



## Effect of Water Soluble Organic Carbon (WSOC) and Polycyclic Aromatic Hydrocarbons (PAHs) emitted by BioChar from the Rubber Sheets Processing Sewage Sludge Combustion

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### ABSTRACT

The total particle mass concentration, size distribution, pH form, polycyclic aromatic hydrocarbons (PAHs) value, the concentration of total Benzo[a]pyrene Toxic Equivalence (BaPTE) and water-soluble organic carbon (WSOC) of smoke particles from the rubber sheets processing sewage sludge biochar burning were studied. In this work, the temperature was measured at 500 mm above the fire base by using K-type thermocouples and a continuously data logger. The result showed that the measured temperature value increased when decreasing the biochar moisture content. The highest average total smoke particle mass concentration values at the initial burning time was found to be 17.53 to 35.27 mg/m<sup>3</sup> and then it was persistently reduced until the 60th minute, when the burning was stopped. The pH of biochar's smoke particles was higher than it was before combustion. The total smoke particle mass concentration, the mass median aerodynamic diameter (MMAD) and PAHs values decreased with increasing burning period and decreased the biochar moisture content. The largest BaPTE emission was observed at the highest moisture content, which was within the range of the greatest particle mass (less than 0.43 micron) in an initial combustion period. This value was about 60% that of a given the total value of BaPTE concentration. Meanwhile, the amount of PAHs, the BaPTE concentration and WSOC were depended on the smoke particle number, moisture content and combustion period.

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## INTRODUCTION

Due to the number of the agricultural industry in Thailand increasing, the sewage sludge of factory wastewater treatment plants also increased. Reviews of bioenergy source which produced from the sewage sludge of factory wastewater treatment plants were discussed as follows. The report of Zsirai (2011) and Begum et al. (2013) showed that the sewage sludge should be accepted a good bioenergy source. Because the energy generation from the sewage sludge produces substantially lower contamination emissions than the energy which produced from fossil-fuel burning. In a study by Photong and Wongthanate (2020), they presented that the sewage sludge has a good potential similar to a source of energy in the future for furnace combustion material and as power generation.

As known, the emission of fine particles from biomass combustion in the atmosphere, for

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instance, biochar burning contributes to photochemical smog and a serious concern for human health (Choosong et al., 2010; Salaudeen et al., 2017; Vicente and Alves, 2018; Turner et al., 2020; Kalasee and Dangwilailux, 2021 and Szatyłowicz, and Hawrylik, 2022). These particles play a particularly important role in human health since this size distribution causes damage to the most critical components of the respiratory system and penetrates deeper than larger particles. For the WSOC and PAHs formation of particulate, they are typical components emitted during the biomass combustion. Some PAHs are known to be carcinogenic and exposure to high levels of them has produced immunosuppressive effects. While WSOC problem was concern due to the presence of natural organic carbon (NOC) in water. NOC interaction with trace amounts of pollutants; such as smoke particles, PAHs, SO<sub>2</sub>; was transferred into a natural water environment (Battaglia et al. 2019; Xu et al. 2020 and Qin et al. 2022).

Investigation into the physicochemical properties of smoke particles from the biochar combustion have indicated that the biochar burning can be a significant source of gaseous matter and fine particles in ambient air, which then leads to serious human health risks and environmental loads. For the effects of WSOC on fine particles pH form, it was divided into two groups: changes to the hydrogen ion activity coefficient and the aqueous phase were diluted by aerosol liquid water associated with the organic fraction.

The information surrounding the physicochemical characteristics of smoke particles in the nano-size range is of particular importance in order to gain an understanding of the impact that burned biomass has on ambient air that can enter the respiratory system and affect the general health of an individual. Currently, Kalasee and Dangwilailux, 2021 have been the only ones studying the effects of smoke particles and PAHs particles from the biochar which produced by palm oil industry sewage sludge burning. Until now, there has been no research on the characteristics of smoke particles, WSOC and PAHs from the sewage sludge in rubber sheets processing biochar combustion.

