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Production of Pyroligneous Acid from Lignocellulosic Biomass and their Effectiveness Against Biological Attacks

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Abstract: Pyroligneous acid which is one of the commercial sources for acetic acid can be produced from high temperature carbonization of lignocellulosic biomass. Acetic acid can be used as a wood preservative to discourage the growth of fungal and molds. However, at higher temperature, organic compounds especially acetic acid in pyroligneous acid degraded except for some phenols. Therefore, effectiveness pyroligneous acid that pyrolysed at different temperature as fungicide and insecticide for used as wood preservative was evaluated. Pyroligneous acids were derived from rubberwood, oil palm trunk and mix hardwood heated at temperature of 300, 400 and 500°C, respectively in an airless container. The yield of pyroligneous acids was calculated and the chemical compounds of the pyroligneous acid were analysed using Fourier Transform InfraRed (FT-IR). For the efficacy of pyroligneous acid tests, rubberwood test blocks were immersed in the pyroligneous acid for 24 h at room temperature. The treated rubberwood test blocks were later tested against mold (*Penicillium* sp.), white rot fungus (*Pycnoporous sanguineus*) and subterranean termites, (*Coptotermes curvignathus*) according to ASTM standard method. The result shows that highest pyroligneous acid yield was found during pyrolysed of lignocellulosic biomass at temperature of 500°C. All the rubberwood test blocks treated with pyroligneous acids were effective against the mold, white rot fungi and termites. Nonetheless, the pyrolysis temperature did not affect the effectiveness of pyroligneous acids against biological agents. Conclusively, pyroligneous acids effective for discourage the growth of mold and white rot fungi as well accelerate the mortality of termites in laboratory condition.

Key words: Pyroligneous acid, termites, oil palm trunk, mold, decay fungi

INTRODUCTION

Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose and lignin. The two main lignocellulosic biomass sources in Malaysia which are wood residues and oil palm trunks that removed during the oil palm replanting activities. The oil palm trunks were not only underutilized but also causes pollution as well (Ratnasingam *et al*., 2008). These biomass can be derived into any chemicals with higher commercial values using different method such as pyrolysis. Pyrolysis of biomass has received special attention since it leads to useful products and simultaneously contributes to diminish environmental pollution arising from wastes accumulation and/or open field burning (Bonelli *et al*., 2001). Pyrolysis is a process of high temperature carbonization of lignocellulosic biomass with absent of oxygen. Pyroligneous acid which is one of the commercial sources for acetic acid is one of the products from pyrolysis. Pyroligneous acid comprises of water (10-20%), a mixture of carboxylic acids among which acetic acid is the most prevalent, several aldehydes and alcohols and pyrolytic lignin (Ninomiya *et al*., 2004). Acetic acid is an important chemical reagent and industrial chemical. Acetic acid can be used as a spray-on preservative to discourage the growth of fungal and molds. Jung (2007) stated the pyroligneous acid inhibited the growth of pathogenic fungus, *Alternaria mali*, which is known to be the agent of Alternaria blotch of apple plants. Pyroligneous acid is also can be used as a fungicide for wood preservative. Bruce and Highley (1991) stated that the pyroligneous acid useful for controlling the wood decay Basidiomycetes by *Trichoderma* sp. Sameshima *et al*. (2002) and Yatagai *et al*. (2002) correlated the termiticidal activity with phenolic and acetic acid contents in the pyroligneous acid produced from charcoal production. Nakai *et al*. (2007) found that pyroligneous acid from pyrolysis of sugi and acacia wood increased the resistance of wood against brown-rot fungi. Femi-Ola *et al*. (2008) stated that many researchers are

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now focused towards the alternative non toxic and biological methods of controlling termites.

As mentioned early, the pyroligneous acid produced from carbonization of wood at temperature above 270°C without oxygen present. However, the content of chemical compounds in pyroligneous acid depends on the biomass composition mainly the three major components, i.e., cellulose, hemi-cellulose and lignin. The interaction of the three major components during pyrolysis show different reactivity depending on temperature that associated to thermal decomposition of each component (Diem *et al*., 2005). The overall pyroligneous acid conversion level related with interactions between the components and minus amounts of mineral matter naturally present in whole biomass samples that catalyze numerous reactions taking place during pyrolysis and affect the final content of pyroligneous acid (Bonelli *et al*., 2001; Liu *et al*., 2008).

