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Article

Effects of Extraction Methods on the Thermal Stability of Extracellular Polymeric Substances-Based Biomaterials from Wastewater Sludge

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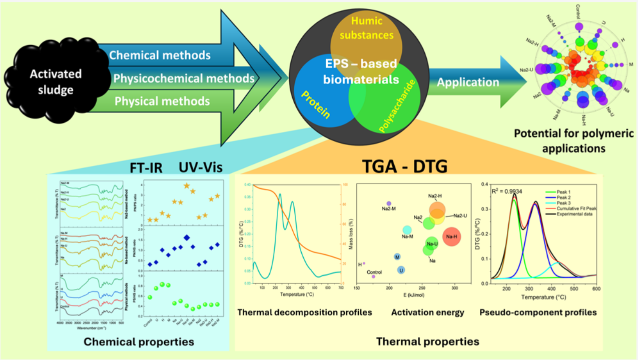
ABSTRACT: Various methods for recovering extracellular polymeric substances (EPS)-based biomaterials from wastewater sludge exist. However, the relationships between extraction methods and properties of biomaterials have not been fully explored. In this study, the thermal properties, including activation energy (AE) and thermal decomposition mechanism, of EPS-based biomaterials extracted by different methods have been determined by thermogravimetric analysis integrated with the deconvolution method. Simultaneously, the chemical properties of these biomaterials, such as the extraction yield, chemical composition, and functional groups, have been monitored to clarify the influences of extraction methods. Notably, proteins and humic-like substances have been found as the major components to determine thermal stability and AE. Moreover, the physicochemical method shows significant effects on enhancing extraction yield and AE, with the NaOH and heat methods proving to be outstanding by delivering the highest AE of 300 kJ/mol and a substantial char formation of 24%. The results have demonstrated the significant impact of extraction methods on the thermal stability of EPS-based biomaterials. Moreover, this finding provides insights into the linkages between the properties of EPS-based biomaterials and extraction methods to guide the selection of appropriate extraction methods tailored to specific applications, including flame-resistant materials.



KEYWORDS: *activated sludge, extracellular polymeric substances, physicochemical extraction, thermal properties, activation energy, thermogravimetric analysis*

1. INTRODUCTION

Activated sludge (AS) processes are the most widely used technologies in biological wastewater treatment plants (WWTPs) worldwide.[1] Nonetheless, the excess amount of wastewater sludge generated from this process poses a critical problem, as sludge disposal covers up to 50% of the total operational costs of treatment.[2] To address this issue, the recovery of value-added materials from wastewater sludge has been considered as a sustainable pathway for improving the environmental and economic sustainability of wastewater treatment following a circular economy concept.[3] The biomaterials in sludge have generally been divided into two types, including intracellular polymerics substances (IPS) and extracellular polymeric substances (EPS).[4] Both IPS and EPS are constituted by similar macromolecule substances, such as proteins (PN), polysaccharides (PS), and humic-like substances (HS).[5] Therefore, the recovery of IPS and EPS separately is still limited by the lack of appropriate quantification and determination of IPS and EPS.[6] Recently, the EPS have gained more interest since they account for high content in sludge (70−90% organic content of sludge) and have shown their potential for various applications. However, most recent EPS extraction methods, for example, alkaline, acid, and ultrasonication, can cause releasing of IPS.[7,8] Studies on extracting the EPS without or minimizing the presence of IPS can be done by using ethylene diamine tetra-acetic acid (EDTA) and cation exchange resin (CER).[6] However, these techniques are toxic, expensive, and less feasible on an industrial scale. In contrast, the EPS properties were influenced by their composition instead of the contamination of IPS. For example, the high aromaticity in both HS and PN can enhance the redox activity of EPS.[9] The high PS and lipid contents can also turn EPS into a water-proofing material.[10] Therefore, according to Feng et al.,[11] the extraction of EPS should be

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developed for specific applications with an ideal structure rather than considering cell lysis or contamination of IPS. Very recently, the EPS from the base−acid method has drawn more attention when they have been widely used in various applications, such as water-proof coating,[12] flame-retardant,[13] and seed coating[14] materials. By using this method, the extracted biomaterials contain the PS, PN, and HS from both IPS and EPS.[15] These biopolymers can interact with cations, resulting in the coating application of extracted EPS. Since the base−acid method has been widely used to recover a mixture of EPS and IPS for material applications, the cognition of the biomaterials from this method has varied over the decade.[16] Therefore, in this study, to be more accurate, we use the term "EPS-based biomaterials" to indicate the extracted biomaterials from the base−acid method. The term will help to clarify that the extracted biomaterials can consist of EPS and other types of biopolymers from sludge, but EPS are the main fraction due to their dominance in sludge.

Extraction is a vital process that can directly impact the properties of EPS-based biomaterials. In the base−acid method, the sludge is first disrupted by using base chemicals, such as sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3), to release biopolymers like PN, PS, and HS.[17] Afterward, the EPS-based biomaterials are collected by precipitation using acid, ethanol, or dialysis.[11] It is apparent that releasing biopolymers in the base treatment step is a key step that is strongly related to the yield and the properties of obtained EPS-based biomaterials. Many studies have indicated that the utilization of physical treatment and chemicals can boost the cleavage process of biopolymers. For example, Cao et al.[8] reported that using ultrasonication can improve the extraction yields of EPS-based biomaterials compared to cation exchange resin methods. According to Lu et al.,[9] the centrifuge methods can release more polysaccharides than using EDTA. The variation in the composition of EPS due to different extraction methods has also been confirmed through various studies.[18,19] However, the effects of these components on the properties of EPS-based biomaterials, especially in thermal stability, have not been reported yet.[20]

The thermal degradation behavior of polymers is also a critical factor for the manufacturing, design, and optimization of polymers and composites.[21,22] Understanding the thermal stability of EPS-based biomaterials not only paves the way for new applications of waste-derived biopolymers but also contributes to more sustainable waste management practices.[23] Activation energy (AE) is a main kinetic parameter that provides information on the thermal degradation of EPS-based biomaterials, which helps to predict the minimum energy required for breaking down the chemical bonds in EPS.[24] The AE value can indicate the thermal stability of materials and the potential for their use as flame-retardant materials.[20] Basically, the thermal properties of materials are influenced by their composition and intermolecular bonds. Gao et al.[25] have claimed that the thermal properties of sewage sludge are affected by the PN and PS, with AE values of 82.28 and 48.34 kJ/mol, respectively. Li et al.[26] have also agreed that PN and PS have a significant impact on the thermal properties of sewage sludge. Their study has also highlighted the differences in the biopolymers' contents and structure between granular and flocculation sludge, which can cause changes in the AE of sewage sludge. In addition, there is a consensus on the fact that PS and PN are the major components that can account for 70−80% of the organic content of EPS-based biomaterials.[27] Some recent studies have also confirmed the abundance of HS, predominantly in the form of humic acid, within sludge and EPS-based biomaterials.[28] In spite of representing only a fraction (6−20%) of the organic components in EPS-based biomaterials, the HS play a role in enhancing hydrophobicity[29] and the adsorption[30] of EPS-based biomaterials. Therefore, based on the existing literature, it can be hypothesized that the extraction methods influence the properties of EPS-based biomaterials through changes in their structure and contents.