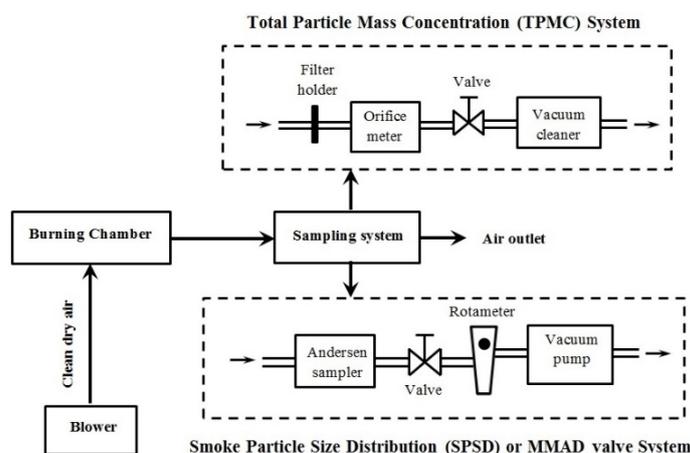
Thus, the objective of this paper was to study the characteristics of smoke particles, WSOC on particle-bound, pH form, and PAHs values in the emissions from the rubber sheets processing sewage sludge biochar burning. The characteristics consist of the smoke particles concentration and the different particles size distribution. WSOC on particle-bound, pH form, PAHs components and the total concentration of BaPTE were discussed to the influence of the temperature, burning times and moisture content, and the contribution of different smoke particle sizes.

## MATERIALS AND METHODS

In this research, the raw materials; the sewage sludge in rubber sheets processing; were produced at a ribbed smoked sheet rubber cooperative factory wastewater treatment plant in Patiu District, Chumphon Province, Thailand. These raw materials were sun dried for approximately three days before under-going pyrolysis at a temperature range from 300 °C to 500 °C for two hours. This is similar to the method used by Archawilawan et al. (2018) and Kalasee and Dangwilailux (2021). A photograph of this biochar from the sewage sludge in rubber sheets processing is presented in Figure 1. The moisture content of this biochar was set at 60% on a dry basis (d.b.) by sprayed with water, collected, and stored in a refrigerator. The biochar samples, which had a moisture content of 20%, 30%, 40%, 50% and 60% d.b., were prepared by using an electric air oven (Shanghai Jingke Scientific Instrument, Minhang, Shanghai, China) at 50 °C, while the completely dried biochar samples were dried at 103 °C in an electric air oven for 24 h until a constant weight was reached (Rattanamechaiskul et al., 2016; Kalasee and Dangwilailux, 2021; Kalasee and Dangwilailux, 2021). In each testing, 10.0 kg of samples which had different moisture content (20%, 30%, 40%, 50% and 60% d.b.) were burned in the combustion room and repeated five times or 175 bits of data (5 run x 7 time x 5



**Fig. 1.** A photograph of samples



**Fig. 2.** Schematic of the experimental set-up

repeat). A centrifugal blower (Nitco, Hessdorf, Germany) was used to set the air speed at 0.2 m/s during combustion.

The acidity of smoke particles can affect the environmental concern. Therefore, smoke particles collected on a quartz-type fibrous filter (ADVENTEC, QR-100, Tokyo, Japan) were dissolved in 5 mL of DI water. The acidity of smoke particles was recorded by an advanced pH meter kit (OAKTON pH 110 instrument, Shanghai, China), while the elemental composition of sample (the carbon, hydrogen, sulfur, and nitrogen) was recorded by a Vario EL III CHNS analyzer (Elementar, Hanau, Germany).

In each experimental condition, the temperature was measured at 500 mm above the fire base by using the K-type thermocouples and a continuously data logger (DataTaker, Melbourne, Australia). The temperature data was noted and discussed after the samples were burned for 10, 20, 30, 40, 50, and 60 min, respectively.

For the total smoke particle mass concentration from the biochar burning, the experimental data was recorded by a vacuum air sampler (Shanghai Yulong Vacuum Pump, Shanghai, China). A quartz type fibrous filter with a diameter of 110 mm (ADVENTEC, Tokyo, Japan) was put on an aluminum filter holder. The total smoke particle mass concentration was noted after the samples had burned for 10, 20, 30, 40, 50, and 60 min respectively, and each testing was taken over 5 min.

The geometric standard deviation (GSD) and the particle size distribution values of the smoke particle from the experimental samples burning were noted and determined by gathering particles which using an eight-stage Andersen air sampler (Andersen impactor Model AN-200, Dylec, Tokyo, Japan). Figure 2 presents an experimental Andersen air sampler which had the

cut-size particle diameters of 0.43, 0.65, 1.1, 2.1, 3.3, 4.7, 7.0, and 11.0 micron, respectively. A constant sampling air flow rate of 28.3 lpm was controlled by using a flow controller system (Dylec, Tokyo, Japan) which consists of a vacuum pump, a control valve, and a rotameter. A quartz type fibrous filter with a diameter of 80 mm (ADVENTEC, Tokyo, Japan) was put on the plate in each stage of the air sampler to collect the smoke particles. The smoke particles size distribution value was recorded and determined under identical provisos similar to the total smoke particles mass concentration. The MMAD value was used to calculate the size distribution value of the smoke particles which set as an aerodynamic diameter at 50% of the cumulative oversize percentage, and the GSD value was calculated by (Hinds, 1982).