This study focus on the efficacy of pyroligneous acid produced at different temperature from rubberwood and mixed hardwood sawdust and oil palm trunk against the white rot fungi, mold and termite under laboratory condition using rubberwood as test block. The rubberwood test blocks were immersed in pyroligneous acid extracted from the three lignocellulolic biomass respectively at three different pyrolysis temperatures. Fourier transform infrared (FT-IR) spectroscopy was employed to analyse the chemical compounds of the pyroligneous acid produced from three lignocellulolic biomass, respectively at three different pyrolysis temperatures.

MATERIALS AND METHODS

This project was carried out from year 2007 until 2009. Three lignocellulosic materials with the most abundant availability in Malaysia i.e., rubberwood, mixed hardwood sawdust and oil palm trunk were selected for this study. The rubberwood and mixed hardwood sawdust were collected from sawn milling factory in Klang, Selangor, Malaysia. Whereas, the oil palm trunk was felled in Ladang Pertanian UPM in Universiti Putra Malaysia, Malaysia.

Production of pyroligneous acids: The oil palm trunk was cut into small pieces before grinded into fines. The oil palm trunk fines, rubberwood and mix hardwood sawdust were sieved to obtain 40 mesh fines for further process. The fines was stored in the conditioning room at temperature 20°C with 65% relative humidity for one week prior to pyrolysis.

After one week, the fines were pyrolysed under temperature of 300, 400 and 500°C, respectively to produce pyroligneous acid. During the pyrolysis process, 80 g of fines were put in the flask and heated in the heating mantle to the require temperature. The pyroligneous acid was collected by condensing the gases from the pyrolysis process. The yields of pyroligneous acid collected were recorded.

Rubberwood test block: Forty five pieces of rubberwood (*Hevea brasiliensis*) block with size of 20 mm in width, 70 mm in length and 7 mm thick were prepared for molds test. Whereas, ninety pieces of rubberwood with the size of 25 mm in width, 25 mm in length and 9 mm in thick were prepared for decay fungi and termites tests. All the test samples were immersed in the pyroligneous acid at room temperature for 24 h in water bath. After 24 h, the rubberwood test blocks were taken out, the excess solution was wiped off and the test blocks were allowed to dry to constant weight under room temperature. The pyroligneous acid retention was calculated based on the gain in weight of the untreated wood.

Fourier transform infrared (FT-IR) spectroscopy test: In this study, the chemical components of pyroligneous acids obtained from different types and temperature of wood biomass were analyzed using Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectroscopy has been used for determination of molecular structures, identification of compounds in biological samples and investigation of complex polymer. FT-IR spectra were recorded in the wavenumber range from 600-4000 cm^{-1} with PerkinElmer Spectrum. A resolution of 4 cm^{-1} and 4 scans/sample was used.

Biological durability evaluation test

Mold tests: Mold resistance test was tested according to standard ASTM D 4445: Standard Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned lumber (Laboratory Method) (ASTM, 2003). The rubberwood test block was placed on a U-shaped glass rod (3 mm in diameter) together with control test block (no treatment). The glass rod was placed on top of the wet papers inside the sterilized Petri dish. Mold (*Penicillium* sp.) was applied onto the wet paper and the petri dish was sealed with cellophane tape to prevent any contaminant with the contact of surrounding atmosphere and incubated for 4 weeks in the incubator. At the end of the testing (after 4 weeks), the number of days for the mold to visually observed (start to grow) on the test block and coverage area by mold on test block were evaluated.

Decay fungi tests: Decay resistance test was tested according to ASTM method D 2017-71 (ASTM, 1978) using cultures of common white rot fungus *Pycnoporous sanguineus* (ASTM, 1978). The rubberwood test blocks were condition to constant weight and steam-sterilized at 100°C, weighed and exposed to *Pycnoporous sanguineus*. After 8 weeks of incubation at 27°C and 70% RH, the surface fungus mycelium was removed, the specimens were dried at 60°C and weight losses were determined as percentage of total rubberwood test block mass.

