Characterization of Synthetic Humin from Solid Hydrolysate and Biochar from Hydrothermal Carbonization Products of Chicken Feather Waste

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Abstract: *Solid hydrolysate and biochar 2:1 are synthetic humus from hydrothermal carbonization of chicken feather waste and contain humin that can be isolated by IHSS method. The recalcitrant humin is obtained in solid form. The yield of isolated humin from biochar 2:1 was 44.5%, and humin from solid hydrolysate was 12.7%. Analysis of humin by FTIR indicated the characteristics of complex functional groups. Based on the XRD and TEM tests, humin is formed from amorphous crystals with <14 nm in size and categorized as a superparamagnetic nanoparticle. The surface morphology of humin from solid hydrolysate is in the form of small spheres attached to larger particles, while humin from biochar 2:1 is smoother and has a larger surface area. This synthetic humin contains the nutrients N, O, Si, Cu, S, Mg, Zn, and K based on the EDX test quantitatively supported by AAS analysis. Characteristics of humin, which contains nutrients, are composed of amorphous crystals with complex functional groups during the hydrothermal carbonization process. Their relatively small heterogeneous molecules are stabilized by hydrophobic interactions and hydrogen bonds to form supramolecular compound associations in hour order. This humin content in synthetic humus is expected to increase its utility as a soil improver.*

Keywords: *humin; humus; biochar; hydrolysate; hydrothermal carbonization*

■ INTRODUCTION

Poultry is one of the largest high-revenue industries in the world that produces large quantities of chicken feather waste, reaching 8.5 billion tons [1-3]. The disposal of chicken feather waste is a serious problem due to its recalcitrant structure, which makes it difficult to decompose and becomes a source of microbiological pathogens that emit a foul odor. This waste has very negative impacts on the overall environment [1,4]. The content of chicken feathers, consisting of 92% keratin, 1.02% carbohydrates, 1.28% lipids, and 0.69% water, is widely used to create value-added products, particularly due to their high protein content [1-3,5-6]. Keratin is a semi-crystalline structural protein from a group of scleroproteins with high mechanical stability and resilience because of the presence of cysteine disulfide bonds, hydrogen bonds, and salt bonds in its structure, resulting in a solid polymer structure [3,5,7].

Kuncaka et al. [8] succeeded in converting chicken feather waste into solid hydrolysate and liquid hydrolysate, which contains less than 0.1% humic acid and 1–10% fulvic acid by the hydrothermal carbonization method using Kuncaka Reactor. Hydrothermal carbonization is a continuous wet thermochemical conversion method that takes 5–240 min under hydro conditions with a temperature of 150–350 °C and an autogenous pressure of 2–6 MPa [8-15]. The formation of humus under natural conditions with biotic and abiotic reactions takes a matter of years [16]. Therefore, this method is another alternative to developing synthetic humus through the concept of the New Road of Synthetic Humification [17], which combines the concepts of terra preta soil [18] and modern humus [19-20].

The hydrothermal carbonization mechanism depends on the composition of the raw materials. The processes of hydrolysis, dehydration, decarboxylation, condensation, polymerization, and aromatization during the hydrothermal carbonization method have decomposed the components of chicken feathers, including keratin, carbohydrates, lipids, and water, into new compounds. The products formed are suspended solids containing a large proportion of carbon (solid hydrolysates), oil-mixed liquids with high water content (liquid hydrolysates) and produce a small amount of CO_2 gas (see Fig. 1) [9,11-15]. Micro-chemical wave hydrothermal treatment is very helpful for the hydrolysis of chicken feather keratin and other matrix components by breaking disulfide bonds in a relatively short time to produce 80% dissolved protein [5,21]. Solid hydrolysate, which consists largely of carbon and is completely enveloped by liquid hydrolysate during the hydrothermal carbonization process, shows promising properties with different morphologies, porosities, and functions in its application as a soil remediation agent [22]. Liquid hydrolysate, on the other hand, tends to be less than optimal for use in certain soils as it is easily carried away by water. Therefore, modifications must be made to impart physical properties to the liquid hydrolysate to maximize its function in soil remediation.