$$GSD = \frac{d_{84.1\%}}{d_{50\%}} = \frac{d_{50\%}}{d_{15.9\%}} = \left[ \frac{d_{84.1\%}}{d_{15.9\%}} \right]^{1/2} \quad (1)$$

In this experiment, the WSOC concentration was measured and analyzed by the TOC-V; total organic carbon analyzer; (Shimadzu Corporation, Tokyo, Japan– limit of detection (LOD) 0.02 mg/L and limit of quantification (LOQ) 6 mg/L). The experimental sample was distilled to deionized water that it was acidified and injected into an oven by using a 0.45  $\mu\text{m}$  Teflon polytetrafluoroethylene (PTFE) syringe filter. Filter blank values in the deionized water used were deducted from the analyzed numbers of the experimental samples.

Following the technique defined in a previous study (Kalasee, and Teekapakvisit, 2020; Kalasee and Dangwilailux, 2021), sixteen forms of PAH compounds were analyzed: Pyrene (Pyr), Fluorene (Flu), Anthracene (Ant), Chrysene (Chr), Naphthalene (Nap), Benzo[k]fluoranthene (BkF), Indeno[1,2,3-cd]pyrene (IDP), Phenanthrene (Phe), Fluoranthene (Flu), Dibenz[a,h]anthracene (DBA), Benzo[b]fluoranthene (BbF), Acenaphthylene (Act), Acenaphthene (Ace), Benz[a]anthracene (BaA), Benzo[g,h,i]perylene (BghiPe) and Benzo[a]pyrene (BaP). These PAH names identified by the Environmental Protection Agency (EPA). In this study, an USE; ultrasonic extraction; technique was used to extract PAHs on the sampled filter. A 5 x 5 mm pieces of the filter sample was ultrasonically extracted twice in liquid solution; ethanol:benzene (1:3, v/v); for 15 min each. After adding 50  $\mu\text{L}$  of dimethyl-sulfoxide (DMSO), the distilled solution was concentrated by a rotary evaporator (Shanghai Jingke Scientific Instrument, Minhang, Shanghai, China) to dispose an ethanol and benzene, and the filtrate was then kept in a refrigerator at -20°C prior to analysis.

Following the technique defined in a previous study (Kalasee and Dangwilailux, 2021), *High-Performance Liquid Chromatography (HPLC)* method with UV detection was described for determination of PAHs in the environmental samples. In this experiment, PAHs were separated and analyzed by using Agilent 1100 Series liquid chromatography or Agilent 1100 LC (Agilent Technologies, California, USA) with ultraviolet absorption detection. The separates and mixtures were squirted into a UPS reversed-phases HPLC packing; C18 columns; (4.6 mm diameter, 250 mm length, particle size 5  $\mu\text{m}$ ; Agilent Technologies, California, USA) with a guard column. An inconstant phase was the acetonitrile-water mixtures which had a flow rate of 1.0–1.2 mL/min. The UV detector wavelength was 254 nm for each PAH. The sixteen PAH-Mix standards which imposed by the United States Environmental Protection Agency (US EPA) was used to prepare the external standard solutions (Rosinska and Dabrowska, 2018; Ciemniak et al., 2019 and Song et al., 2020). The qualitative analysis method of PAH was based on the comparison of the retention time and the values of UV spectra with reference standards by the software data program analysis of Chemstation.

For the PAH carcinogenic potency, the total BaPTE were determined by the multiplying mass concentration of individual PAH components ( $PAH_i$ ) with its respective toxic equivalency factors ( $TE_i$ ). The total BaPTE concentration value was determined by Nisbet and Lagoy (1992).

$$BaPTE = \sum_i (PAH_i \times TE_i) \quad (2)$$

The QC/QA measures of samples investigations are essential for the high data quality results (Singh et al., 2013). For the system calibration, the standard solutions were prepared from the stock solution, which was the ten times dilution of the 16 PAHs mix standard (Supelco catalog number 4-8743, U.S.A). The solvent used for dilution was acetonitrile. Calibration curves using PAHs standard solution in the range of 0.52-10.52  $\mu\text{g mL}^{-1}$  Nap were prepared by diluting the standard PAH from stock solution with acetonitrile. While, the reference samples (blank) were prepared, retained and analyzed in the same manner as the real samples to precision and evaluate analytical bias. Limit of detection (LOD) was considered as the lowest concentration that could provide a signal to noise ratio (S/N) more than 3. To determine the limit of detection, the PAH standard solutions were prepared for concentrations in the range of 0.0105-10.5200  $\mu\text{g mL}^{-1}$  of NaP. The limit of detection for all PAHs or the concentrations of PAHs gives the S/N of more than 3.