This study aims to investigate the effects of the extraction methods on the thermal properties of EPS-based biomaterials from AS. The thermal properties of EPS-based biomaterials are examined through their decomposition behavior and activation energy. Additionally, variations in EPS-based biomaterials composition are analyzed to provide insights into how extraction techniques influence thermal stability. First, EPS-based biomaterials are recovered by different processing techniques, including physical, chemical, and physicochemical methods. The thermal properties of EPS-based biomaterials are measured using thermogravimetric analysis (TGA) because it is an effective method for investigating thermal stability as well as for calculating the AE of materials. The AE values of EPS-based biomaterials and their pseudocomponents calculated from the deconvolution method are determined by Kissinger (KS), Flynn−Wall−Ozawa (FWO), and Kissinger−Akahira−Sunose (KAS) methods. Simultaneously, the changes in their components (PN, PS, and HS), yield, and functional groups are characterized and analyzed to elucidate the influence of extraction methods. Based on the results, the key components for the thermal characteristics of EPS-based biomaterials are determined through correlation analysis between chemical and thermal properties. The potential applications of EPS-based biomaterials and the limitations of various extraction methods are also discussed.

2. MATERIALS AND METHODS

2.1. Materials. The AS (flocculent sludge) was supplied by Mangere WWTP (Auckland, New Zealand), which has been designed for treating 350,000 m^3 of wastewater per day. The secondary treatment of this plant uses a biological nutrient removal technique with eight reactor compartments, including four anoxic and four aerobic. Wastewater sludge was collected in the thickening tank, where primary and secondary sludges were mixed before feeding into an anaerobic digestion process. The fresh sludge was stored at 4 °C before being sterilized at 121 °C and 221 kPa for 30 min using the 385ELC-D autoclave (Tuttnauer, Australia) to reduce the possible risk of microorganisms for humans and the environment.[31] The sterilization by autoclave process is one of the common methods using heat and steam to remove bacteria in many industries. According to Lu et al.,[32] using an autoclave process could not only increase the solubilization efficiency of AS by up to 20% but also release PN and PS from tight-bound EPS to slime EPS and supernatant. Subsequently, the sterilized sludge was stored in sealed bottles at 5 °C for further use. The sterilized sludge characteristics are shown in Table S1. All of the chemicals and reagents are listed in Text S1.

2.2. EPS-Based Biomaterials Extraction Methods. The EPS-based biomaterials were extracted by three types of methods, including physical, chemical, and physicochemical methods, in which the conditions were chosen based on the existing literature.[7,33,34] The weight of AS used in all methods was 3 g of total solid, and the reaction volume of each method

Table 1. Summary of EPS-Based Biomaterials Extraction Methods and Their Conditions[a]

				conditions	
group categories	methods	abbreviation	pH	chemical	parameters
physical methods	control	control	5.64 ± 0.04	–	–
	ultrasonication	U	5.60 ± 0.04	–	40 W, 5 min
	heat	H	5.63 ± 0.02	–	80 °C, 30 min
	microwave	M	5.64 ± 0.05	–	900 W, 150 s
Na-based methods	NaOH	Na	13.21 ± 0.15	1 w/v %	–
	NaOH and ultrasonication	Na-U	13.10 ± 0.13	1 w/v %	40 W, 5 min
	NaOH and heat	Na-H	13.01 ± 0.12	1 w/v %	80 °C, 30 min
	NaOH and microwave	Na-M	13.20 ± 0.12	1 w/v %	900 W, 150 s
Na2-based methods	Na_2CO_3	Na2	10.30 ± 0.13	1 w/v %	–
	Na_2CO_3 and ultrasonication	Na2-U	10.10 ± 0.04	1 w/v %	40 W, 5 min
	Na_2CO_3 and heat	Na2-H	10.01 ± 0.09	1 w/v %	80 °C, 30 min
	Na_2CO_3 and microwave	Na2-M	10.22 ± 0.10	1 w/v %	900 W, 150 s

[a] – not applicable.

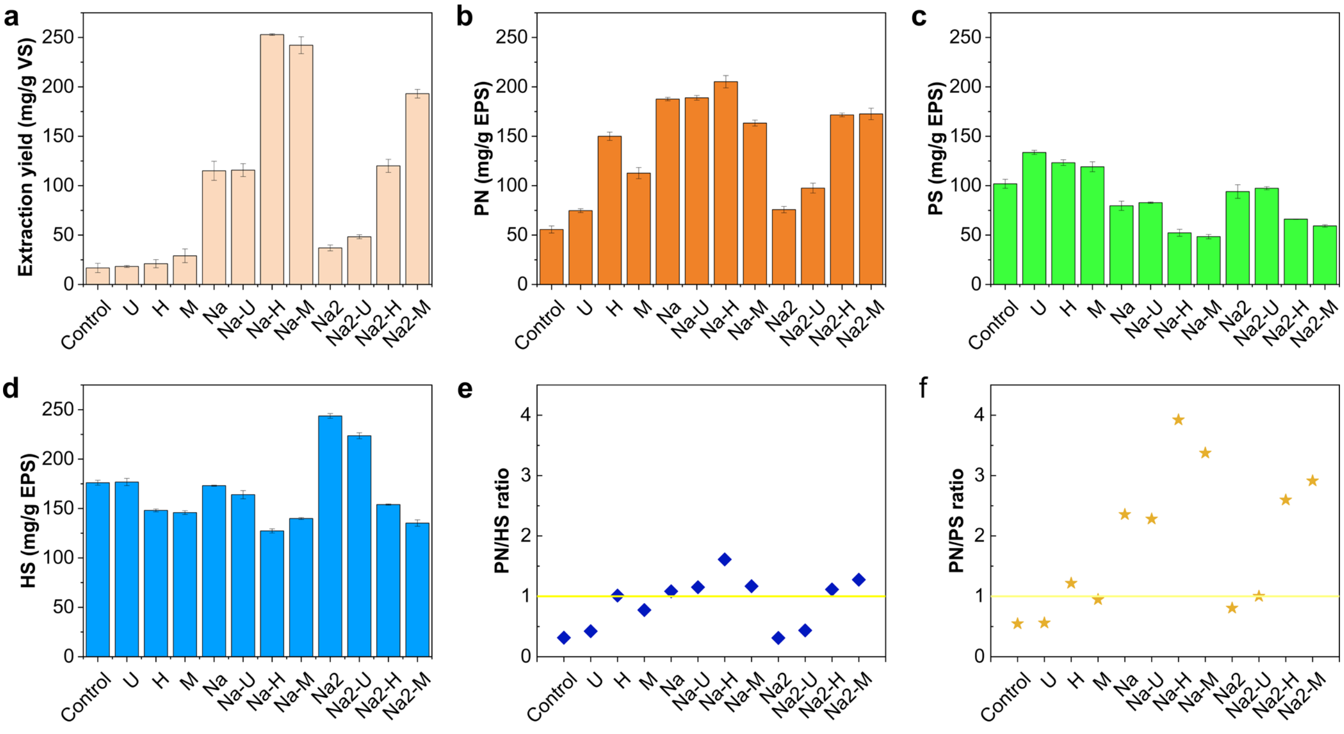

Figure 1. Extraction yield (a), PN (b), PS (c), HS (d), and PN/HS ratio (e) and PN/PS ratio (f) of EPS-based biomaterials recovered by different methods.

was 100 mL. The total solids were identified by the different masses of samples before and after drying at 105 °C for 24 h.

2.2.1. Physical Method. AS sample was added to distilled water until reaching 100 mL before being treated with several physical techniques, such as ultrasonication (U, 40 W, 5 min), heat (H, 80 °C, 30 min), and microwave irradiation (M, 900 W, 150 s).

2.2.2. Chemical Method. AS samples were mixed with distilled water before addition of NaOH or Na_2CO_3. The concentrations of NaOH and Na_2CO_3 were controlled at 1% (w/v). The mixtures were slightly shaken to ensure the dissolution of NaOH and Na_2CO_3.

2.2.3. Physicochemical Methods. AS samples were mixed with 1% (w/v) NaOH or Na_2CO_3 and subsequently treated using the physical techniques.