According to the modern definition, humus is a complex and heterogeneous mixture of relatively small organic components stabilized by hydrophobic interactions and hydrogen bonds [19-20]. Therefore, the method of partial hydrothermal carbonization (PHTC) generates associations of supramolecular compounds from relatively small heterogeneous molecules (see Fig. 2), which are stabilized by hydrophobic interactions and

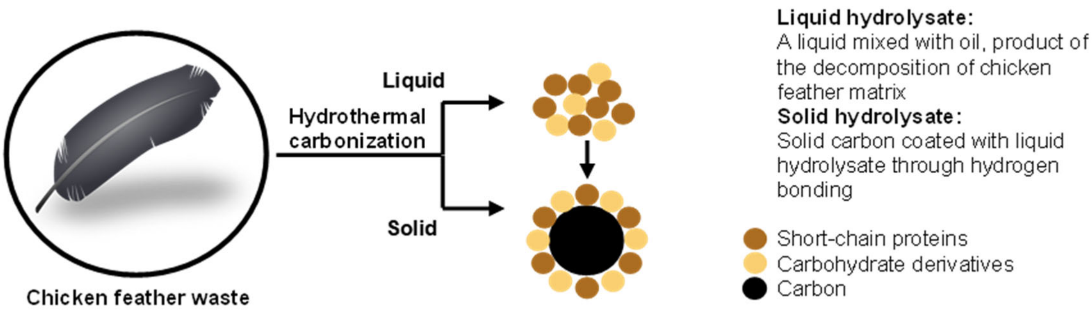

Fig 1. Hydrolysate formation from the hydrothermal carbonization process [8]

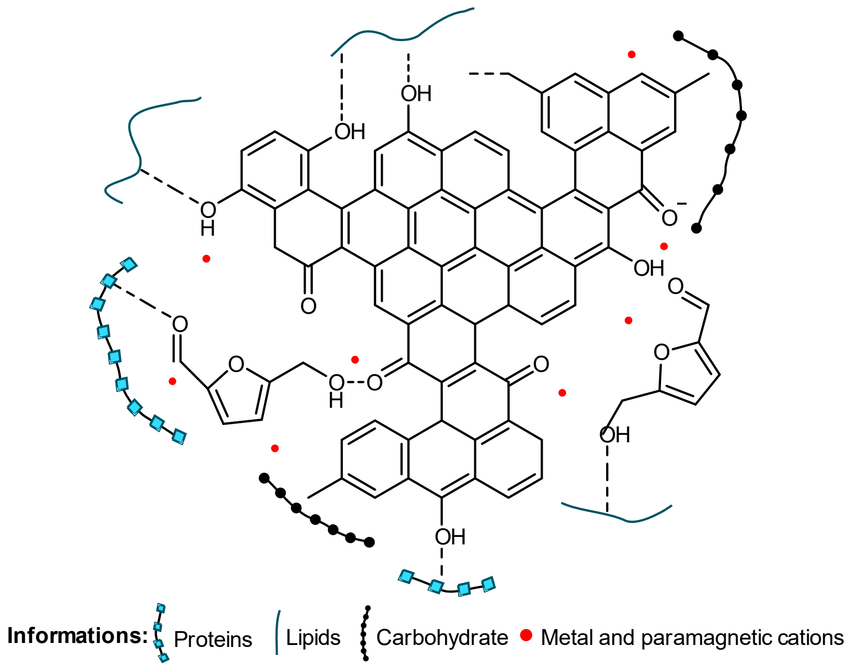

Fig 2. Hypothetical association model between supramolecular humus molecules [17]

hydrogen bonds only in hour order [8,16-17]. Fertile soil contains at least 5% organic humus material, which is recognizable by its dark color [23]. The important role of humus is to control the ecological and environmental functions of the soil, which contributes to the biological, chemical, and physical properties of the soil and soil fertility, as it can store water and nutrients available to plants and release the energy needed [23-25]. Natural humus consists of specific humin substances with amorphous structural properties, dark brown to black color, polydispersity, high molecular weight in the range of 20,000–300,000 g mol^{-1}, and high thermodynamic stability. Humic substances consist of humic acid, fulvic acid, and humin due to their solubility in acidic and base solutions [26-28].

