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PRESERVATION OF RUBBER WOOD AGAINST BIOLOGICAL DECAY BY SALTS DERIVED FROM LEACHATE

Muhammad Syazwan, Bin Azmi, Lee Choon Wai
Mohd Firdaus Bin Yhaya, Norli Ismail, Husnul Azan Bin Tajarudin*
University Sains Malaysia, School of Industrial Technology
Bioprocess Technology Division
Penang, Malaysia

Hamidi Abdulaziz, Husnul Azan, Bin Tajarudin
University Sains Malaysia, Solid Waste Management Cluster
Science and Engineering Research Centre
Engineering Campus
Penang, Malaysia

Hamidi Abdulaziz
University Sains Malaysia, School of Civil Engineering
Engineering Campus
Penang, Malaysia

Kok Kwang Ng
National University of Singapore
Centre for Water Research, Department of Civil and Environmental Engineering
Singapore

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ABSTRACT

Wood preservatives are typically chemicals that granted antimicrobial properties to timber, while leachate is the liquid that percolated through accumulation of waste materials. *Clostridium butyricum* NCIMB 7423 was used to ferment leachate with 1.15 L working volume under anaerobic condition at 37°C and pH 6.5.This research focuses on preserving rubber wood by acids and salts derived from the fermented leachate. First and second set of treatment were performed to study fungal resistance of wood treated by acids and salts respectively. Biological decay test was conducted using *Phanerochaete chrysosporium* to determine the percentage of mass loss for different preservatives. The salt concentration of 0.5 M sodium acetate and sodium butyrate shows satisfactory result to preserve rubber wood. Thus, the salts derivative could be converted

into rubber wood preservative. This research will reflect to material from waste to wealth and support sustainable technology.

KEYWORDS: Wood preservation, leachate, *Clostridium butyricum*, waste to wealth, anaerobic fermentation.

INTRODUCTION

Malaysia has a rich tropical rainforest that currently supplies over RM 1.7 billion of wood products for exports (Malaysian Timber Industry Board: Export E-Statistics, 2016). Monocultural plantations such as rubber tree plantations, provide alternate source for wood timber (Ratnasingam et al. 2011). Rubber trees, scientifically known as *Hevea brasiliensis* are suitable for latex harvesting at 5 years of age and remains productive for the next 28 years (Henderson 1977). After that period, it has to be removed for replanting due to uneconomical production. However, rubber trees persist being an asset because it can be turned into timber for production of furniture and indoor building components (Killmann and Hong 2000). Thus, the timber as agricultural byproduct is relatively inexpensive compared to timber from natural forest. Nevertheless, major drawbacks such as low dimensional stability and vulnerability to biodegradation have hindered wood processing industries to expand its commercialisation in the world's market.

Inherent susceptibility of wood to biodegradation has promote the creation of wood preservative to increase its durability and service duration. To date, wood preservatives can be categorised into four groups: water-borne preservatives, oil-borne preservatives, light organic solvent preservatives (LOSPs), and nano-sized-particle preservatives. (Barnes and Murphy 1995, Clausen et al. 2011). These preservatives are toxic chemicals that penetrate and retain in wood structure, thus giving protection against biotic agents. While, acetic and butyric acids have evinced antiseptic action to bacteria and fungi (Pundir and Jain 2011). This is due to its undissociated molecules of acetate and butyrate that passively diffuse into microbial cell and once dissociate, it causes disruption of cellular membrane and pH of internal environment (Theron and Lues 2007).

Cellulose, hemicellulose and ligninare the components that impart strength and rigidity in wood. White-rot fungi are capable of degrading simply lignin or simultaneously with cellulose, thus causing structural damage in wood (Witomski et al. 2012).

P. chrysosporium, a white-rot fungus has been utilised for fungal pretreatment to delignify and hydrolyse lignocellulosic biomass in the production of fuel (Bak et al. 2009). Similar fungus has been used to infect rubberwood in production of biogas (Okino et al. 2009). The responsible enzymesfor degradation are lignin peroxidase (LiP), laccase, manganese peroxidase, versatile peroxidase, cellulase, xylanase and other hemicellulases (Dashtban et al. 2010).

Landfill leachate is a contaminated liquid from accumulation of moisture from waste materials in the landfill. In Malaysia, the leachate treatment lacks of efficient and sustainable integrated process while conventional biological treatment requires high cost, longer operation time and large footprint. A possible approach to resolve the issue is by implementing an industrial membrane bioreactor that can treat leachate biologically and produce valuable chemical products. In this work, the novelty is on the application of volatile acids and salts derived from the landfill leachate as wood preservative. This research employs "waste to wealth" and sustainable concept whereby the waste material can be converted to useful products and potentially harmless substances concurrently.