After extraction, the treated mixtures were centrifuged at 4000 rpm for 20 min to collect the supernatant. The supernatant samples were continuously precipitated by adding HCl 1 M until pH 2.2 to obtain EPS-based biomaterials in the acidic form.[35] The EPS-based biomaterial precipitates were collected from the mixture by centrifugation at 6000 rpm for 30 min. The collected EPS-based biomaterials were rinsed with distilled water before drying in an oven at 50 °C overnight. All of the extraction experiments were repeated at least twice on the same sludge sample. For comparison purposes, we defined all of the methods with the presence of NaOH as "Na-based methods," and corresponding to the case of Na_2CO_3, we named "Na2-based methods." Further, the phrase "physical method" is used to indicate methods that do not use an alkali for extraction. The abbreviations of obtained EPS and the groups of extraction methods are shown in Table 1. The pH values of the extraction mixtures were recorded by a pH meter (HI5521, Hanna Instruments, Australia).

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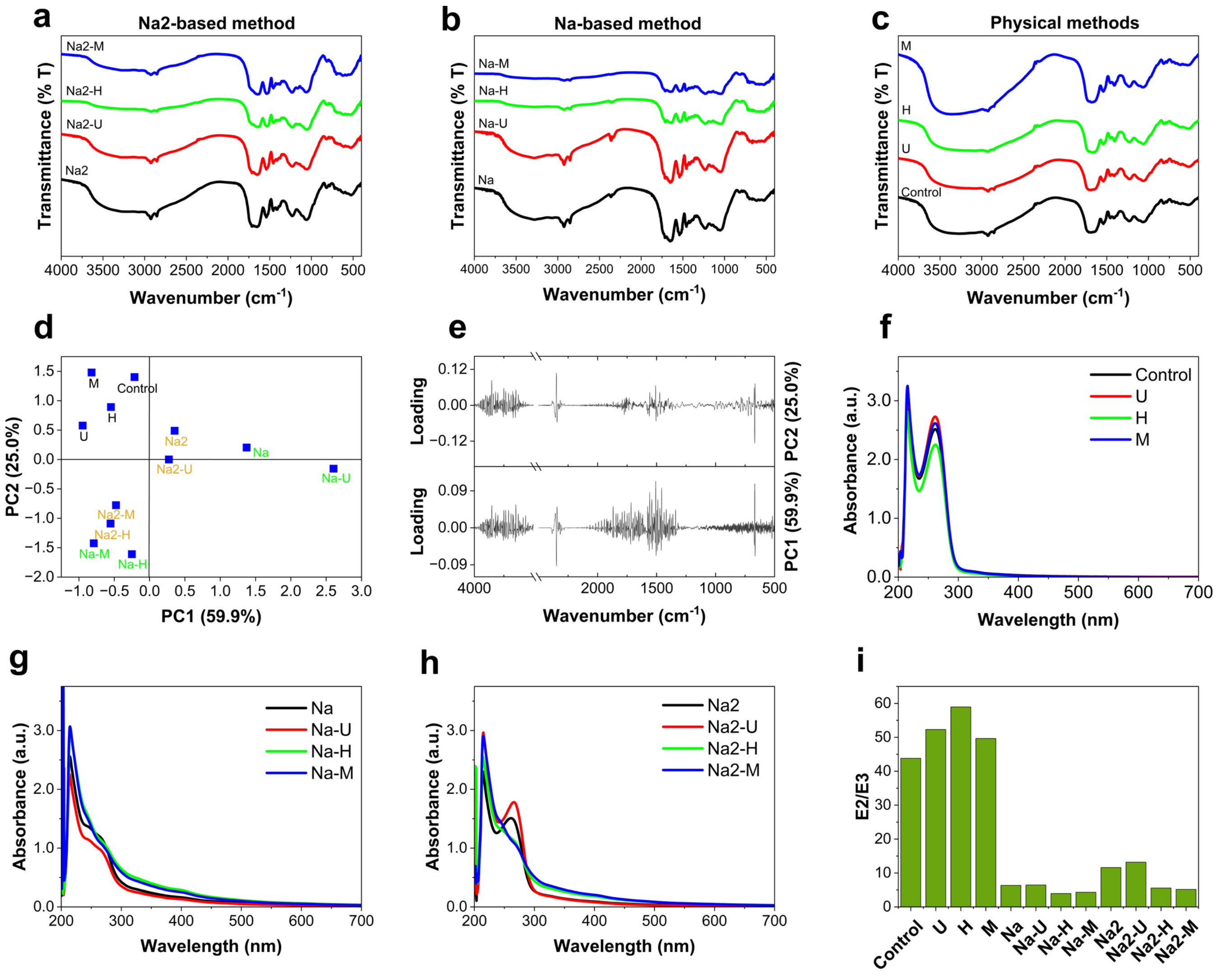

Figure 2. FT-IR (a−c), PCA score scattering (d), PCA loading (e), UV−vis (f−h), and E2/E3 ratio (i) of EPS-based biomaterials obtained by different extraction methods.

2.3. Analytical Methods. The extraction yield was expressed as a weight of volatile solids (VS) of EPS-based biomaterials per unit weight of VS of AS (mg/g of VS). The total solids, volatile solids, and ash contents of AS and EPS-based biomaterials were determined by the standard methods described in APHA.[36] PN, HS, and PS contents were determined using the modified Lowry method[37] and phenol−sulfuric acid method.[38] The chemical functionals and aromatic groups of all materials were identified by Fourier transform infrared spectroscopy (FT-IR) and ultraviolet−visible spectroscopy (UV−vis), respectively. In order to explore the similarities and differences in the FT-IR spectra of each EPS-based biomaterial sample, principal component analysis (PCA) was used. The detailed information is shown in Text S2−S3.

The thermal decomposition of EPS-based biomaterials was analyzed using TGA equipment (Q5000, TA Instruments, USA) with a heating rate of 5, 20, and 30 °C/min. Around 10 mg of the sample was heated from ambient temperature to 700 °C under nitrogen with a flow rate of 50 mL/min. The AE of each EPS-based biomaterial was determined by FWO and KAS methods. Due to the complexity of the thermal decomposition process, the deconvolution method was used to describe this process under multiple steps. Detailed information is provided in Text S4−S5.

3. RESULTS AND DISCUSSION

3.1. Effects of Extraction Methods on Chemical Properties of EPS-Based Biomaterials. *3.1.1. EPS-Based Biomaterials Extraction Yields and Composition.* The extraction yield and composition of EPS-based biomaterials in various methods are presented in Figure 1. In general, the extraction yields of EPS-based biomaterials mainly depend on the impacts of chemicals or physical techniques on the flocculation matrix of AS. Physical treatment disrupts the interaction of EPS and other components in the flocculation matrix. In contrast, the chemical treatment immediately changes the osmotic pressure in organism cell walls, leading to the efflux of biopolymers.[39] Notably, the energy level and pH values also influence the EPS-based biomaterial yield, which has not been mentioned in the previous study. The extraction yield of the M method (900 W) reaches the highest yield of 29.0 mg/g of VS, while the U method (40 W) yields less (18.3 mg/g of VS). Due to high functional group contents, the extracted biomaterials tend to reflocculate, resulting in a little increase in the yield of the U method; however, the high