While, the reference levels (blanks) of WSOC were measured automatically for 30 min by passing the sample through a Teflon PTFE membrane filter. The reference levels ranged between 17 and 24  $\mu\text{g C}$  per 1 L of water during the study period. The uncertainty in the WSOC measurements was estimated to be between 3 and 10%.

A completely randomized design (CRD) was used to analyze the experimental data. The probability value (p-value) at 95% confidence interval was the significance evaluation terms.

## RESULTS AND DISCUSSION

Table 1 presents the carbon, hydrogen, sulfur, and nitrogen components of biochar sample. The carbon content of the biochar was  $29.27 \pm 3.12\%$ . The result showed that carbon value remained the same level with the carbon value of rice husks (Hata et al., 2014 and Ahiduzzaman and Islam, 2016). It suggested that the biochar from the sewage sludge in rubber sheets processing had a good potency to use as bioenergy source.

During pyrolysis process, the loss of volatile and tar matters takes away a lot of Hydrogen and Nitrogen contents. The components of remaining Hydrogen and Nitrogen were respectively  $2.15 \pm 0.18\%$  and  $3.57 \pm 0.11\%$  through pyrolysis at  $500^\circ\text{C}$ . This result was similar to the Ali et al., (2022) report. The Sulfur content of the biochar was in the ranges of  $4.82 \pm 0.24\%$ . In this study, the components of remaining Sulfur had a high value. This was because the natural rubber sheet was produced by the addition of a coagulant, for example wood vinegar, acetic acid, formic acid and sulfuric acid to the fresh Para rubber latex in product processing (Kalasee and Dangwilailux, 2021). While, the pH of the biochar ranged from  $5.19 \pm 0.28$ . The pH levels of biochar were similar to the reports of Angin, 2013; Lehmann and Joseph, 2015; Mensah and Frimpong, 2018; Wongrod et al., 2018; Reza et al., 2020 and Merdun et al., 2023. The low pH of biochar might be from the low pyrolysis temperature (Yuan et al., 2011; Heitkotter and Marschner, 2015; Ferreira et al., 2019; Vu and Do, 2021 and Ali et al., 2022) and the acids addition in the RSS production.

**Table 1.** Components in the tested biochar samples

Samples	Component (%)			
	C	H	S	N
Bio-char from sewage sludge in rubber sheets processing	$29.27 \pm 3.12$	$2.15 \pm 0.18$	$4.82 \pm 0.24$	$3.57 \pm 0.11$

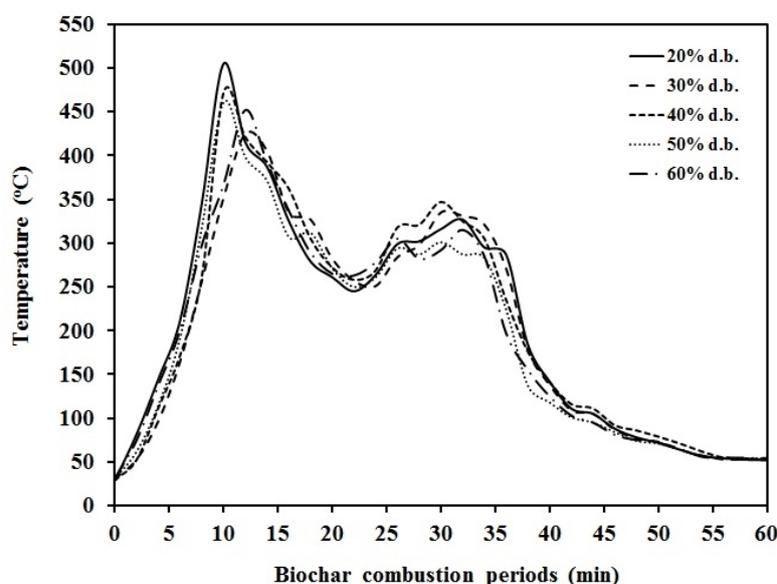


Fig. 3. The temperature values of the biochar combustion.

At 500 mm above the fire base, Figure 3 presents the biochar burning had three phases: preheating, degassing and charcoal. In the preheating phase, the temperature values increased very rapidly from the initial burning period to about 10-20 min after the beginning of the combustion period. In the degassing phase, the temperature values decreased during the first part, and it rose during the second part after most of volatile and tar particles dissolved (Kalasee and Dangwilailux, 2021). Finally, the temperature values reduced to 49-51°C at the charcoal phase. In addition, Figure 3 shows the average highest temperature value of about 505 °C was found at the preheating phase, in the lowest biochar moisture content (20% d.b.). In the degassing phase, 20% d.b., was the shortest because 20% d.b. had the lowest biochar moisture content value and had the least volatile and tar particles. In the charcoal phase, the results presented that 20% d.b. took the longest time because it had the most biofuel after combustion in the first and second phases of the biochar burning stage. The result showed that when reducing biochar moisture content, the measured temperature value increased. This result was similar to the Kalasee and Dangwilailux, (2021) report.

