydrate (Min-ECH). This study applied hot water and ultrasound shock pretreatments before incubation to investigate its effect on the Min-ECH of paddy soil.

2. MATERIAL AND METHODS

2.1. Soil sampling and analysis

Soil samples were collected from a paddy field in Hoa Vang, Da Nang, Viet Nam in May 2020. The paddy field runs a long-term rice paddy cultivation (more than 100 years). The region was located in an area with tropical monsoon climate, high temperature, and little fluctuation. The average annual temperature is about 25.6°C, with the highest in June (29.2°C), and the lowest in February (21.2°C). The paddy field size was 20 m × 60 m in size. Soils were sampled with three replicates, with each replicate mixed up with three soil cores. We collected soil depth at 15 cm as this is the plow layer, and the root of rice plant almost penetrated to this depth. A total of 5 treatments, with 3 replicates comprising 15 soil samples, was set up for the experiment. Collected samples were air-dried and sieved using a <2 mm diameter sifter before analysis. The soil was classified as alluvial soil. SOC was analyzed according to the Walkley–Black analysis method (Walkley, 1947). Soil pH (H_2O) values were determined by mixing soil samples with distilled water at a ratio of 1:10 (soil/water). The total carbohydrate content was determined using Dubois method (Albalasmeh et al., 2013; DuBois et al., 1956). More detail of soil properties is presented in Table 1.

2.2. Pretreatments and anaerobic incubation experiment

Before incubation, all soil samples were subjected to pretreatment by hot water, ultrasound, mixed hot water, and ultrasound with untreated soils, as shown in Fig. 1.

After pretreatment, samples were divided into two parts: one part was directed extracted and measured carbohydrate and counts for ECH, and the other part was subjected to a four week anaerobic incubation. With minor modification, anaerobic incubation was obtained following previous research (Cheng et al., 2016; Cheng et al., 2007; Kautsar et al., 2020). After pretreating soils as shown in Fig. 1 (control, hot water, mixed hot water, ultrasound, and mixed ultrasound treatments), soils were subjected to anaerobic incubation. All soil sample bottles were capped with a plastic stopper in which air could be ventilated. The bottles were incubated at 25°C for four weeks in the dark. The incubated soil was then immediately extracted by shaking for 30 minutes on a reciprocal shaker.

After incubation, samples were extracted for measuring incubation extracted carbohydrates (Incu-ECH). The mineralized soil (Min-ECH) was calculated by subtracting Ini-ECH from Incu-ECH, as presented in Fig. 1.

Table 1. The initial properties of the paddy soil in this research.

Parameters	Values
SOC (g kg^{-1} soil)	16.4
Soil moisture (%)	4.7
Sand %	32
Silt (%)	38
Clay (%)	30

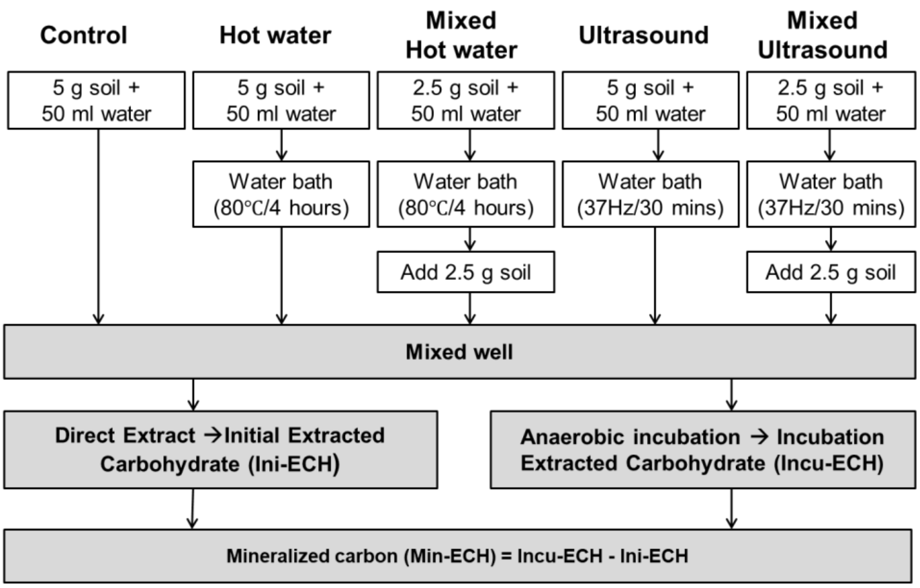

Figure 1. The proposed schematic procedure for pretreatment and incubation experiment.