Humin is a component closely related to inorganic colloids in soil [29]. Humin, by definition, is a term used to distinguish it from other fractions in humus, it has no clear chemical structure, and is a constituent of soil organic matter that is insoluble in acidic or base solutions [25,29-30]. Humin consists of a complex and heterogeneous mixture of organic components that form an amorphous compound with a molecular weight of 20,000 to 300,000 g mol^{-1} and consists of 80 to 90% soil organic carbon. The humin content in humus largely determines soil fertility and the function of the biosphere, especially in agriculture and sustainable ecosystems [25-27]. Humin in hydrothermal carbonization products is in the form of microspherical precipitates that tend to agglomerate [31]. The humin core is formed by the simultaneous and complex reactions of dehydration, rehydration, rearrangement, condensation, and aromatization of the carbohydrates contained in chicken feathers. Humin formation involves

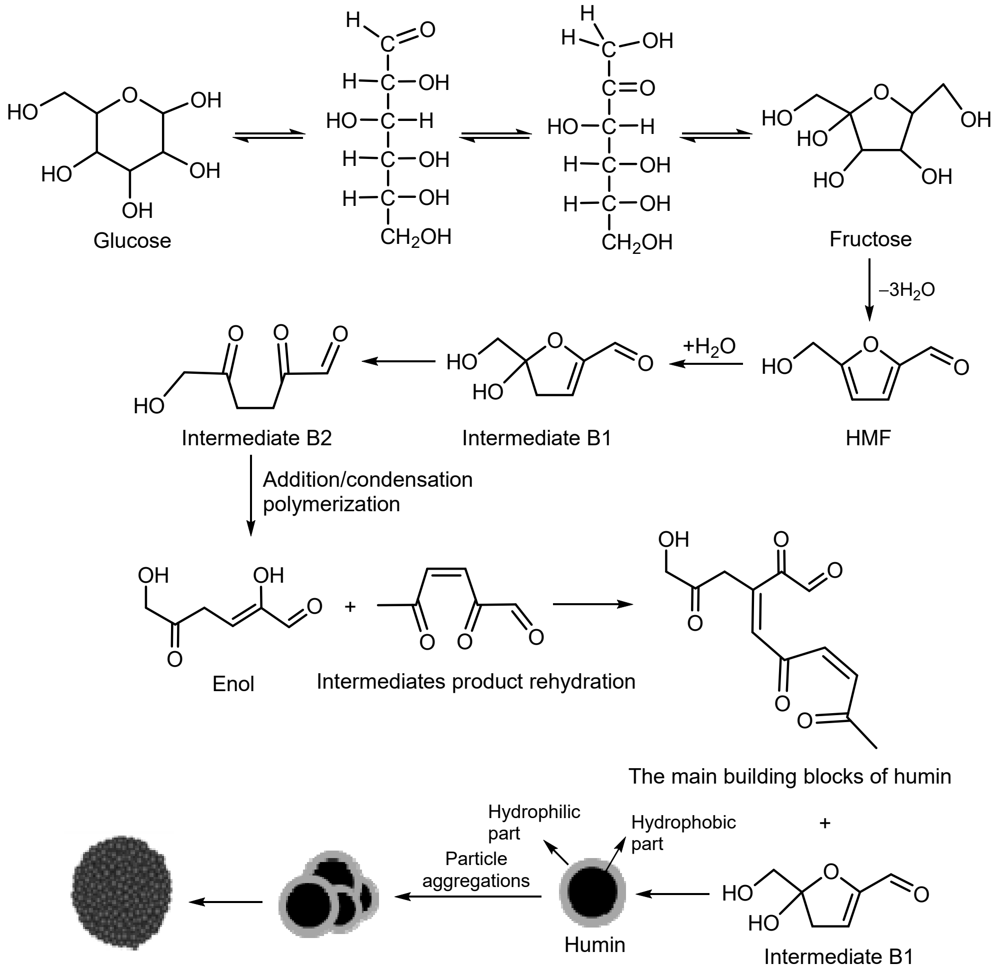

Fig 3. Mechanism of humin formation [32]

dissolved oligomers, the growth of primary particles, and the physical absorption of dissolved molecules in a hydrothermal carbonization system, including a short-chain protein that simultaneously undergoes aggregation of particles into large complex molecules (Fig. 3) [31-32].