Figure 4 shows the total smoke particle mass concentration values from the biochar burning. It presents the average highest total smoke particle mass concentration values at the start of the burning time was found to be 17.53 to 35.27 mg/m<sup>3</sup> and then it was persistently reduced until the 60th minute, when the burning was stopped. For this reason, the increasing of burning period affected the biochar inner water reduced after water evaporation resulting in the degree of incomplete combustion decreased. In addition, this result presented that the smoke particle mass concentration was enhanced exponentially as the moisture content of biochar was increased. This is not unexpected since the presence of water in the biochar retards completeness of the combustion. As the water amount in the biofuel is increased, combustion effectiveness is decreased as more heat is needed to vaporize the water. This water vapor may help the agglomeration of smoke particles to form soot. Furthermore, a relatively high amount of the evaporated water cannot be transferred through the porous structure inside the fuel. Pressure is built up and cracking of the particles occurs and thus, more fly-ash can crack from the ash with higher mass of moisture (Sarker et al., 2014; Ma and Ye, 2015; Wang and Ma, 2018).

For the pH of smoke particle from the biochar combustion, the results showed that the pH value was found to be 6.21 to 6.83. It was higher than that found before combustion (pH values

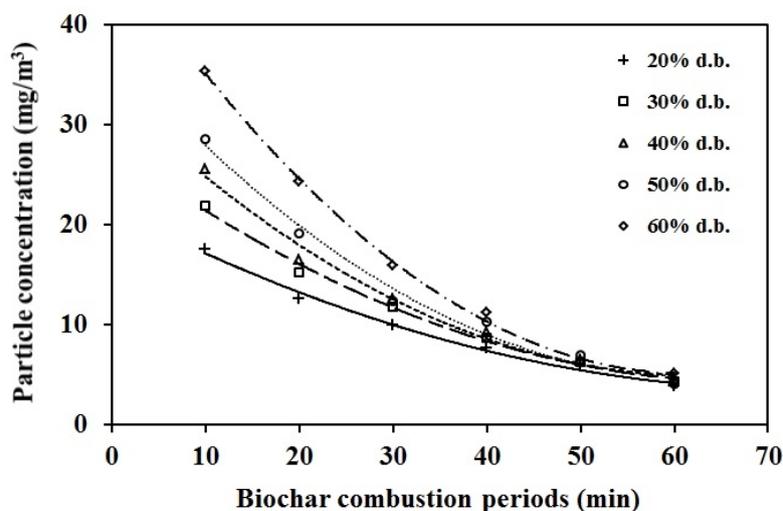


Fig. 4. The total smoke particle mass concentration values from the biochar combustion.

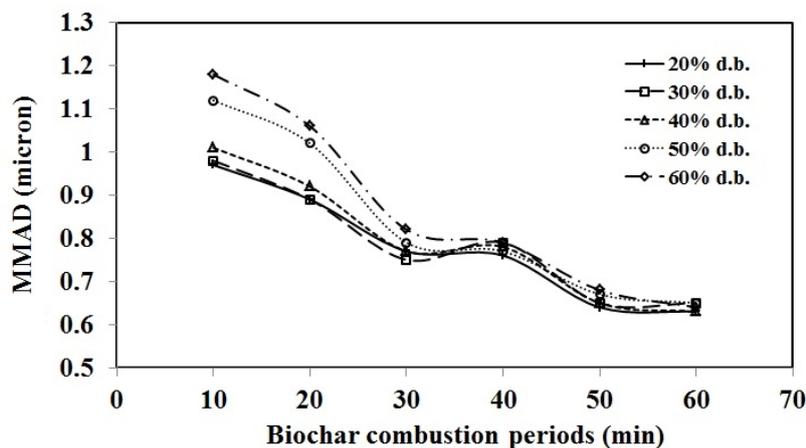


Fig. 5. MMAD values from the biochar combustion.

range between 5.98 and 6.15). This result probably caused by the elimination of organic materials while alkaline salts were retained. Another reason for the decreased acidity may be that the organic nitrogen attendant as amine functionalities transforms into pyridine-like compounds. Thereafter, the amount of acidic surface functional clusters decreased as the percentage of oxygen lost when increasing temperature (Yuan et al., 2011; Imam and Capareda, 2012; Kim and Sharma, 2012; Chen et al., 2014; Krutof and Hawboldt, 2018; Madsen and Glasius, 2019; Ali et al., 2022).