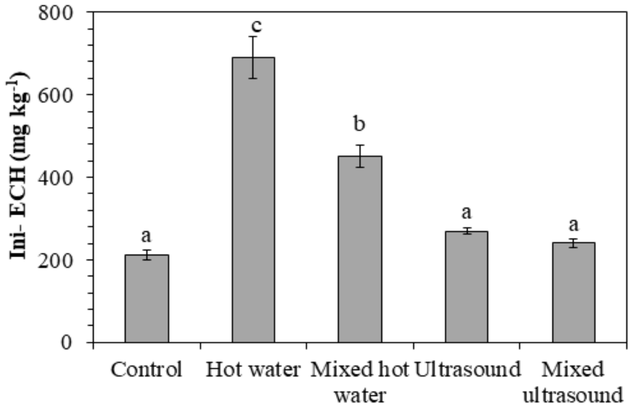

Figure 2. Initial extracted carbohydrate content using different pretreatment methods

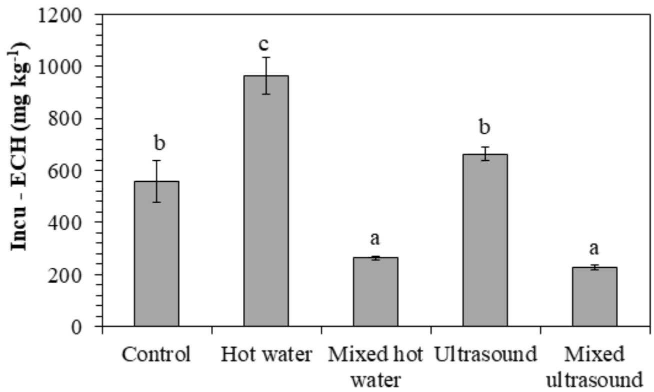

Figure 3. Incubated carbohydrate content using different pretreatment methods after 4 weeks of anaerobic incubation

Table 2. Percentages of Min-ECH, Ini-ECH, and Incu-ECH to the bulk soil organic carbon (SOC).

Treatment	Ini-ECH	Incu-ECH	Min-ECH
	%		
Control	1.3	3.4	2.1
Hot water	4.2	5.9	1.7
Mixed hot water	2.7	1.6	-
Ultrasound	1.6	4.0	2.4
Mixed ultrasound	1.5	1.4	-

2.3. Statistical analysis

Experiments were performed thrice, and the results were presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) coupled with Tukey's test was obtained using SPSS version 20 to determine the differences of mineralized carbohydrate potential among treatments.

3. RESULTS

3.1. Content of initial extracted carbohydrates (Ini-ECH)

The initial ECH was determined before conducting anaerobic incubation. As shown in Fig. 2, In-ECH ranged from 211 to 691 mg kg^{-1}, with the highest amount shown in hot water treatment (691 mg kg^{-1}), followed by mixed hot treatment (451 mg kg^{-1}), while the remaining three treatments are similar to each other (211–269 mg kg^{-1}, $p > 0.05$).

3.2. Content of Incubation mineralized carbohydrate (Incu-ECH)

The Incu-ECH was determined after four weeks of anaerobic incubation (as designed in Fig. 1), which is shown in Fig. 3. The amount of Incu-ECH ranged from 229 to 961 mg kg^{-1}. Similar to Ini-ECH, hot water treatment showed the highest ECH (961 mg kg^{-1}); however, the second amount showed in control and ultrasound (558 and 663 mg kg^{-1}). The lowest ECH addressed to two mixed soil treatments (264 and 229 mg kg^{-1} for mixed hot water and mixed ultrasound, respectively).

3.3. Contribution of ECH to SOC

Soil carbohydrate is an essential part of SOC, which serves as a primary food for soil microorganisms to turn over the inert soil organic matter into becoming a labile pool (Lowe, 1978). Therefore, we calculated its portion contribution from Ini-ECH, Incu-ECH, and Min-ECH to SOC, presented in Table 2. The ECH extracted from the incubation experiment showed the highest contribution (1.4–15.9% of SOC) followed by In-ECH (1.3%–4.2% of SOC). Interestingly, the Min-ECH/SOC ratios were similar among non-mixed soil treatments (1.7%–2.4% of SOC), while both mixed soil treatments showed negative Min-ECH led to uncountable values for contribution.