MATERIAL AND METHODS

Leachate fermentation

Leachate was obtained from aerobic treatment pond of Pulau Burung Sanitary Landfill (PBSL), a municipal solid waste landfill equipped with semi-aerobic system. Prior to fermentation, the leachate was pretreated using physicochemical adsorption method to reduce indigenous inhibitors i.e. heavy metals and volatile fatty acids. Limestone particles of industrial grade with the size of 46 -125 micron was used as the adsorbent. Anaerobic fermentation of pretreated leachate was performed in a membrane reactor with 1.15 L working volume (Minifors, Infors HT, Switzerland) using *C. butyricum* NCIMB 7423 at 37°C and pH 6.5. The leachate fermentation products that include mixture of acetic and butyric acids were the potential preservative for the wood treatment. There was no presence of *C. butyricum* or any biotic contaminants in that product since it was filtered by the 0.22 micron ceramic membrane and always kept in sterile condition before the wood preservation study.

Fungal isolation and maintenance

Local fungal isolate, *P. chrysosporium* was acquired from Bioprocess Technology Division, School of Industrial Technology, Malaysia. The isolate was maintained on potato dextrose agar (PDA) (CM0139, OXOID Ltd., United Kingdom) medium slants at 4°C until usage (Oxoid manual). Before operation, fungal isolate of pure culture was subcultured on new PDA plate at 37°C for seven days. The lactophenol cotton blue wet mount was used to stain and observe the fungi (Leck 1999). Dye was formulated from phenol, methyl blue, lactic acid, and distilled water. Any contamination was detected based on morphological characteristic and microscopic analysis using standard taxonomy and procedure (Guarro et al. 1999).

Wood treatment

Malaysian rubber wood was cut into smaller wood fragments using saw at the size of 20 x 20 x 10 mm (R x T x L). The wood blocks were submerged in respective preservatives with the volume sufficient to cover up the whole blocks for duration of treatment of 3, 7 or 21 days at 31 ± 10°C. The studied preservatives were acetic acid, butyric acid, acetate salt, and butyrate salt. After respective treatment period, triplicate of wood blocks were withdrawn from the solution. The efficiency of treatments was evaluated by measuring preservative retention. Total chemical retention ($kg{\cdot}m^{-3}$) was determined by weighing each sample before and after treatment to determine solution retained in the wood blocks. The wood samples were removed from preservative solution as according to concentration and treatment duration. They were blot dried to remove remaining solution from the wood surface and weighed to determine gross retentions for each sample. The retention was calculated by the following formula:

$$R = 10\frac{GC}{V}(kg/m^3)$$

where: G - the grams of preservative solution absorbed by the sample,
C - the grams of preservative chemical in 100 g of preservative solution,
V - the volume sample in cubic centimetres.

Fungal decay test

P. chrysosporium was cultured on a PDA plate and incubated at 37°C for 7 days. This was to ensure the fungi were matured before being transferred to liquid medium. The mycelium

was cultured in liquid broth to obtain fungi in intact form. The broth used was Japan Industrial Standard (JIS) broth which composed of glucose 25 g/L, malt extract 10 g/L, peptone 5 g/L, KH_2PO_4 3 g/L, $MgSO_4 \cdot 7H_2O$ 2 g/L (Hermiati et al. 2013). The acid-treated and salt-treated wood blocks were left to decay with the presence of white-rot fungus mycelium inside sterile universal bottles. Then, the inoculated wood blocks were incubated at 26°C for 8 weeks. After the decay period, the test blocks were removed from the universal bottles and fungus was carefully brushed off the blocks' surface. Next, the blocks were oven dried at 70°C until constant weight was achieved. The percent mass loss in the individual test blocks from the decay fungi were then calculated using the formula,

$$\text{Mass loss (\%)} = \left(\frac{M_i - M_f}{M_i}\right) \text{x}100$$

where M_i and M_f are the initial mass of wood block before and after decay test respectively.

All the results obtained in this study were analysed statistically with analysis of variance (ANOVA) test and Duncan' test using IBM's SPSS software, version 22.