For the smoke particles sampling by using an eight stage Andersen sampler, the results presented that the average MMAD value of smoke particles was found to be 0.62 to 1.18 micron at various burning periods and moisture content. Figure 5 presents the decreased the burning periods and rise in moisture content increased the MMAD value. As shown in Figure 5, for the highest moisture content (60% d.b.), the MMAD values decreased rapidly at 10 to 30 min after the biochar's initial burning. This result indicated that the vapor from biochar burning caused the size of smoke particles to be larger than normal from addition and impaction. After that the MMAD values decreased until the combustion was finished. This result agreed with the Hata et al., (2014) and Kalasee and Dangwilailux, (2021) reports. In addition, the size distribution of the biochar burning suggested a single-mode behavior (Figure 6). They contained major

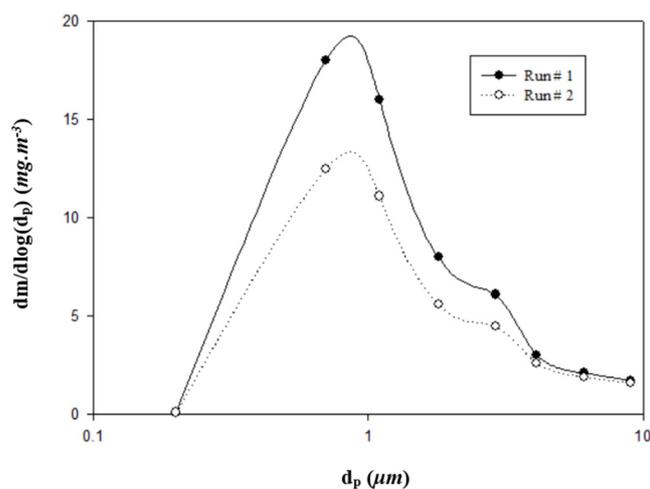


Fig. 6. Size distribution of smoke particles from the biochar burning

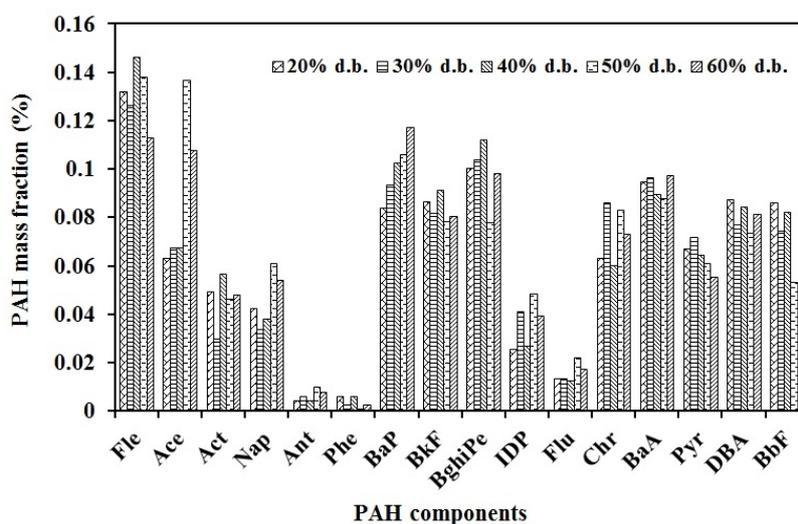


Fig. 7. The sixteen forms of PAH compounds in smoke particle samples from biochar burning for different moisture content (ng/g fuel).

particles in an accumulation mode, similar to the results of Kalasee and Dangwilailux, (2021) and Kalasee and Dangwilailux, (2021) reports.

In this study, the mass fraction profile of the sixteen forms of PAH compounds in smoke particle samples from biochar burning for different moisture content is shown in Figure 7. The result presented that PAH components from the biochar combustion could be categorized by two major types: 2–3 rings and 4–6 rings, respectively. The 2-3 rings PAH compounds are Fluorene (Fle), Acenaphthene (Ace), Acenaphthylene (Act), Naphthalene (Nap), Anthracene (Ant) and Phenanthrene (Phe), While Benzo[a]pyrene (BaP), Benzo[k]fluoranthene (BkF), Benzo[g,h,i]perylene (BghiPe), Indeno[1,2,3-cd]pyrene (IDP), Fluoranthene (Flu), Chrysene (Chr), Benz[a]anthracene (BaA), Pyrene (Pyr), Dibenz[a,h]anthracene (DBA) and Benzo[b]fluoranthene (BbF) are 4-6 rings of PAH compounds. It agreed with the reports of Sun et al., 2010; Jiao et al., 2015; Kalasee and Dangwilailux, 2021. The effect of burning times and fuel moisture content on the concentration of 2–3 rings and 4–6 rings PAH compounds were