Humin is a fraction that arranges humus and can be isolated from other fractions, that are, humic acid and fulvic acid, due to different solubility. According to the International Humic Substance Society (IHSS), humin can be isolated using sodium hydroxide (NaOH) to produce humin precipitate, while humic acid and fulvic acid are in the solution phase [19,26,33-34]. A NaOH solution for a long time under suitable conditions can hydrolyze weak ester bonds, facilitate oxidation reactions in samples, and increase the concentration of carboxylic acid groups [28,35]. Isolation using the alkaline method followed by DMSO was able to isolate up to 93% of the total organic carbon in clay [33]. Humin morphology depends on the substrate and catalyst of the raw materials in which it is formed. SEM and TEM images are analyses that can preserve the solid morphology of humin and provide the average size of humin particles. Analysis with XRD provides a microcrystalline structure that can be used to analyze the humin formation process [31]. The aim of isolating and characterizing humin, which is a recalcitrant fraction, is to understand the organic carbon components in soil because they have an important role in maintaining soil fertility and quality, especially in the fields of agriculture and sustainable ecosystems [25-26]. Understanding the natural humin components can be fundamental for developing synthetic humin to contribute soil humin requirements, especially on agricultural soils that are low in organic matter [8].

■ EXPERIMENTAL SECTION

Materials

The samples used in this study were solid hydrolysate and biochar 2:1 prepared under the auspices of CV Humus Yogyakarta. The chemicals used in the study were NaOH from Merck, dimethyl sulfoxide (DMSO) pro-analysis from Merck, 98% sulfuric acid (H_2SO_4) from Merck, and distilled water.

Instrumentation

Humin isolation was performed using laboratory glassware (pyrex) and a magnetic stirrer (Thermo Scientific Cimarec$^+$). The isolated humin was characterized using a Fourier transform infrared spectrophotometer (IR Prestige-21 Shimadzu), X-ray powder diffraction (PanAnalytical E'xpert Pro), transmission electron microscopy (JEOL JEM 1400), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX JED-2300), and atomic absorption spectroscopy (Perkin Elmer 3110).

Procedure

Sample preparation

The hydrothermal carbonization process of chicken feather waste produces solid hydrolysate and liquid hydrolysate. The solid hydrolysate was dried and ready for analysis. The liquid hydrolysate is combined with biochar, and the mass ratio used is liquid hydrolysate (2) and biochar (1) to produce a product called biochar 2:1.

Humin preparation

A total of 11 g of solid hydrolysate and 8 g of biochar 2:1 were reacted with 0.1 M NaOH, respectively. The ratio of sample mass to solvent volume was 1:10. The mixture was stirred with a magnetic stirrer for 4 h and then settled overnight. The precipitate of synthetic humin was separated from the filtrate by centrifugation at 5,000 rpm for 10 min, then washed with distilled water and dried at room temperature. Synthetic humin was washed with a mixture of DMSO and 98% H_2SO_4 (94:6, v/v). The mixture was stirred with a magnetic stirrer for 14 h, then diluted to pH 2 with distilled water and separated from the filtrate by centrifugation. Synthetic humin was washed with distilled water until clear and dried at room temperature.

Sample analysis

Synthetic humin isolated from solid hydrolysate and biochar 2:1 were analyzed by FTIR, XRD, TEM, SEM-EDX, and AAS, respectively. Both samples were tested and analyzed to determine the physical and chemical properties of synthetic humin from the hydrothermal carbonization of chicken feather waste.

■ RESULTS AND DISCUSSION

Isolation of Humin Synthetic

Like natural humus, synthetic humus is also expected to contain humin and aims to carry out soil remediation using a host-guest chemistry concept through hydrogen bonds because it can accelerate the fulfillment of humus in the soil. The humin fraction was isolated from synthetic humus using the IHSS method, that is, a 0.1 M NaOH solution followed by DMSO:H_2SO_4 (94:6, v/v) to produce dark-colored microspherical solids that were insoluble at all pH conditions after the isolation process [26,33-34].

The yield of synthetic humin in Table 1 shows a significant difference in the mass. Modification of liquid hydrolysate and biochar with the mass ratio 2:1, then referred to as Biochar 2:1, are successfully aggregated into synthetic humus compounds with complex hydrogen bonding reactions. The hollow biochar matrix acts as a host for the liquid hydrolysate, increasing the aggregation of each particle [9]. In addition, mixing biochar with liquid hydrolysate in agricultural applications will increase their effectiveness as a soil remediation. Biochar plays a role in duplicating soil organic carbon, and its functional groups have cation exchange capacity, so it can improve chemical properties and soil biology [9,36]. Solid hydrolysates, which consist mainly of carbon and are completely enveloped by liquid hydrolysate during the hydrothermal carbonization process, show promising properties as soil remediation agents with a humin yield of 12.7% [22].