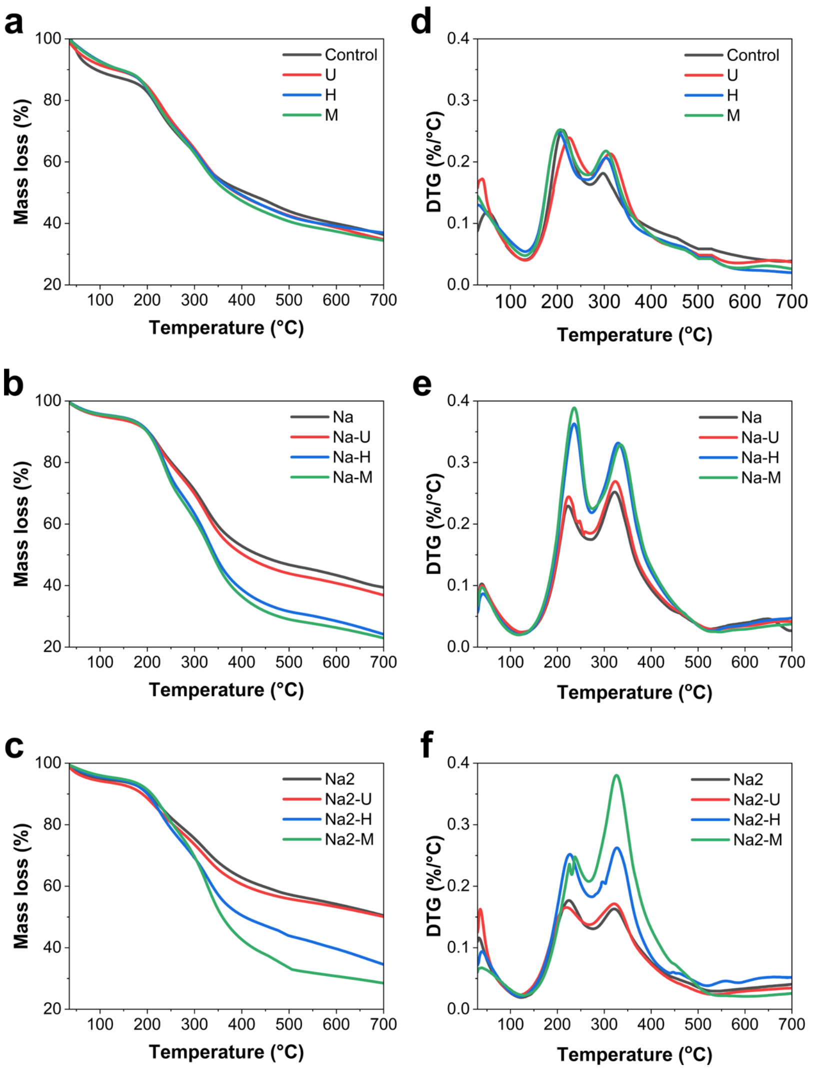

Figure 3. TGA (a−c) and DTG (d−f) curves of EPS-based biomaterials obtained by different extraction methods at a heating rate of 5 °C/min.

energy of the H and M methods can prevent this process.[39] The Na-based methods (pH 13) lead to a higher extraction yield than the Na2-based methods (pH 10) as the high pH value and strong basic properties of NaOH can enhance the cell lysis process and digest many floc components.[40] In addition, the extraction yields of the methods, including Na-H (252.9 mg/g of VS), Na-M (242.2 mg/g of VS), and Na2-M (193.1 mg/g of VS), are higher compared to results from some previous studies.[17,41] The combination of alkaline and thermal treatments (heat and microwave) demonstrates the potential for increasing the extraction yield of EPS-based biomaterials from AS.

The chemical compositions of EPS-based biomaterials are illustrated in Figure 1b−e. The PS and HS are dominant components in EPS-based biomaterials from the physical method, with contents of 101.9−133.6 mg/g and 145.8−176.1 mg/g, respectively. PS and HS are located on the outer layer of AS; thus, they can easily be extracted with low energy.[30] In contrast, PN are predominantly found in the inner layer of EPS, where they bond with cells and other components through nitrogen functional groups and hydrogen bonding.[42] The extraction techniques using high-energy methods (e.g., heat and microwave) and alkaline solutions are more effective in extracting PN compared to physical methods. For instance, the Na-H method has the highest PN content (205.2 mg/g). However, in high temperatures (∼150 °C), some PN can degrade into ammonia[43] or react with PS through the Maillard reaction.[44] Therefore, it is visible that the PN and PS contents of Na-M are lower than those of the Na-H method. As can be observed in Figure 1e, the Na-based methods extract EPS with high PN content (PN/PS ratio of 2.4−3.4 and PN/HS ratio of 1.08−1.61). The Na2 and Na2-U methods produce HS enrichment biomaterials with values of 243.7 and 223.6 mg/g, respectively. The high PN/PS ratio when using the heating method and alkali is also consistent with some existing studies.[27,45] These results highlighted the significant effects of extraction methods on the chemical composition of EPS-based biomaterials.

3.1.2. Functional Groups of EPS-Based Biomaterials in Different Extraction Methods. The functional groups of EPS-based biomaterials from various extraction methods have been determined by FT-IR and UV−vis spectroscopic methods, as shown in Figure 2. The FT-IR spectra of all of the biomaterials have similar functional groups. The broad peak at 3400 cm^{-1} is attributed to the hydroxyl group (−OH) and amino acidic group (−NH). The peaks at 2850 and 2920 cm^{-1} correspond to C−H stretching groups of PS, PN, and HS.[46] The amide I bands are represented by C−O and C−N stretching at 1620−

Table 2. TGA and DTG Data of EPS-Based Biomaterials Obtained by Different Extraction Methods at a Heating Rate of 5 °C/min[a,b]

	Zone I				Zone II				Zone III				
methods	T_{peak} (°C)	DTG (%/°C)	content (%)	$T_{offset1}$ (°C)	T_{peak} (°C)	DTG (%/°C)	content (%)	$T_{offset2}$ (°C)	T_{peak} (°C)	DTG (%/°C)	content (%)	$T_{offset3}$ (°C)	final residue (%)
control	47.3	0.16	12.7	143.3	222.2	0.25	21.4	283.1	314.4	0.18	25.0	572.2	36.5
U	44.0	0.17	9.5	141.9	227.1	0.24	22.4	283.0	317.0	0.21	27.4	554.0	35.2
H	42.1	0.13	10.0	142.5	213.5	0.25	21.4	272.3	316.7	0.21	29.0	582.9	37.0
M	41.4	0.14	10.1	146.4	222.6	0.25	23.8	283.0	314.4	0.22	28.2	584.6	34.5
Na	40.3	0.10	5.6	131.1	225.2	0.23	19.0	276.7	324.2	0.25	29.5	526.4	39.4
Na-U	40.7	0.10	5.6	129.6	224.7	0.24	19.9	277.1	322.3	0.27	31.7	537.1	37.0
Na-H	42.7	0.09	4.8	126.3	237.4	0.36	26.6	277.6	332.4	0.33	38.3	541.4	24.2
Na-M	41.2	0.10	5.0	128.5	237.2	0.39	28.8	279.8	336.9	0.33	38.1	529.5	23.0
Na2	34.6	0.11	5.5	128.4	224.8	0.18	16.2	279.7	324.2	0.16	22.4	544.8	50.5
Na2-U	36.9	0.09	5.0	128.1	224.8	0.21	19.2	279.7	325.4	0.22	27.6	534.1	41.7
Na2-H	39.8	0.09	5.1	129.6	228.3	0.25	21.3	278.2	327.8	0.26	30.6	528.0	35.3
Na2-M	41.5	0.07	4.8	132.7	237.7	0.25	19.5	275.0	329.3	0.38	43.6	539.2	28.5

[a] T_{peak}: the temperature at the DTG curve reaches the peak. [b] $T_{offset1}$, $T_{offset2}$, and $T_{offset3}$: the temperature end Zone I, II, and III, respectively.