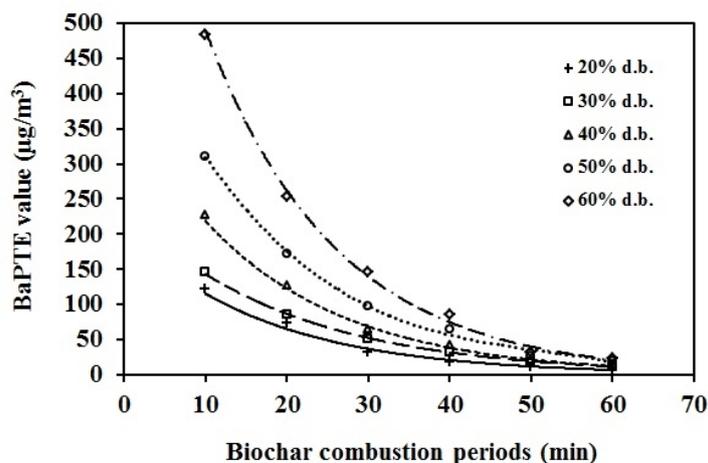


Fig. 8. BaPTE value in the samples from biochar burning for different moisture content

similar to the effect of total smoke particle and PAH concentration. This result presented a long combustion period resulting in the low concentrations of PAHs, in part, from the decomposition of the PAHs. It was similar to the Smit and Meincken, (2012); Meincken and Funk (2015); Schiemann et al., (2019); Kalasee, and Teekapakvisit, (2020); Kalasee and Dangwilailux, (2021) reports. At the beginning of burning times, the total smoke particle concentration of 4–6 rings PAH values was higher than the 2–3 rings value. Thereafter, the total concentration of the 4–6 rings PAH value continually decreased until it was lower than the 2–3 rings PAH value in the final burning periods. It probably caused by the evaporation and decomposition of the 4–6 rings PAH compounds (Tekasakul et al., 2008 and Kalasee and Dangwilailux, 2021).

For the highest moisture content (60% d.b.) of bio-char combustion, the result showed that the particle size less than 0.43 micron was found to greatest the PAHs value. It was found to be 195.62, 147.25, 72.69, 27.14, 11.23 and 8.67 mg/m<sup>3</sup> after the biochar was burned for 10, 20, 30, 40, 50 and 60 min, respectively. Then, the quantity of PAHs continually decreased when the particle size was increased and the moisture content of biochar decreased. For this reason, the quantity of PAHs was depended on the smoke particle number, moisture content and combustion period.

The quantity of total BaPTE concentration of the smoke particle from the biochar combustion is presented in Figure 8. It showed that the increased of combustion period and decreased moisture content of biochar affected the total BaPTE concentration value decreased. The largest BaPTE emission was observed at moisture content of 60% (d.b.), which was within the range of the greatest particle mass (less than 0.43 micron) in an initial combustion period. It was found to be 283.28 µg/m<sup>3</sup> at 10 min after combustion. This value was about 60% that of a given the total value of BaPTE concentration (482.65 µg/m<sup>3</sup>) at the beginning of combustion (10 min), as well as all sample conditions (Figure 9). This result suggested that the particle size had a significant effect on the amount of BaPTE, as did the moisture content and combustion period. In addition, the result showed that the total smoke particle concentration of 4–6 rings PAH values was higher than the 2–3 rings value at the initial combustion period. Afterward, the 4–6 rings PAH values continuously decreased until the combustion was finished, synonymous with the BaPTE values. For this reason, this result indicated that PAHs with higher molecular weights or the 4–6 rings PAH compounds had a high influence on carcinogenic potencies.

Figure 10 and Figure 11 presents the results of WSOC per unit of biochar mass and mass ratios of WSOC in sized smoke particles, respectively. The WSOC decreased as the smoke