Termite tests: Termite resistance test on the rubberwood test blocks were tested according to standard ASTM D3345-74 using the subterranean termites, *Coptotermes curvignathus* (ASTM, 1980). Test blocks were placed in the center of a cylindrical plastic container (50 mm in diameter and 38 mm in height) with 1 g of subterranean termites. The test blocks were set upon 1 g of washed sand and covered with a wet 42.5 mm Whatman filter paper circle as a food source and to maintain humidity. The containers were maintained at 25°C and 80% RH for 4 weeks. Termite activity in each bottle was observed and rated after week 1 and week 4 of the testing period. At the end of the testing period, the percentage of weight loss due to termite attack and termites mortality rate were calculated.

RESULTS AND DISCUSSION

Yields of the pyroligneous acids: The yield of pyroligneous acid produced from three lignocellulosic biomass at three different temperatures was shown in Table 1, as shown in the table, an increase in the temperature increases the pyroligneous acid yields. The highest pyroligneous acid yield was obtained from pyrolysed oil palm trunk at the temperatures of 500°C. The pyrolysis temperature has an important effect on the yield of pyrolysis products from the various types of biomass. Encinar *et al.* (2000) stated that the pyrolysis of *Cynara cardunculus* L. carried out in a fixed-bed reactor at the temperature between 300 and 800°C gave maximum pyroligneous acid yield at 500°C. In another study, the slow pyrolysis of the straw and stalk of the rapeseed plant in a tubular reactor under the conditions of static atmosphere was carried out in the temperature range of 350-650°C and the maximum oil yield (about 18%) was obtained at 650°C (Onay *et al.*, 2001). Demirbas (2006) stated that the pyroligneous acid obtained from the shell pyrolysis at lower temperatures (670-810 K) contain many highly oxygenated polar components that help dissolve the phenolic fractions in water. At elevated carbonization temperatures, the amount of these oxygenated organic components decreased which result in a greater heating value.

Table 1: Yield of pyroligneous acids from different lignocellulosic biomass and different temperature (w/w)

	Temperature of pyrolysis (°C)		
Treatments	300 g	400 g	500 g
Rubberwood pyroligneous acids	0.053	0.069	0.098
Oil palm trunk pyroligneous acids	0.050	0.086	0.129
Mixed hardwood pyroligneous acids	0.029	0.080	0.115

FT-IR analysis: Table 2 showed the possible chemical compounds based on the functional group from FT-IR analysis. The broad band of the hydroxyl stretching group with wave number of 3600-3200 cm^{-1} from FT-IR spectrum indicates that the present of water impurities and other polymeric O-H in the pyroligneous acids (Islam *et al.*, 2003). The spectrum also showed that the band of C-H stretching with wave number of 3000-2800 cm^{-1} indicates the present of alkanes groups in the pyroligneous (Islam *et al.*, 2003; Tsai *et al.*, 2007).

Cellulose and hemicelluloses decomposition products, such as carboxylic acids, ketones, phenol, aldehydes and alcohol, were represented by C = O (A chemical group consisting of carbon and oxygen) stretching group with the wave number of 1750-1625 cm^{-1} by FT-IR spectrum. The C = C stretching vibrations from the FT-IR spectrum indicates of alkenes and aromatics compounds present in the pyroligneous acids (Beis *et al.*, 2002; Acikgoz *et al.*, 2004). The wave number below 1500 cm^{-1}, all the bands were very complex and had their origin in a variety of vibrational modes. The pyroligneous acids were acidic as the oxygenated functional groups of O-H; C = O; C-O and aromatic compounds shows in the FT-IR results and had the potential as a chemical feedstock.

Weight gained rubberwood test blocks: Table 3 showed the weight gained of rubberwood test blocks immersed in pyroligneous acid for 24 h at room temperature, as shown in table, the gained in weight of all the treated blocks was range from 3.64-4.90%.

Biological durability

Mold tests: The result for the efficacy of pyroligneous acid as preservative chemical in rubberwood against mold *Penicillium* sp. is presented in Table 4. All the rubberwood test blocks treated with pyroligneous acid discourage the growth of the mold with the pyroligneous acid produced from oil palm trunk showed the most encouraging result against the mold.