1720 cm^{-1}. The stretching vibration of C−H and N−H at 1510−1530 cm^{-1} indicates the amide II structure.[45] The amide III bands are caused by the vibration of C−N stretching at 1210 cm^{-1}, and this peak cannot find the AS spectrum. Furthermore, the peak at 1027 cm^{-1} corresponds to the C−H plane bending group of PS.[27]

Due to the similarity of EPS-based biomaterial spectra, PCA has been applied to distinguish the FT-IR results among the biomaterial samples (Figure 2d−e). The PCA shows that PC1 and PC2 accounted for 85% of the variance in the FT-IR spectrum, with 59.9 and 25% of the total variability explained by PC1 and PC2, respectively. Utilizing the second derivative of FT-IR spectra, the PCA method categorizes the EPS-based biomaterials into four distinct groups.[47] With the presence of chemicals during extraction, the position of EPS-based biomaterials is shifted to increase the PC1 score and decrease the PC2 score, indicating significant changes in the functional groups. Despite using different heating methods, the alkaline and heat-microwave methods are clustered together, suggesting similar integrative effects on functional groups of EPS-based biomaterials. The scores of Na-U and Na2-U closely resemble those of Na and Na2, implying negligible changes in their functional groups.

Compared to the FT-IR method, UV−vis spectra clearly indicate changes in the electron transition of aromatic components in EPS-based biomaterials from different extraction methods (Figure 2f−h). Overall, most spectra are dominated by two peaks at 210−220 nm and 260−270 nm. The peak at 210−220 nm is present due to the electron transition (n → π*) in the carboxyl, carbonyl, ester, and amide groups in EPS-based biomaterials.[48] The peak at 260−270 nm can be attributed to the transition of $\pi \rightarrow \pi$* electron in aromatic groups of amino acids and HS.[48] These peaks can be observed in the physical, Na2, and Na2-U samples, Figure 2f,g, whereas the peaks at 260−270 nm of other methods have disappeared in Na-H, Na-M, Na2-H, and Na2-M. The reason can be due to the reaction of NaOH and PN under long heating treatment and high energy from the microwave, resulting in the degradation of aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine.[49] The base media of Na_2CO_3 and the energy of ultrasonication are not strong enough to hydrolyze PN during the treatment. In addition, the ratio of E_2/E_3 (250 and 365 nm) is demonstrated to have a negative correlation with molecular weight.[35] The highest E_2/E_3 of the physical methods reveals the low molecular weight of extracted components in EPS-based biomaterials. The low ratio in Na- and Na2- based methods signified that there is a high-molecular-weight compound extracted. The E_2/E_3 values of Na-H, Na-M, Na2-H, and Na2-M methods reached the lowest values, suggesting that the heating and microwave have positive effects on extracting high-molecular-weight EPS-based biomaterials.

3.2. Effects of Extraction Methods on Thermal Properties.

3.2.1. Thermal Gravimetric Analysis of EPS-Based Biomaterials. The TGA and DTG curves of the recovered EPS-based biomaterials at different heating rates are illustrated in Figure 3. The shapes of DTG and TGA curves have been unchanged at various heating rates between 20 and 30 °C/min, indicating that the reaction mechanism is not affected by increasing heating rate (Figure S1). The thermal decomposition data collected from the TGA and DTG graphs at 5 °C/min are also shown in Table 2. Generally, the thermal degradation processes of all biomaterials can be divided into three zones. The dehydration reaction and degradation of volatile compounds mainly occur in Zone I, within the temperature range of 30−140 °C.[50] All EPS-based biomaterials in Na-and Na2-based methods have a peak temperature of 52−72 °C with a mass loss of 4.8−5.6 wt %, whereas the biomaterials from the physical methods exhibit slightly higher mass loss of 9.5−12.7 wt.%. The higher residue content in Zone I of the control and U samples than those of H and M samples indicates the impacts of heating on reducing the hydrogen bonds of EPS and water, which are mainly in charge of moisture content in EPS-based biomaterials. In the range of 140−300 °C (Zone II), 16.2−28.8 wt.% mass loss of these biomaterials can be associated with the degradation of PS and PN I.[51] Zone III includes one peak and shoulder in a range from 300 to 600 °C, presenting the decomposition of HS[52] and PN[53] with mass losses of 22.4−43.6 wt.%. Similarly, Kim et al.[54] reported that EPS-based biomaterials extracted from granular sludge using the Na2-H method decompose between 150 and 600 °C, with a char yield of 32% at 700 °C. This is comparable to the results from the current study, where EPS-based biomaterials obtained using the same method decompose between 140 and 600 °C and have a char yield of 35.3% at 700 °C. Remarkably, the DTG curve shapes of EPS-based

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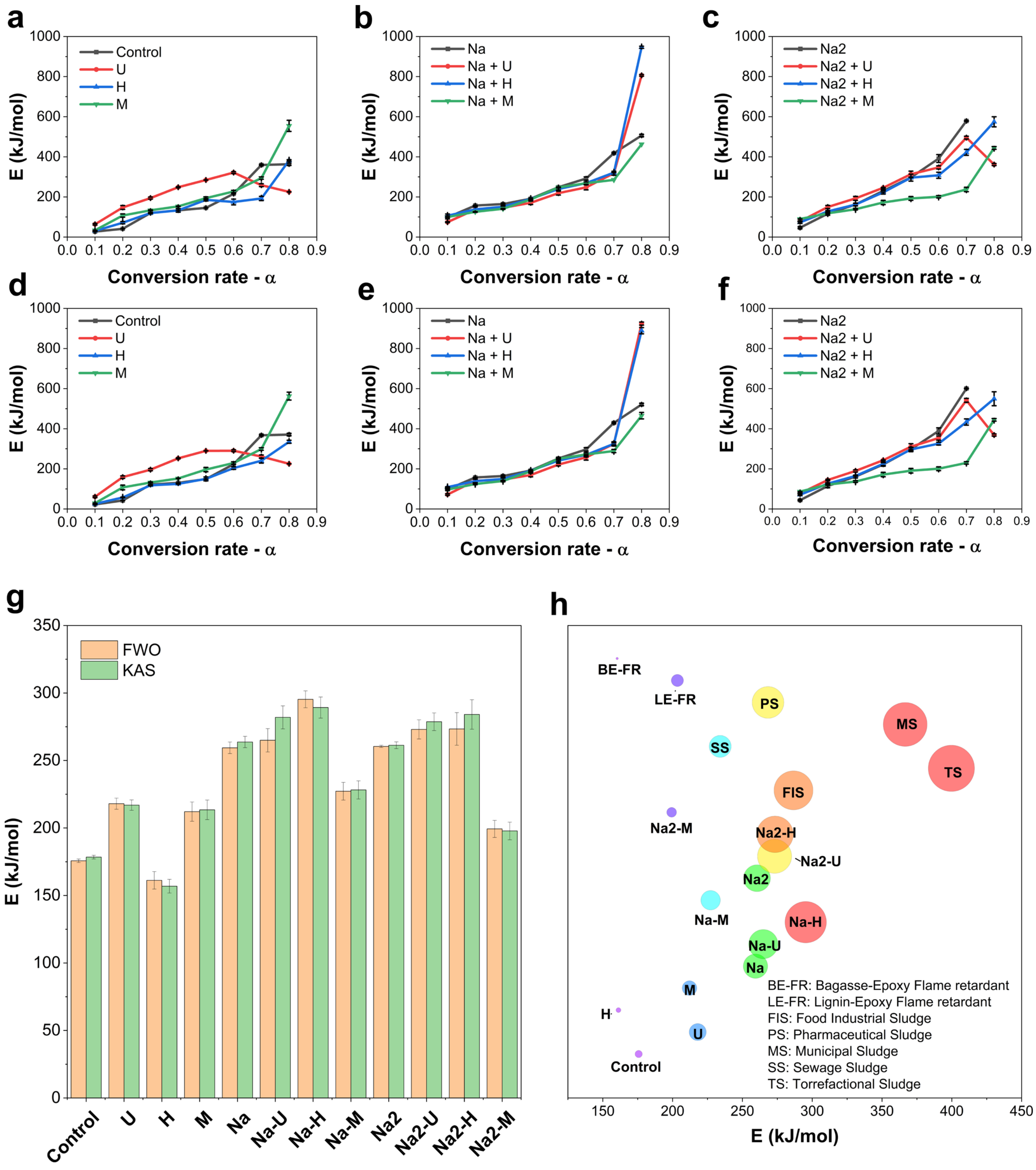