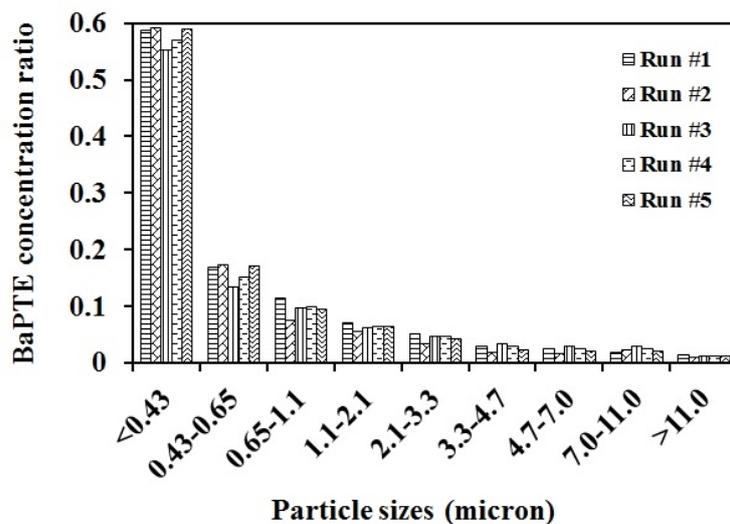


Fig. 9. BaPTE value mass fraction in different smoke particle sizes at the highest biochar moisture content (60% d.b.) and 10 min after combustion

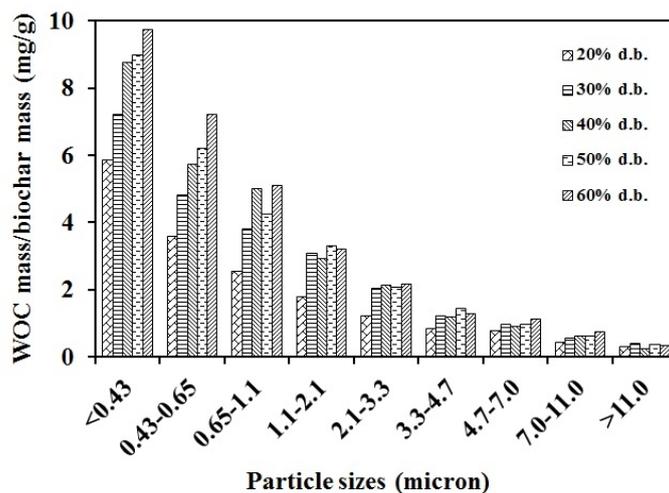


Fig. 10. WSOC mass value per unit of biochar mass value in different smoke particle sizes

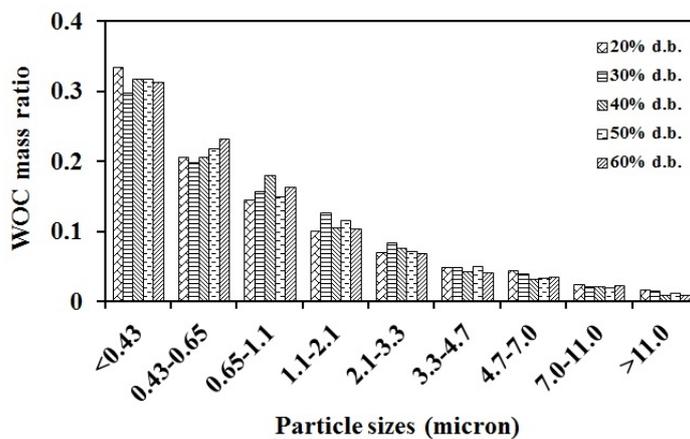


Fig. 11. WSOC mass value ratio in different smoke particle sizes

particle size increased. The smoke particle size less than 0.43 micron made the maximum contribution of 35%–50% of total emissions, indicating the importance of micro-particles as an ambient WSOC carrier. For this reason, the gaseous WSOC might be absorbed by micro-particles or solubility incidence (Chughtai et al., 1996; Hata et al., 2014). The results suggested that for WSOC, the smoke particle size had a strong influence effect on the number of WSOC, as did the moisture content and combustion period.

## CONCLUSION

In this work, the carbon content and the pH of the bio-chars was  $29.27 \pm 3.12\%$  and  $5.19 \pm 0.28$ , respectively. The average highest temperature value of about  $505\text{ }^{\circ}\text{C}$  was found at the lowest biochar moisture content. The pH of biochar's smoke particle was higher than it was before combustion. The average MMAD value of smoke particles was found to be 0.62 to 1.18 micron at various burning periods and moisture content. The decreased the burning periods and rise in moisture content increased the MMAD, total smoke particle mass concentration and PAHs value. In addition, the average highest total smoke particle mass concentration values at the start of the burning time was found to be 17.53 to 35.27  $\text{mg}/\text{m}^3$  and then it was persistently reduced until the 60th minute, when the burning was stopped. The smoke particle size of biochar combustion had a significant effect on the amount of PAHs and BaPTE, as did the moisture content and combustion period. While, WSOC decreased as the smoke particle size increased. Future work on this knowledge has been improved. The chemical compositions of biochar, atmosphere-relative humidity, pressure, clean air speed value, oxygen and nitrogen concentrations in atmospheres have been considered and analyzed.

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## CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/ or submission, and redundancy has been completely observed by the authors.

## LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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