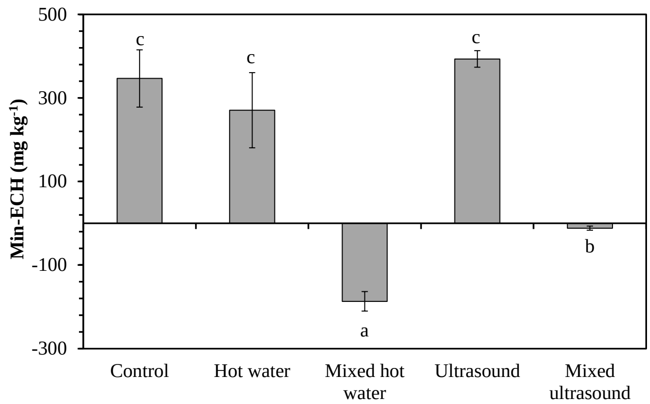

Figure 4. Soil carbohydrate mineralization using hot water and ultrasound pretreatments

Table 3. Loss of Min-ECH of mixed soil treatments (mixed hot water and mixed ultrasound) over their relative unmixed soil treatments.

	Loss of Min-ECH (mg kg^{-1})	Calculated description
Mixed hot water	496	Mixed hot water over relative un-mixed treatments
Mixed ultrasound	382	Mixed ultrasound over relative un-mixed treatments

Table 4. ECH and Min-ECH in this study versus previous relative research.

References	Soil types	Extracted condition	ECH	Incubation condition	Min- ECH by incubation	CO_2 emission by incubation
			mg C kg^{-1}		mg C kg^{-1}	
Present study	Paddy	25°C	211	25°C / 4 wks	347	-
		80°C	691	25°C/ 4 wks	271	-
		Ultrasound	269	25°C/ 4 wks	393	-
Lee et al. (2007)	Forest, agriculture	20°C	54-129	20°C/ 2 wks	55-105	173-520
		4°C	78-102	4°C/ 4 wks	-	246-258
		– 20°C	98-141	– 20°C/ 4 wks	-	291-435
		– 80°C	84-105	– 80°C/ 4 wks	-	312-519
Chantigny et al. (2014)	Agriculture	25°C	25-160	-	-	-
		80°C	50-250	-	-	-
Ghani et al. (2003)	Dairy farm	80°C	1073-2072	-	-	-
Li et al. (2007)	Paddy	25°C	900-1500	-	-	-
Cheng et al. (2016)	Paddy	-	-	25°C/ 4 wks	-	408-541
Cheng et al. (2007)	Agriculture	-	-	25°C/ 4 wks	-	100-650
Kautsar et al. (2020)	Paddy	-	-	25°C/ 4 wks	-	930-1420
Tang et al. (2021)	Paddy	-	-	25-26°C/ 4 wks	-	638-650
Tang et al. (2016)	Paddy	-	-	– 5°C/ 4 wks	-	226-1109
		-	-	5°C/ 4 wks	-	233-933
		-	-	15°C/ 4 wks	-	88-668
		-	-	25°C/ 4 wks	-	69-494

Note: - : No measurement

4. DISCUSSION

ECH is usually low, accounting for only 20%–50% of the total extracted SOC (Ghani et al., 2003; Nguyen-Sy et al., 2020; Nguyen-Sy, Tan, et al., 2021). In our research, hot water treatment could extract more than 300% than control treatment. That means, that extracting at high temperature could attract more ECH than extracting organic carbon at room temperature (Table 2). Interestingly, after incubation, ECH of hot water treatment is higher than control at only 75% (much lower than 300% as the initial extraction). Hence, this research aimed to enhance the potential of ECH through an incubation experiment. As expected, the Ini-ECH was highest in hot water treatment (Fig. 2). It was surprising that the ECH in ultrasound was not significantly higher than control because several research reported that ultrasound helps in degrading the soil texture. Therefore, our result proves that the most labile carbon (ECH) was not sensitive to be regulated by ultrasound as the stable carbon pools but more sensitive by high temperature. The dominance of extracted carbon and carbohydrate of hot water over cold water has been widely reported in several studies (Ghani et al., 2003; Hamkalo & Bedernichek, 2014; Nguyen-Sy et al., 2020; Wu et al., 2020). Incubation experiments are commonly used to investigate soil mineralization. During this process, microorganisms interact with the SOM, and part of the stable pool of SOM is converted into labile pools. Therefore, it is reasonable to expect the ECH after incubation to be enhanced than the initial extracted ECH. We hypothesized that hot water and ultrasound could promote the breakdown of stable SOM to labile SOM in mixed soil treatments. However, ECH of mixed hot water and mixed ultrasound were lower than control treatment (Fig. 3), and it could be attributed to the negative effect of temperature (80°C) and ultrasound (37Hz) on soil microorganism activity. In our study, the content of SOC was low (1.64%), which was similar to recent research by (Rendana et al., 2021), which indicates that clay–silt paddy soil has low SOM content (2.63%–3.42%). Therefore, the content of labile carbon was also low.