The morphology and properties of synthetic humin are dependent on the substrate and hydrothermal carbonization operating conditions. Therefore, analytical techniques such as FTIR, XRD, SEM, TEM, and AAS were used to determine the physical and chemical properties of synthetic humin.

Physicochemical Properties of Synthetic Humin

Based on analysis of the data obtained, the two synthetic humins provide a relatively similar FTIR spectrum pattern. According to the FTIR spectrum that is shown in Fig. 4, the broadband at 3,400 cm^{-1} indicates the overlap between the OH and NH groups. The stretching vibration of the N–H group originates from a short-chain protein containing nitrogen in the form of NH_2, amplified by the bending N–H vibration at 1,635 cm^{-1}, which indicates an amide functional group [37]. The wavenumber also shows stretching vibrations of the hydroxyl and carboxyl O–H groups [8], and O–H vibrations of water molecules, silicates, and carbohydrate derivatives [26], which is correlated with the hydrophilic surface of polyaromatic compounds [8,37].

The wave numbers at 2924 and 2854 cm^{-1} are asymmetric and symmetrical C–H stretching vibrations which indicate the aliphatic structure of the methylene ($-CH_2-$) and methyl ($-CH_3$) groups [8,26,38-39] found in the polyaromatic structure of lipids [8,12,35]. The group

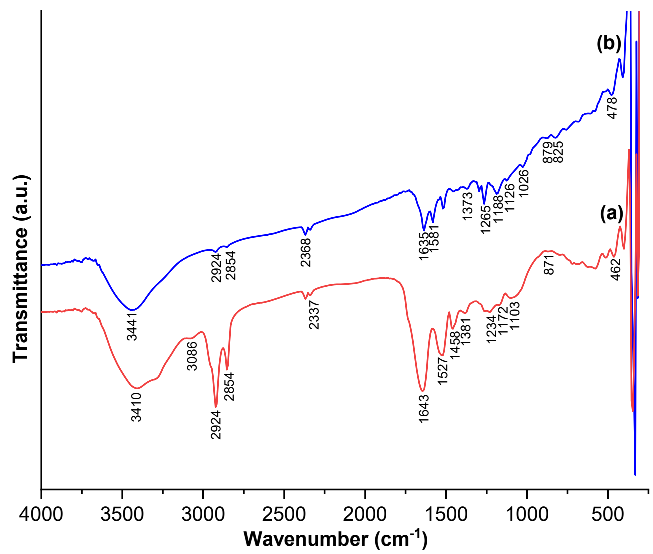

Fig 4. FTIR spectra of (a) humin from solid hydrolysate and (b) humin from biochar 2:1

Table 1. The yield of isolated synthetic humin

Sample	Sample weight (g)	Humin weight (g)	Humin yield (%)
Biochar 2:1	8	3.6	44.5
Solid hydrolysate	11	1.4	12.7

was strengthened by the C–H bending vibration of CH_3 at 1,400 cm^{-1}. The 2,300 cm^{-1} band shows the O–H vibration of the –COOH carboxylate group of the amide on the protein [4].

The 1,600 cm^{-1} band shows the bond vibration of the C=C group of symmetrical and asymmetric stretching alkenes in the polyaromatic structure [8] of short-chain proteins [12] reinforced by the aromatic C–H bending vibrations at 850 cm^{-1} [8,12]. This absorption proves that HTC products form a polyaromatic structure that can play a role in binding nutrients in the soil [8].

Short bands at 1,000 to 1,250 cm^{-1} show stretching vibrations of the C–O group [8,12,26] polycondensation products on the surface of polyaromatic polysaccharides [8,12,39] and shows the stretching vibration of the aromatic C–N groups of the protein. The 870 cm^{-1} band shows the Si–O mineral group from inorganic materials [26], while the 450 cm^{-1} band shows Fe–O mineral groups in synthetic humin [8,39].