Table 2: FT-IR analysis for functional group in different types of pyroligneous acid and temperature treated wood

Types of treatment	Temperature of Pyrolysis (°C)	Wave No. (cm^{-1})	Functional group	Class of compound
Rubberwood pyroligneous acids	300	3600-3200	O-H	Polymeric, water impurities
		3000-2850	C-H	Alkanes
		1750-1625	C = O	Ketones, aldehydes, carboxylic acids, esters
		1500-1450	C-H	Alkanes
		900-750	O-H	Aromatics
	400	3000-2800	C-H	Alkanes
		1750-1625	C = O	Ketones, aldehydes, carboxylic acids, esters
	500	3040-2845	C-H	Alkanes
		1755-1725	C = O	Ketones, aldehydes, carboxylic acids, esters
OPT pyroligneous acids	300	3600-3200	O-H	Polymeric, water impurities
		3100-2800	C-H	Alkanes
		1610-1540	C = C	Alkenes, aromatic compound
		830-625	C = C	Alkenes, aromatic compound
	400	3600-3200	O-H	Polymeric, water impurities
		1900-1650	C=C	Alkenes, aromatic compound
		750-630	C=C	Amino, aromatic compound
	500	3600-3200	O-H	Polymeric, water impurities
		1205-805	O-H	Aromatics, NH groups
Mix hardwood pyroligneous acids	300	3600-3200	O-H	Polymeric, water impurities
		3000-2800	C-H	Alkanes
		1750-1625	C=O	Ketones, aldehydes, carboxylic acids, esters
		1300-1000	C-O	Esters
	400	3600-3200	O-H	Polymeric, water impurities
		3000-2800	C-H	Alkanes
		1750-1625	C=O	Ketones, aldehydes, carboxylic acids, amide
	500	3600-3200	O-H	Polymeric, water impurities
		3000-2800	C-H	Alkanes
		1750-1625	C=O	Ketones, aldehydes, carboxylic acids, esters

Table 3: Pyroligneous acids content in different types of pyroligneous acid and temperature treated wood

Types of treatment	Temperature of pyrolysis (°C)	Gain in weight (%)
Rubberwood pyroligneous acids	300	3.64
	400	3.76
	500	4.37
OPT pyroligneous acids	300	4.14
	400	3.75
	500	4.63
Mix hardwood pyroligneous acids	300	4.90
	400	4.26
	500	4.83

Table 4: Days of molds start to grow on different type of pyroligneous acid and temperature

Type of treatment	Temperature of pyrolysis (°C)	Days
Untreated	-	1 -2 days
Rubberwood pyroligneous acids	300	4 - 5 days
	400	no mold growth
	500	no mold growth
Oil palm trunk pyroligneous acids	300	22 - 24 days
	400	15 - 18 days
	500	No mold growth
Mix hardwood pyroligneous acids	300	8-11 days
	400	15-18 days
	500	4-8 days

The result also shows that, as pyrolysis temperature increase, the pyroligneous acid produced were effective against the mold. This may be caused by the different chemical composition obtained between the types of pyroligneous acid pyrolysed with different temperature. Nakai *et al.* (2007) found that the temperature were an important factor in characterization of the components and the effectiveness of the liquids in controlling wood-degrading fungi was generally higher at higher temperature.

All the rubberwood test blocks treated with pyroligneous acid had less than 10% of block surface area were covered by mold after 4 week of testing period except for control board that showed approximately 75% of block surface area being covered with mycelium. The rubberwood blocks treated with pyroligneous acid produced from oil palm trunk prevented mold growth effectively. Normally, at moisture contents greater than 20%, mold establishment can occur on unseasoned wood in 24-48 h if temperatures permit and rapid drying of the

Table 5: Percentage of weight loss of rubberwood treated with temperature in fungi test

Type of pyroligneous acid	Temperature of pyrolysis (°C)	Weight loss (%)	Resistance class
Untreated	-	26.74	Moderately resistant
Rubberwood Pyroligneous Acids	300	8.64	Highly resistant
	400	11.49	Resistant
	500	13.61	Resistant
OPT Pyroligneous Acids	300	15.31	Resistant
	400	11.24	Resistant
	500	10.82	Resistant
Mix Hardwood Pyroligneous Acids	300	10.81	Resistant
	400	13.80	Resistant
	500	11.14	Resistant

wood does not occur. Consequently, the mold will grow on the surface of wood and covered with more surface area at the end of the testing period. This may explained why the control blocks more susceptible to mold growth.

Decay fungi tests: After 8 weeks of being exposure to fungus *Pycnoporous sanguineus*, all untreated test blocks and blocks treated with pyroligneous acid showed fungus colonization visually on the surface of the blocks. The percentage of weight loss and resistance level of untreated test blocks and blocks treated with pyroligeneous acid from rubberwood, oil palm trunk and mixed hardwood pyroligneous acids, respectively were shown in Table 5.