ECH generated during anaerobic incubation (Min-ECH) of control treatment was similar to hot water and ultrasound treatments, indicating that pretreating soil with hot water and ultrasound did not enhance nor inhibit ECH potential. There have been numerous studies on soil carbon mineralization affected by temperatures but mainly focused on CO_2 emission (Chantigny et al., 2014; Cheng et al., 2016; Cheng et al., 2007; Lee et al., 2007; Li et al., 2007; Tang et al., 2021; Tang et al., 2017) (Table 4) or incubation carbohydrate but without pretreatment (Lee et al., 2007; Liu et al., 2019). To our knowledge, this research is the first report to investigate the effects of heat and ultrasound shocks before implementing conventional anaerobic incubation.

The Min-ECH represents the amount of ECH generated during a four-week anaerobic incubation (as designed in Fig. 1) and is shown in Fig. 4. Min-ECH of non-mixed soil treatments, including control, hot water, and ultrasound, showed positive values, were similar, and ranged from 271 to 393 mg kg^{-1}, while mixed soil treatments, including mixed hot water and mixed ultrasound, showed negative values and varied in the range of from −187 to −12 mg kg^{-1}. As mentioned above, two trends regulated the Min-ECH in hot water and ultrasound treatments: (Min-ECH positive) some living organisms and fresh residues in soil were broken down by hot water and ultrasound, and (Min-ECH negative) some soil microorganisms were deactivated by stress condition (high temperature and ultrasound). Thus, the two trends were merged and, interestingly, did not change Min-ECH compared to control treatment. However, it is complicated for the negative result for Min-ECH of mixed hot water and mixed ultrasound treatment. The negative values indicated that some carbohydrates from Ini-ECH were decomposed and lost during the incubation process. As shown in Table 3, mixed hot water lost 496 mg kg^{-1}, higher than mixed ultrasound at ~30% compared with estimated unmixed soil treatments. This may be due to the microorganism in mixed soil treatments. Since half of the soil in mixed soil treatments was untreated (same as control), microorganism in this part was not affected by hot water and ultrasound treatment. Therefore, microorganisms from untreated soil might take acts of decomposing part of Ini-ECH and Min-ECH to become carbon dioxide and methane (CO_2 and CH_4) and lost through volatilization. In this experiment, we did not obtain carbon emissions through incubation; however, some other studies reported that CO_2 and CH_4 emissions through four weeks of anaerobic incubation of rice paddy soil might occupy about 1.6%–2.0% SOC (Cheng et al., 2016; Cheng et al., 2007; Kautsar et al., 2020). This value is similar to the Min-ECH in control, hot water, and ultrasound treatments.

Interestingly, the loss of ECH by mixed soil treatment (382–496 mg kg^{-1}) is also close to a similar study by Cheng et al. (2016) (Table 4), which showed that carbon decomposition was about 417–630 mg kg^{-1} with a similar experiment on eight paddy soil samples. This is more convincing in order to explain the loss of Min-ECH from mixed soil treatments. However, due to this limitation of this study, further studies focused on carbon dioxide and methane emission are needed to confirm our findings.

5. CONCLUSION

This research investigated the effects of pretreating soil by hot water and ultrasound on the carbohydrate mineralized potential from long-term rice soil. Despite extracted carbohydrate being likely enhanced by hot water and ultrasound-assisted methods, final incubation ECH was declined by mixed soil treatment (53%–59% compared to control). Furthermore, the Min-ECH was not affected by hot water or ultrasound-assisted methods; meanwhile, the combination of untreated and treated soil tends to decrease ECH enormously after incubation. To deeply understand the mechanism of these preliminary results, further research on soil microorganisms is needed.

Acknowledgments

This research is funded by University of Technology and Education - The University of Danang under project number T2021-06-01.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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