Synthetic humin from biochar 2:1 and solid hydrolysate characterized with an FTIR spectrophotometer aims to qualitatively identify the constituent functional groups. Based on these data and compared with other references of natural humin in soil, both of these spectra FTIR is humin like the natural humin [36,39-41]. It is known that humin consists of polyaromatic structures derived from biomolecular derivatives such as proteins, carbohydrates, and lipids, which result from the hydrolysis of chicken feather waste [9,11-15]. The aromatic structure of humin is expected to enhance the role of synthetic humus as a soil amendment. The main structure of humin is not known with certainty and requires further research in the field of soil chemistry. Hu et al. [42] studied the FTIR spectra of humin formed from several biomass substrates and showed that humin consists of carbonyl, hydroxyl, and aromatic structures. Thus, carbohydrates in cellulose which are converted to humin, undergo dehydration reactions, rearrangements, aromatizations, and aldol condensations. Therefore, humin from synthetic humus resulting from the hydrothermal carbonization of chicken feather waste generally has functional groups like natural humin in nature.

The nature of the carbon framework that composes humin was analyzed using XRD. The humin diffractogram in Fig. 5 shows that the carbon structure of synthetic humin biochar 2:1 and solid hydrolysate is amorphous carbon, indicated by the appearance of weak peaks at 5–45°. The carbon structure formed in humin from synthetic humus, which is produced by hydrothermal carbonization of chicken feather waste, no longer shows the crystallinity of the chicken feather structure [4] but has been hydrolyzed to form new aggregates that are identical to natural humin such as in peat soil [42-43], in the form of an amorphous carbon structure [28,31]. The weak vibration of Fe in the FTIR test is also shown by the humin diffractogram that insignificant peaks of Fe are equally distributed in the humin structure which is composed of amorphous carbon. Based on the FTIR and XRD tests, it can be concluded that humin in solid hydrolysate and biochar 2:1 has a polyaromatic compound with an amorphous carbon structure like natural humus.

Surface Morphology of Synthetic Humin

Characterization by SEM and TEM is used to observe humin morphology without destroying its original state. TEM and SEM images help estimate the average size of humin particles. After the hydrothermal carbonization reaction, humin can be observed in the

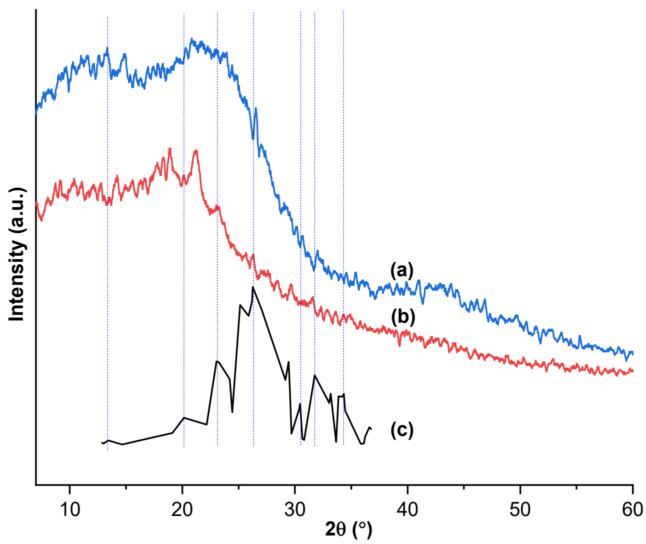

Fig 5. Diffractogram of humin from (a) biochar 2:1, (b) solid hydrolysate, and (c) JCPDS of Fe

range of 0.2–20 nm as amorphous spherical bulk particles [31]. The TEM projection in Fig. 6 shows the size and distribution of synthetic humin particles. The projection of dark and light colors shows the density of the particles in the compound. The TEM projection in Fig. 6 analysis with Image-J shows the size of both of the humins is less than 14 nm projected through the dark black part and is coated by polyaromatic amorphous carbon. The Fe element contained in complex aggregates of humin structures coated by polyaromatic amorphous carbon with a size smaller than 20 nm is classified as nanoparticles that can be superparamagnetic properties [44-45].

The difference in the results of the TEM analysis for these two types of synthetic humin is the interaction between the particles. Humin from biochar 2:1 shows separated particles, while humin from solid hydrolysate shows stacked particles. This is possible because the crystallinity of humin from solid hydrolysate is more amorphous than humin from biochar 2:1, so there is less ordering and interaction between the particles. Furthermore, the results of this analysis also show that the surface area of humin from biochar 2:1 is larger than humin from solid hydrolysate [9]. Humin particles that separate from each other show better aggregation than particles that stack aggregate each other.