Figure 4. Variation of AE in different conversion rates in the FWO method (a−c), KAS method (d−f), and average AE (g) of EPS-based biomaterials; a comparison of AE values between EPS-based biomaterials and other materials (h).

biomaterials from Na-H and Na-M methods are very close together, indicating the similar effects of these physical methods on the thermal properties of EPS-based biomaterials, as shown in PCA analysis.

3.2.2. Activation Energy of EPS-Based Biomaterials. The AE at different conversion rates and the average AE of the extracted EPS-based biomaterials from various extraction methods are depicted in Figure 4. The R^2 values of all samples, ranging from 0.9395 to 0.9979, signify the high degree of accuracy in the two methods used (Table S3). The consistency between AE values derived from KAS and FWO verifies the reliability of these results.

The low AE values at conversion rates of 0.1−0.2 can be attributed to the degradation of moisture and volatile compounds. The variation of the AE following conversion rates highlights the complexity of the decomposition process.

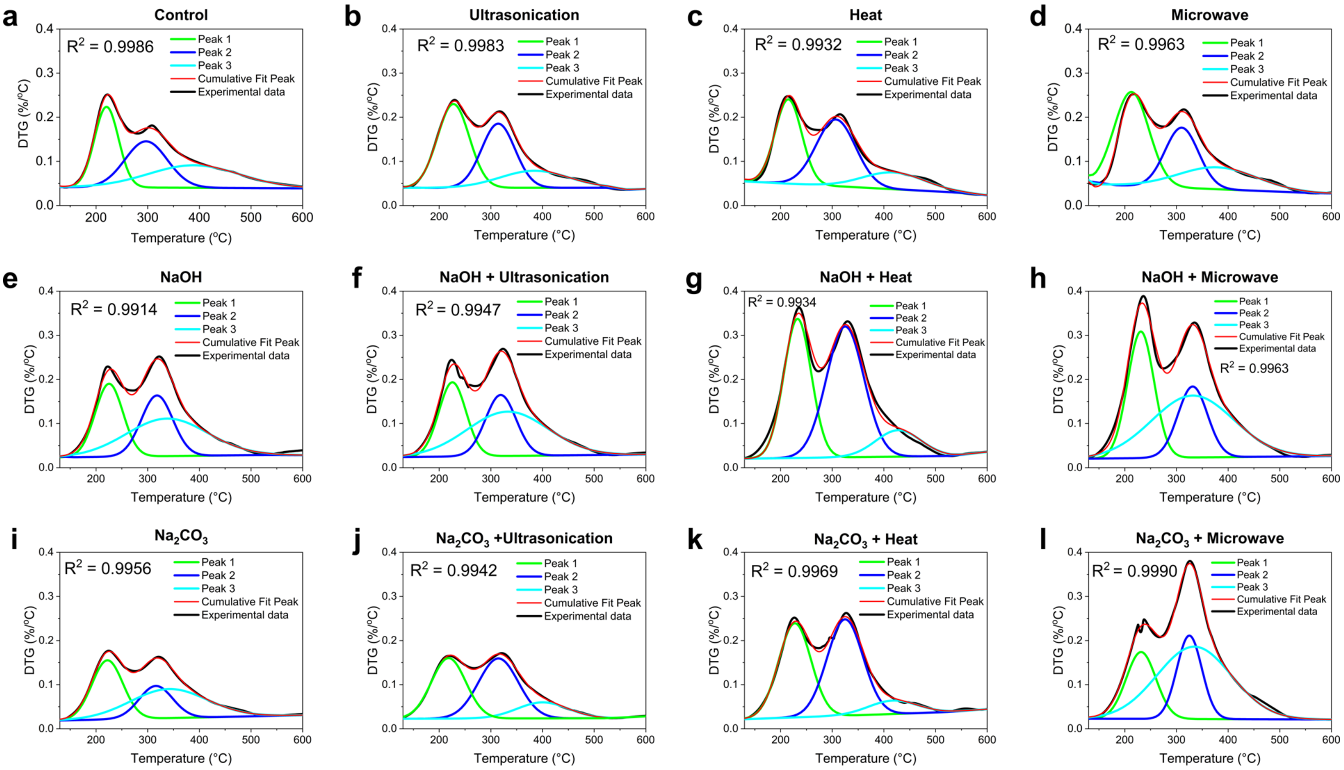

Figure 5. Deconvolution of DTG curves of EPS-based biomaterials obtained by different extraction methods under a heating rate of 5 °C/min.

The AE of all EPS-based biomaterials tends to increase in the α range from 0.2 to 0.5, corresponding to the decomposition of Zone II. The difference in the AE in Zone III (α = 0.6−0.8) demonstrates the effects of extraction methods on the thermal performances of EPS-based biomaterials. It is detectable that the EPS-based biomaterials from the Na-H method rise to the highest AE values of 946.4 kJ/mol for FWO and 889.0 kJ/mol in KAS from α = 0.7−0.8. Also, in this α range, Na and Na-M samples increase to about 506.1 and 462.9 kJ/mol, respectively. The sudden changes in AE values can be explained by the degradation of denatured PN due to the reaction of NaOH and PN. This finding has also been reported by Maurya et al. (2016), who claimed that the denatured PN can increase the AE values of deoiled microalgal biomass to 1254 kJ/mol from FWO and 1298 kJ/mol from KAS methods. On the other hand, these samples from the extraction methods using Na_2CO_3 do not show a significant improvement in AE. Hence, it can be inferred that the combination method using NaOH and physical techniques is more effective for increasing the AE than the control method. In particular, the average AE value of EPS-based biomaterials from the Na-H method achieves the highest values of 295.3 kJ/mol in FWO and 289.2 kJ/mol in KAS methods.

The AE values of EPS-based biomaterials were compared with several types of sludge, as illustrated in Figure 4h. The biomaterials extracted using Na-based methods, Na2-H, and Na2-U methods have AE values similar to those of the food industry[55] and pharmaceutical sludges,[56] suggesting comparable energy requirements for their degradation. Li et al.[57] demonstrated that the high stability of sludge can be influenced by the EPS content. They found that removing EPS decreased the AE required for the hydrolysis of the sludge. This indicates that Na-based methods effectively extract components that have a high thermal stability from sludge. Combined with the observed thermal decomposition behavior, it is evident that EPS-based biomaterials play a significant role in sludge thermal stability. Furthermore, municipal sludge (220−914 kJ/mol)[58] and torrefied industrial sludge (177−689 kJ/mol)[50] exhibit higher AE values than the EPS-based biomaterials in this study, indicating that these sludge types are promising sources for extracting EPS-based biomaterials with high thermal stability. Therefore, further research should be conducted to determine the optimal extraction methods for specific thermal properties of different sludge types.