RESULTS AND DISCUSSION

Tab. 1 gives the retention results of acids and salts treatment for duration of 3, 7 and 21 days. For various preservative concentration, average preservative retention in ascending order was as follows: acetic acid, butyric acid, acetate salt solution and butyrate salt solution. However, average preservative retention for duration of treatment was inconsistent. It was recorded hat cold soaking method may give highly variable result. Nevertheless, when adequate retention and penetration levels are achieved, their service life should be equivalent to wood treated by pressure (Lebow 2010).

Tab. 1: Preservatives retention of acids and salts for 3, 7 and 21 days of treatment duration.

Type of preservatives		Retention ($kg \cdot m^{-3}$)		
		3 days	7 days	21 days
Acetic acid	0.5 M	1.88	1.92	1.95
	1.0 M	2.09	2.24	2.22
	1.5 M	2.39	2.25	2.32
	2.0 M	2.37	2.40	2.25
Butyric acid	0.5 M	2.59	2.70	2.79
	1.0 M	3.06	2.91	3.08
	1.5 M	3.50	3.31	3.31
	2.0 M	3.48	3.35	3.30
Acetate salt solution	0.5 M	2.72	-	-
	1.0 M	3.19	-	-
Butyrate salt solution	0.5 M	3.50	-	-
	1.0 M	4.05	-	-

All rubber wood blocks used in this study should be classified as sapwood. To date, there is no standard level of preservative retention as no research has been reported using similar wood preservatives. Average preservative retention ranged from 1.88 $kg \cdot m^{-3}$ for acetic acid with 3 days cold soaking to 4.05 $kg \cdot m^{-3}$ for butyrate salt solution with 3 days cold soaking. Prolong exposure

of acids to rubber woods has affected the preservative retention to some extent. Longer treatment duration typically increased the retention but it is exceptional case for acetic acid and butyric acid greater than 1.5 M. This is probably due to different in efficiency of preservative diffusion or saturation state for that preservatives have been reached as early as 3 days.

Generally, white rot fungi is more infectious than brown rot fungi in hardwood species (Eaton and Hale 1993). Rubberwood as tropical hardwood shares the same weakness (Nagaveni et al. 2005). Average mass loss of wood blocks treated by acetic acid and butyric acidis presented in Figs. 1 and 2, correspondingly. All wood blocks treated by either acetic or butyric acids have recorded higher mass loss percentage compares to non-treated wood. Formerly, wood acetylation process had a major problem when 50 percent of acetic acid used in treatment was entrapped inside the treated timber. Its presence was undesired due to capability of corroding metal fasteners and hydrolysing the wood components, hence impairing wood strength (Carraher and Sperling 2013). Moreover, high concentration of organic acids also contribute to significant mass loss and damage wood mechanical properties (Sundqvist et al. 2006). This study may evince that acidity of acetic acid and butyric acid have facilitate the fungal degradation of wood blocks rather than preserving or protecting it from fungal infection.

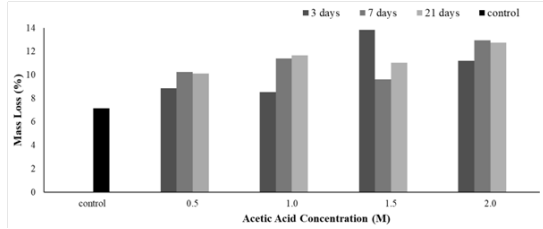

Fig. 1: Average mass loss of rubber wood treated with acetic acid.

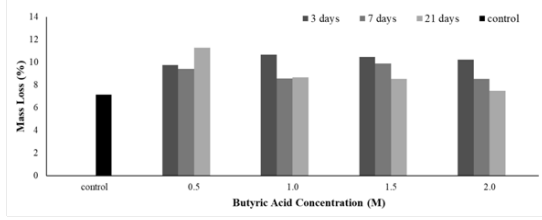

Fig. 2: Average mass loss of rubber wood treated with butyric acid.

Two-way ANOVA test and Duncan's results of acetic and butyric acids decay test are given in Tabs. 2 and 3. From both table it can be concluded that there are no statistical differences between treatment duration of 3, 7 or 21 days. The results indicate that at concentration of 2.0 M acetic acid, the fungal decay achieved the highest percent mass loss. While at lower concentration of acetic acid, it is vice versa. The interaction between acetic acid concentration and treatment duration is also significant for percent mass loss.

Tab. 2: Two-way ANOVA test and Duncan's test results for acetic acid treatment (α≤0.05).

Source	dF	F	p	Duncan's test
Acetic acid concentration	4	56.696	.000	Control[a] (7.153)
Treatment duration	2	2.313	0.116	0.5[b] (9.734)
Concentration x Duration	8	10.417	.000	1.0[c] (10.533)
				1.5[d] (11.499)
				2.0[e] (12.304)

Means within column followed by same letter is not significantly different. Values in parenthesis are means of percent mass loss for groups in homogenous subsets.