The resistant classes were based on weight loss of test blocks as according to Standard ASTM D-2017-71. Untreated test blocks showed moderate resistance to fungus, meanwhile test blocks treated with Rubberwood pyroligneous acid pyrolysed at temperature 300°C showed the highest resistant against the white rot fungi. While, other treated blocks showed only resistant to white rot decay. Phenolic components are expected to be primarily responsible for any antimicrobial activity as stated by Suzuki *et al.* (1997). He suggested that the phenolic compounds of 4-ethyl-2-methoxyphenol and 4-propyl- 2-methoxyphenol that contained inside pyroligneous acid might have some preservation effects. Most phenolic compounds have disinfectant properties which may explain why lignin-rich fractions are more effective preservatives than the whole bio-oil.

Termite tests: Mean percentage weight loss of treated and untreated rubberwood test blocks caused by subterranean termites, *Coptotermes curvignathus* was shown in Fig. 1 and the mortality and visual attack ratings were given in Table 6.

All the treated blocks have the weight loss approximately 10%. Whereas, untreated blocks had higher amount of weight loss with value of 25%. According to Nakai *et al.* (2007), the complex structure of pyrolysis liquid from carbonization of wood biomass

Table 6: Average termite attack and mortality rating for rubberwood test blocks treated with different types of pyroligneous acids

		Termite bioassay	
Treatment	Temperatures of pyrolysis (%)	Attack rating[a]	Mortality rating[b] (%)
Untreated	-	7	52.68
RW pyroligneous acids	300	10	100
	400	10	100
	500	10	100
OPT pyroligneous acids	300	10	100
	400	10	100
	500	10	100
Mix HW pyroligneous acids	300	10	100
	400	10	100
	500	10	100

[a]Termite attack rating scale: 0, failure; 4, heavy; 7, moderate attack; 9, light attack and 10, sound. [b]Mortality rating: 0-33%, slight; 34-66%, moderate; 67-99%, heavy and 100%, complete

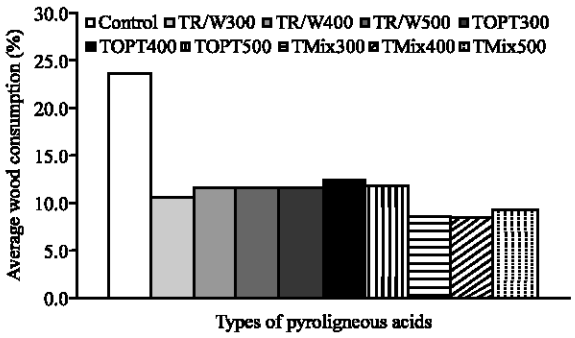

Fig. 1: Percentage of weight loss of rubberwood of different type of pyroligneous acid and temperature in termite test

might be expected to protect wood from fungal and termite attack. Previous studies by Sameshima *et al.* (2002) and Yatagai *et al.* (2002) correlated the termiticidal activity of wood vinegars with phenolic and acetic acid content from charcoal production. In these studies, the low mean weight loss of treated blocks with pyroligneous acid might caused by the present of phenolic and acetic acid content.

The summary of ANOVA shows that no significant different was observed on the mean percentage weight

loss for the variables used in this study. The pyoligneous acid pyrolysed from any lignocellulosic biomass at different temperature equally effective against termicidal activities except for treated block using mixed hardwood pyroligneous acid that showed better resistance against termicidal activities.

As shown in Table 6, Pyroligneous acids pyrolysed from different lignocellulosic biomass at different temperature were equally effective against subterranean termites where the entire termite in the testing bottles had 100% mortality compared to untreated wood where the termite's mortality rate about 50%.

CONCLUSIONS

The highest pyroligneous acid yields were obtained at 500°C for all three types of lignocellulosic biomass. Temperature was an important factor in the yield production but not an important factor in the effectiveness of controlling wood biological attack. The pyroligneous acid from pyrolysis process of lignocellulosic biomass may have a potential source of a number of valuable chemicals. The FT-IR analysis revealed probable compounds such as ketone, aldehyde, phenols and carboxylic acids with water impurities. Some of those chemicals may have importance in developing new wood preservatives against biological degradation and also as a chemical feedstock.

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