3.2.3. Pseudocomponents of EPS-Based Biomaterials. The deconvolution method can be used to understand the reaction mechanisms by breaking down the DTG results into several pseudoreactions. In this study, the temperature range of 150−600 °C is selected for the deconvolution method because of the devolatilization of the main components of EPS-based biomaterials. The DTG results are deconvoluted with the Gaussian function, and the results of the samples at a heating rate of 5 °C/min are shown in Figure 5. The deconvolution results of the heating rates of 20 and 30 °C/min are illustrated in Figure S5. The correlation coefficient (R^2) values of all samples are above 0.99, implying that the calculated curves fit well with the experimental curves and that the number of pseudocomponents (PSC) assumed for these biomaterials is reasonable.

As shown in Figure 5, the DTG curves of EPS-based biomaterials consist of three peaks, in which two peaks close to 230 and 330 °C are associated with the peaks of DTG, and a peak varies with different extraction methods. According to Campo et al.,[51] the third PSC could present the high thermal resistance of PN II due to the high bonding energy of this chemical component. Conversely, Li et al.[26] suggest that the peaks could be associated with dead microorganisms. In order to gain insight into the effects of the chemical components on

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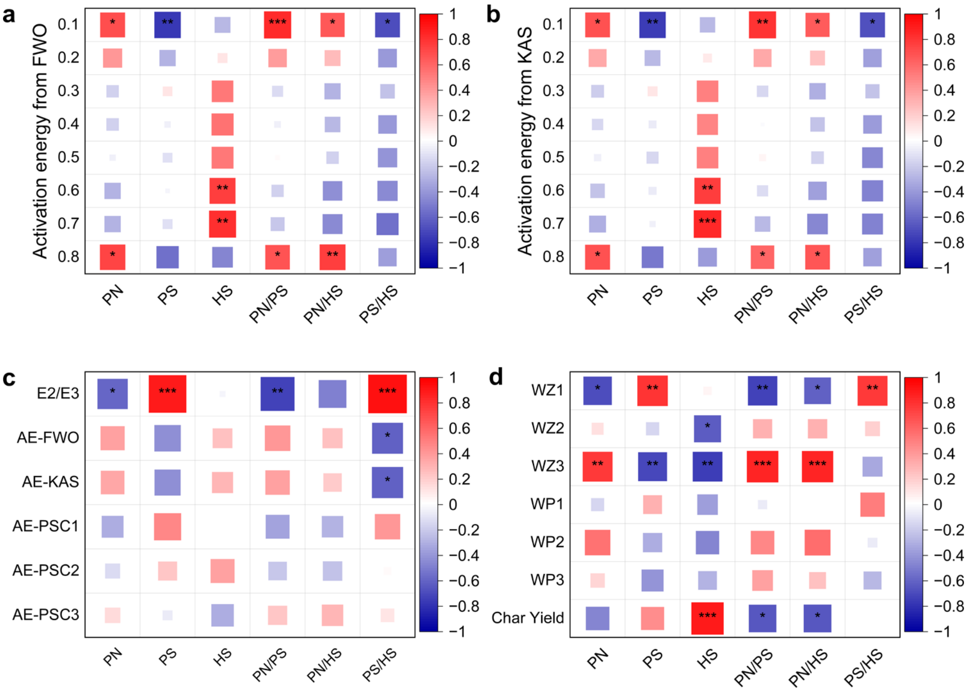
Table 3. Deconvolution Data of the DTG Curve at 5 °C/Min and Activation Energy of Pseudocomponents of EPS-Based Biomaterials

	Pseudocomponent 1				Pseudocomponent 2				Pseudocomponent 3				
methods	T_{peak} (°C)	DTG (%/°C)	AE (kJ/mol)	R^2	T_{peak} (°C)	DTG (%/°C)	AE (KJ/mol)	R^2	T_{peak} (°C)	DTG (%/°C)	AE (KJ/mol)	R^2	average AE (kJ/mol)
control	220.36	0.18	219.85	0.9946	297.01	0.10	157.05	0.9995	389.97	0.05	143.61	0.9916	173.50
U	227.49	0.19	266.23	0.9985	314.35	0.15	290.78	0.9955	384.33	0.04	159.74	0.9956	238.92
H	215.05	0.19	161.58	0.9795	307.05	0.15	175.33	0.9992	418.79	0.04	315.33	0.9953	217.41
M	212.87	0.21	138.83	0.9940	310.22	0.14	250.65	0.9972	379.20	0.05	155.34	1.0000	181.61
Na	225.28	0.17	182.86	0.9981	318.12	0.14	239.14	0.9992	337.41	0.09	51.39	0.9931	157.80
Na-U	225.57	0.17	181.72	0.9999	319.09	0.14	233.59	0.9973	332.39	0.10	44.98	0.9980	153.43
Na-H	232.80	0.32	167.88	0.9957	324.95	0.30	198.61	0.9845	427.71	0.06	375.72	0.9944	247.40
Na-M	231.10	0.29	116.05	0.9924	331.41	0.16	186.62	0.9902	331.41	0.14	186.54	0.9902	163.07
Na2	222.08	0.13	145.60	0.9982	315.87	0.07	291.12	0.9908	340.97	0.07	62.61	0.9998	166.44
Na2-U	218.22	0.14	119.30	0.9990	322.23	0.14	296.77	0.9862	400.14	0.04	288.09	0.9928	234.72
Na2-H	227.47	0.21	171.16	0.9979	324.69	0.22	287.51	0.9918	417.23	0.03	200.58	0.9944	219.75
Na2-M	231.54	0.15	134.27	0.9918	325.02	0.19	183.34	0.9866	334.55	0.16	143.48	0.9998	153.70



Significant level: * p≤0.05; ** p≤0.01; *** p≤0.001
-WZ1, WZ2, WZ3: overall mass loss at Zone I, II, III, respectively.
-WP1, WP2, WP3: overall mass loss of PSC1, PSC2, PSC3, respectively.

Figure 6. Pearson correlation heatmap between EPS-based biomaterials composition and their thermal properties, including AE from FWO (a), from KAS (b), from pseudocomponent analysis (c), and mass loss in different stages (d).

the thermal decomposition of EPS-based biomaterials, the Kissinger method was selected to calculate the activation energy of each PSC. The variation of activation energy of each PSC in the different extraction methods is listed in Table 3.

Overall, the thermal degradation of PSC1 happens at 220−230 °C with AE values of 116.1−219.9 kJ/mol. The results are highly related to the thermal degradation of PS; for example, Li et al.[59] have reported that a decomposition peak of PS at 230 °C was associated with the pyrolysis of sodium alginate, and the AE was recorded as 188 kJ/mol. Further, Li et al.[26] have indicated that the AE values of 139.9 kJ/mol for granule sludge and 264.0 kJ/mol for flocculent sludge at about 230 °C can be relevant to the decomposition of PS. On the other hand, the DTG peak (297−330 °C) and AE (157.1−296.8 kJ/mol) values of PSC2 can relate to the decomposition of PN.[60] The bond energy value of N−O, usually major in PN, is 230 kJ/mol. Li et al.[52] have also reported the AE of humic acid from soil in this stage is 241.7−466.7 kJ/mol. The pyrolysis process of PSC3 reaches a peak at 348−448 °C and ends at approximately 600 °C in all methods. The variant trends in AE values are found in PSC3, which implies that different reactions occurred depending on the extraction methods. According to Bach and Chen,[60] the AE values of PN, lipid, and carbohydrate in this stage are 208.8, 48.5, and 40.4 kJ/mol, respectively. Therefore, the higher AE found in this stage is mainly attributed to the PN content. For example, the Na-H sample (PN/PS = 3.9) has an AE value of 375.7 kJ/mol, while the AE value of the Na2 sample (PN/PS = 0.8) is 62.6 kJ/mol.