Tab. 3: Two-way ANOVA test and Duncan's test results for acetic acid treatment (α≤0.05).

Source	dF	F	p	Duncan's test
Butyric acid concentration	4	6.231	.001	Control[a] (7.153)
Treatment duration	2	2.625	.089	0.5[aa] (10.123)
Concentration x Duration	8	1.485	.204	1.0[aa] (9.3078)
				1.5[aa] (9.630)
				2.0[aa] (8.747)

Means within column followed by same letter is not significantly different.Values in parenthesis are means of percent mass loss for groups in homogenous subsets.

Salt treatment was performed along with acid treatment due to several advantages. These include avoiding strong odor released by the volatile acids and potential damage to wood mechanical structure. As presented in Fig. 3, salt treatment using sodium acetate and sodium butyrate solution have shown promising result of wood preservation. It was found that at 0.5 M of acetate and butyrate salts inhibited the growth of white rot fungus.

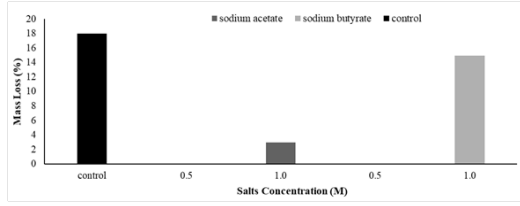

Fig. 3: Average mass loss of rubber woodtreated with sodium acetate and sodium butyrate.

There was also no mold growth on the surface of treated wood. This could be due to the fact that sodium acetate has a strong antifungal property, while sodium butyrate has an antibacterial and potential antifungal property (Stiles et al. 2002, Nguyen et al. 2011).

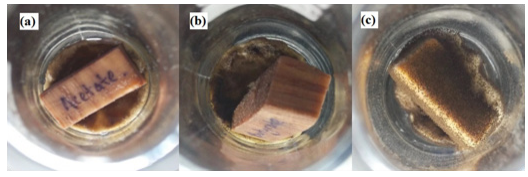

Fig. 4: Wood blocks treated by salts after 8 weeks; (a) acetate-treated wood (0.5M), (b) butyrate-treated wood (0.5M), (c) control.

Fig. 4 presents the result of salt treatment, in which after 8 weeks there was no fungus grown on wood blocks treated by 0.5 M sodium acetate and butyrate. There is no AWPA standard to refer for above ground or ground contact preservation as there is no similar study or application has been performed. However, salts retention at 2.72 $kg \cdot m^{-3}$ and 3.5 $kg \cdot m^{-3}$ for respective sodium acetate and sodium butyrate were sufficient to prevent fungi growth. It should be noted that further leaching and penetration test should be performed for complete study and commercial application. Considering rubber wood as easily penetrated and treated wood even with non-pressure treatment, it should not hinder the salts applicability as wood preservative in the near future.

CONCLUSIONS

Results indicate that acetic acid and butyric acid derived from fermented leachate were ineffective to preserve wood in which higher percent mass loss was recorded in comparison to control. Decay test was not significantly affected by treatment duration while the acid concentration shown significant effect for percent mass loss. Acetate and butyrate salts were proven to preserve rubber wood at concentration of 0.5 M. Thus, by converting acetic and butyric acids derived from landfill leachate into acetate and butyrate salts, leachate can be fermented and potentially turned into a valuable wood preservative.

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Muhammad Syazwan, Bin Azmi, Lee Choon Wai
Mohd Firdaus, Bin Yhaya, Norli Ismail, Husnul Azan, Bin Tajarudin*
University Sains Malaysia
School of Industrial Technology
Bioprocess Technology Division
11800 Penang)
Malaysia
*Corresponding author: azan@usm.my
Phone: +6046536194

Hamidi Abdulaziz, Husnul Azan Bin Tajarudin
University Sains Malaysia
Bsolid Waste Management Cluster
Science and Engineering Research Centre
Engineering Campus
14300 Nibong Tebal
Seberang Perai Selatan
Penang
Malaysia

Hamidi Abdulaziz,
University Sains Malaysia
School of Civil Engineering
Engineering Campus
14300 Nibong Tebal
Penang
Malaysia.

Kok Kwang Ng
National University of Singapore
Centre For Water Research
Department of Civil and Environmental Engineering
Engineering Drive 2
Singapore 117576