The surface morphology analysis of synthetic humin using SEM in Fig. 7 shows humin as a disordered agglomerate [31]. Synthetic humin from biochar 2:1 exhibits a surface morphology like a solid plate with a combination of surfaces that are part smooth and part rough, forming an indentation. Due to its relatively large surface area, this synthetic humin has formed a new aggregate between the liquid hydrolysate and the biochar host, which forms its physical framework, according to TEM image analysis. Therefore, the surface morphology of this synthetic humin shows relatively the same characteristics as biochar, in the form of a porous solid with mesoporous size and a better surface area than

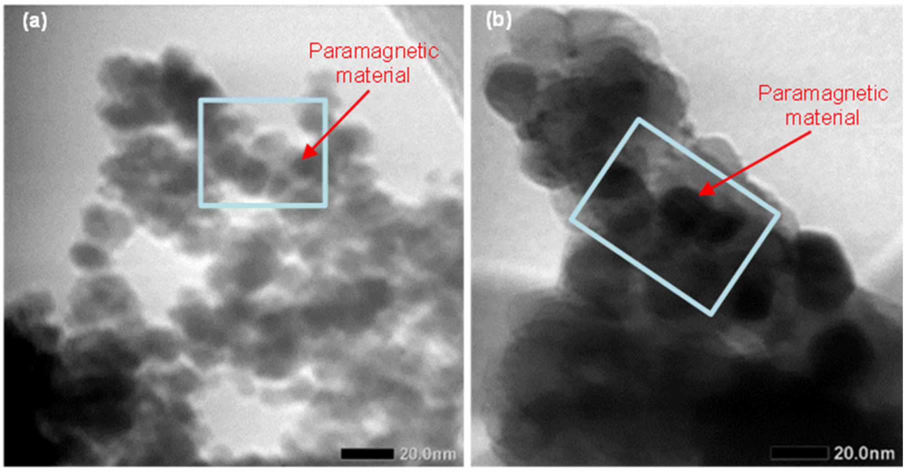

Fig 6. TEM image of synthetic humin from (a) biochar 2:1 and (b) solid hydrolysate

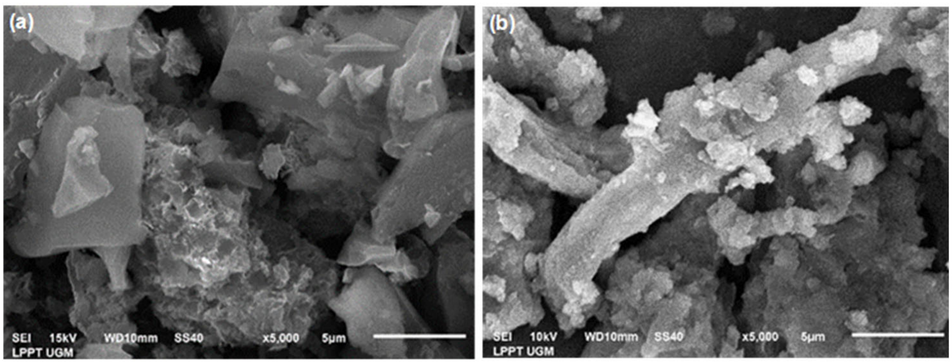

Fig 7. SEM of humin from (a) biochar 2:1 and (b) solid hydrolysate

Table 2. Synthetic humin in EDX analysis

Element	Humin from biochar 2:1		Humin from solid hydrolysate	
	% Mass	% Atom	% Mass	% Atom
C	42.35	53.48	53.77	59.30
N	9.39	10.17	29.46	27.86
O	27.04	25.64	14.74	12.20
Si	17.82	9.63	0.22	0.10
Cu	1.33	0.32	1.07	0.22
S	-	-	0.73	0.30
Mg	0.15	0.10	-	-
Zn	1.09	0.25	-	-
K	0.26	0.10	-	-

humin from solid hydrolysate [39]. Synthetic humin from solid hydrolysate showed that the surface morphology was in the form of an irregular spherical agglomerate of microparticles attached to a larger surface. It is theoretically possible that these clumps originate from soft polymers that make up carbohydrates and proteins, which decompose into small fragments and form carbon globules on the surface [15].