3.3. Correlation of EPS-Based Biomaterials Composition and Thermal Properties.

In this study, Pearson

correlation analysis was conducted to evaluate the potential relationship between the chemical and thermal properties of EPS from various extraction methods (Figure 6).

Notably, the PS and HA are positively correlated with the AE value of EPS-based biomaterials (red square) because they contain many recalcitrant linkages, such as C−N, N−O, and C≡C. In particular, the PN content, PN/HS, and PN/PS ratios are positively correlated with AE in the α range of 0.1, suggesting that a higher PN content can enhance thermal resistance at lower temperatures. The Pearson correlation analysis also indicates that HS increases the AE of EPS-based biomaterials in the range of 0.6−0.7. The HS content has a strong correlation with the char yield after the pyrolysis process, implying that the HC content can promote the formation of pyrolysis residues. The results are also consistent with the study of Li et al.,[52] who reported that the char yield of humic acid from the soil is around 44.8−45.7% at 800 °C. In addition, the PS content is significantly positively correlated with the mass loss at Zone 1 (Figure 6d), indicating that the PS can be highly degraded in this stage. The weak correlation between EPS-based biomaterial components, the average AE from different methods, and the AE of PSCs has confirmed the complicated pyrolysis process. Based on the current findings and the existing literature, the potential of PN and HS in enhancing the thermal properties of EPS-based biomaterials can be highlighted, with extraction methods influencing the concentration of these components.

3.4. Application and Limitations. Current research has identified that EPS-based biomaterial properties, especially thermal decomposition, are affected by extraction methods. The properties are ranked to elucidate the effects of extraction methods, as shown in Figure 7.

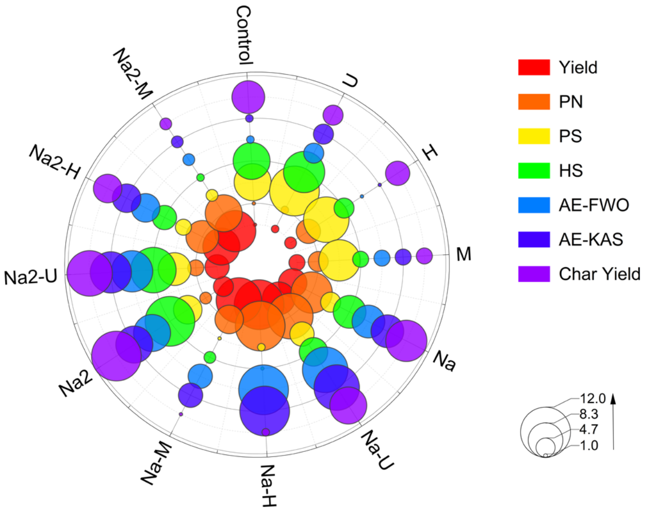


Figure 7. Ranking of EPS-based biomaterials on various parameters of each extraction method.

All of the EPS-based biomaterials from the physicochemical methods have higher AE values (200−300 kJ/mol) than that of lignin (166−182 kJ/mol)[61] and phytic acid (39.7−55.1 kJ/mol),[62] which have already been reported as a flame retardant. With this high thermal stability, the biomaterials can be used for many thermal treatment processes compared with other biopolymers. Therein, high thermal stability is one of the vital factors when looking for a polymer for packaging applications. Moreover, these AE values are higher than those of other biobased flame retardants, such as lignin-epoxy (158.94 kJ/mol for KAS method)[63] and bagasse-epoxy composites (160.3 kJ/mol for FWO method)[64] (Figure 4h). Furthermore, the char yields of EPS-based biomaterials from all extraction methods are higher than 17.11% of phytic acid.[62] Char formation can directly impact the fire properties of materials.[65] Most biopolymers, such as PN, cellulose, and chitin, have demonstrated their potential as flame retardants, creating char as a protective layer against heat and fire.[66] Since AE and char yield are the key parameters to determine thermal stability and fire retardancy, it can be noted that the Na-H extraction technique shows significant improvement in the yield, VS, and PN contents compared to other methods. At the same time, the EPS-based biomaterials from this technique exhibit the highest AE value, with a char yield of 27.83% at 700 °C. These research outputs suggest excellent thermal properties of EPS-based biomaterials from the Na-H method and potential applications as a flame retardant.

The ideal extraction procedure of the EPS is supposed to minimize cell lysis, not disrupt the polymer structure and efficiency.[37,67] However, the concept of the "ideal" technique seems to be inappropriate when put together with the circular economy concept. For example, adding many steps to reduce the effects of cell lysis during extraction can increase energy consumption. At the same time, residues with high bacteria still require additional treatments prior to disposal into the environment. Due to the limitation in extracting "pure" EPS, the application of EPS has been hindered. In comparison, alginates from algae and lignin from lignocellulosic biomass have been intensively studied over the decades due to simple extraction methods. The EPS-based biomaterials from the base−acid method not only have better thermal stability than lignin, as shown in this current study, but also have gel-formation properties as alginates.[35] In addition, Zheng et al.[4] have also claimed that the recovery of EPS-based biomaterials can also contribute to the transition to the circular economy in sewage management. Nevertheless, a poor understanding of their properties and complex extraction methods has led to an underrating valuation of EPS-based biomaterials as compared to the cases of lignin and alginates. Therefore, instead of focusing on minimal cell lysis or high EPS purity, the new extraction method from sludge should be effective, safe, and suitable for specific EPS applications. In fact, the combination of NaOH and conventional heating is also a common method in biomass pretreatment and is feasible for scaling up this technology to a larger scale (i.e., pilot scale). Le et al.[68] have utilized the Na−H method to produce silica and lignin from rice straw biomass. They show the possibility of silica and lignin recovery process in the waste stream and increase energy efficiency when integrated into the main system of bioethanol production from rice straw.[69] This work can be considered one of the role models demonstrating the potential of the Na-H method for extracting EPS-based biomaterials from AS on a larger scale. Oppositely, the Na-H method is a time-consuming process with a retention time of 30 min, but it takes only 5 min for Na-U and Na2-U and about 2.5 min for Na-M. This finding denotes that applying other physical methods instead of conventional heating can be more promising in extracting EPS-based biomaterials. However, the main limitations of these technologies are the feasibility of the upscaling operation and the capital cost.[11]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c10329.

> Chemicals and reagents (Text S1); chemical characterization (Text S2); statistical analysis (Text S3); activation energy (Text S4); deconvolution method (Text S5); the characteristics of sterilized sludge (Table S1); TGA and DTG data of EPS-based biomaterials extracted by different methods at different heating rates (Table S2); regression coefficients from the FWO and KAS methods of EPS-based biomaterials obtained by different extraction methods (Table S3); TGA and DTG curves of EPS-based biomaterials obtained by different extraction methods at heating rates of 20 °C/min and 30 °C/min (Figure S1); plots for determination of activation energy of EPS-based biomaterials obtained through different extraction methods by FWO and KAS (Figure S2); deconvolution results of EPS-based biomaterials obtained by different extraction methods at heating rates of 20 °C/min and 30 °C/min (Figure S3) (PDF)

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Notes

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ABBREVIATIONS

AS, activated sludge; AE, activation energy; DTG, derivation of derivative thermogravimetric; EPS, extracellular polymeric substances; FT-IR, Fourier transform infrared spectroscopy; FWO, Flynn−Wall−Ozawa; HS, humic-like substances; KS, Kissinger; KAS, Kissinger−Akahira−Sunose; PCA, principal component analysis; PC, principal component; PSC, pseudo-component; PN, protein; PS, polysaccharides; TGA, thermal gravimetric analysis; UV−vis, ultraviolet−visible spectroscopy; VS, volatile solids

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