EDX analysis in Table 2 showed that the major constituent of synthetic humin material is polyaromatic amorphous carbon, as shown in XRD analysis with >50% carbon atomic percentage and >42% mass fraction. The presence of Fe elements, which are nanoparticle-sized and covered in large quantities of polyaromatic amorphous carbon, cannot be detected during EDX analysis with a voltage of 15 kV and a magnification of 5,000×. Modifying liquid hydrolysate with biochar results in different macro and micronutrient compositions. Synthetic humin from biochar 2:1 contains the macronutrients C, N, O, Mg, and K and the micronutrients Cu and Zn, while humin from solid hydrolysate contains the macronutrients C, N, O, and S and the micronutrient Cu. The macronutrients N, O, Mg, K, and S are nutrients that plants require in large amounts, while the micronutrients Cu and Zn are required in small amounts.

The presence of paramagnetic particles in this synthetic humin can act as an oxygen binder when humin is applied to soil as a slow-release agent. This oxygen group explains the high affinity for water so that it can be used to increase the water storage capacity of the soil [9,37]. In addition, the redox reaction of synthetic humin Fe with an environment rich in Fe minerals will play an important role in the biogeochemical cycles of pollutants. Electron transfer can take place even in the presence of oxygen, therefore, humin will be a promising material for soil remediation [8,46]. The presence of macro and micronutrients in the humin also shows that synthetic humus has components that can increase the need for soil humus so that it can provide nutrients to the soil for plant growth and fruit formation.

Quantitative Test of Humin Content

The analysis of the humin fraction with AAS aims to provide quantitative information to complement the FTIR, XRD, TEM, and SEM-EDX analysis data in the form of elements that are sources of macro and micronutrients. The results of the analysis with AAS in Table 3 showed that synthetic humin from solid hydrolysate and biochar in a ratio of 2:1 contained the elements Ca, K, Si, Fe, Mn, Cu, and Zn.

The highest element concentration in humin from biochar 2:1 and humin from solid hydrolysate is Fe. This proved the presence of Fe in humin on FTIR and XRD analysis. The Fe concentration in humin from solid hydrolysate is more than biochar 2:1. This is possible because the solid hydrolysate, chelates more iron than the liquid hydrolysate during the hydrothermal carbonation process [8]. This Fe content in synthetic humin is still within the normal limits according to the Regulation of Indonesian Minister of Agriculture Number 70 of 2011 on Organic Fertilizers, Biological Fertilizers, and Soil Improvers, so it is good humin [47].

Table 3. Mineral concentration of humin from AAS analysis

Element	Humin from biochar 2:1 (mg kg^{-1})	Humin from solid hydrolysate (mg kg^{-1})	Regulation of Indonesian Minister of Agriculture (ppm)
Ca	70.31	2.18	-
K	16.01	1.99	-
Si	3.86	5.09	-
Fe	912.12	3408.74	Max 9000
Cu	3.74	26.38	-
Zn	9.63	23.48	Max 5000

The Si content in humin also proves that humin can bind inorganic minerals, including Si and Fe. The presence of the elements Ca, K, Cu, and Zn in the EDX characterization data was also verified by AAS analysis. This proves that the decomposition of keratin, carbohydrates, and lipids from chicken feather waste by the hydrothermal carbonization method can release macro and micronutrients that are useful in the soil. So biochar 2:1 and solid hydrolysate contains synthetic humin which is intended to be used as a soil remediation by accelerating the formation of soil humus.

■ CONCLUSION

This study shows that the problem of natural humus degradation, which is much faster than its formation process, can be overcome by the formation of synthetic humus, which has the same properties as natural humus and can be produced in an hour order. Conversion of the components keratin, carbohydrates, lipids, and water from chicken feathers into synthetic humus produces solid hydrolysate and liquid hydrolysate, which contain humin-like natural humus. The biochar-modified liquid hydrolysate ran into humus aggregation, which gave a humin yield of 44.5%, while the humin yield of the solid hydrolysate was 12.7%. Analysis of the physicochemical properties of synthetic humin shows that the complex association compounds between short-chain protein derivatives, carbohydrates, fats, and inorganic minerals form aggregation of polyaromatic amorphous carbon complex compounds. The humin surface morphology of the solid hydrolysate was in the form of spherical microspheres like natural humin, while the humin surface morphology modified with biochar showed a combination of biochar as a host with a larger surface area. Based on FTIR, TEM, SEM-EDX, and AAS tests, synthetic humin exhibits superparamagnetic nanoparticle properties covered by amorphous carbon, Si and Fe minerals, also contains the macronutrients N, O, S, Mg, and K and the micronutrients Cu and Zn. The humin content in this synthetic humus complements its properties like natural soil humus, which can be used as a soil remediation agent and soil humus accelerator.

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