CONVERSION OF WASTE PAPER-BASED BAIT FORMULATION FOR BIOGENIC PRODUCTION BY TERMITES IN TROPICAL LAND

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ABSTRACT

Termites are well-known decomposers in tropical forest ecosystems. This study explores their usefulness in bioconversion of waste papers-based food sources. The aim was to determine the physical and chemical characteristics of biogenic products converted by termites from waste paper-based bait matrices in tropial forest land. The tested hypothesis was that the existing termites effectively convert the bait matrices into nutrient-rich organic materials, potential for the improvement of soil properties. For the purpose of the study, wastes of cardboard, office papers, and decayed pine wood were equally mixed based on their oven-dried weights. The mixture formulation enriched with nutrients was prepared in a closed design of PVC-pipe (with the target density of 0.5 g cm^{-3}, moisture content of 50-70%). The physical and chemical properties of biogenic structure resulted by termites were determined at 2, 4, and 6 weeks. The formulated mixture with the bait design successfully attracted termites *Macrotermes sp.*, which utilized the mixture as a food source and converted it into valuable biogenic products, enhancing physical and chemical properties of soil.

Keywords: Bioconversion, biogenic products, *Macrotermes sp.*, termite baits, waste papers

INTRODUCTION

Various types of paper wastes comprise a large proportion of biodegradable solid waste around the world. It is approximately 17% of the total amount of municipal solid waste generated from 161 countries, which is currently over 3.5 million ton per day and is projected to grow to over 6 million ton per day by the year 2025 (Hoornweg and Bhada-Tata 2012). A number of options is available to manage increasing amount of lignocellulosic wastes, but most of these materials are still deposited in landfills and simply burned. The increased amount of such waste indicates the important of its recovering and reprocessing into a range of products. In general, recycling of the paper has been becoming an interest in many countries, particularly in Europe having been reaching the highest recycling rate of 71.7% (ERPC 2014). Mixed paper waste can also be recovered to produce quantify thermal energy and used to increase the quantity and quality of fired brick (Erdincler and Vesilind 1993, Shibib 2015). In addition, various biomaterials, such as biofuel, chemical compounds, and fertilizers, can be produced by recovering and reprocessing such waste through chemical pretreatment

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and biological degradation (Kishino et al. 1998, Ohkuma 2003, Fox and Noike 2004, Scharf and Boucias 2010). However, due to the high variability in types and constituent compounds, waste paper products are not automatically suitable for biological decomposition, including by termites (Lenz et al. 2011). Thus it is important to attract termites by formulating a specific paper-based food source, applying it with appropriate bait design, and evaluating the potential for efficient bioconversion process in the field.

Biological degradation of organic materials naturally occurs through enzymatic processes by many microorgnisms. It involves a variety of different enzymes produced by different species of orgnisms such as fungi, bacteria, protozoa, algae and yeasts. These organisms are proved to have limitations for a practical application (El-Sheekh et al. 2009, Dwivedi 2012). This study employed termites since they are adaptable and capable in degrading lignocellulotic materials in a reasonable period of time with the aid of their associated microbial symbionts. With their digestive capability and huge abundance, termites greatly contributed to the physical and chemical modifications of soils (Ohkuma 2003). Bioconversion or biological degradation by termites is an ecological process performed for nutrient recycling, habitat creation, and soil formation. Termites are decomposers of lignocellulosic wastes and producers of organic nutrients (Haritos et al. 1993, Itakura et al. 2006). Both aspects of the termite's ecological roles can be utilized by baiting termites with food source made out of cellulosic waste products and allowing their conversion into nutritional supplements for soil amendment. Termites produce a wide range of organic-minerals or biogenic structures such as galleries, casts, sheeting and nests from food sources by mixing and impregnating them with their saliva (Mora et al. 2003). Their productivity depends on the types of food sources, termite species, and their living environment. Effects of variations in the living environment of termites have been studied for termites' productivity and their impact on the soil (Al Houty 1998, Burtelow et al. 1998, Lachnicht et al. 1997, Gilot 1997). However, effects of food sources and potential of utilizing waste paper products have not been studied so far. The current research was designed to investigate how to formulate attractive food source for termites out of mixing paper wastes and decayed wood and how to attain useful decomposed materials. The addition of decayed wood was due to the fact that it contained an attractant for termites (Esenther et al. 1962).

Biogenic conversion of paper wastes by termites can produce organically rich materials, which leads to increased land productivity (Paoletti et al. 2003). However, various chemicals, such as fillers, coatings, adhesives, toners, dyes, and inks, are added in the production process of paper products, which also decrease cellulose fiber length and may contain some hazardous substances (Haritos et al. 1993, Yoshimura et al. 1993, Pivnenko et al. 2015). Thus, accessibility of termites may be limited for utilizing waste paper products as a food source. This is the first study that looked at the formulation of waste paper products as termite baits with a particular composition and design in relation to the physical and chemical properties of bioconversion products in the field. It was thought that termites will readily consume the baits more effectively in the field, avoiding some limititations including the presence of metal ions in the printed paper waste. This is due to the fact that although the metals cannot be destroyed, microorganisms can act on heavy metals through sorption to cell surface by physicochemical mechanisms, organic acids excretion, insoluble sulfides or polymeric complexes formation, intracellular accumulation, and enzyme-catalyzed transformation (Lloyd and Lovley, 2001). In the case of termites, it was found that the high activity of food digestion and energy production in their gut caused significant high concentration of metal ions such as Mn, Zn, and Fe, particularly deposited in mandibles for hardening the exoskeleton (Yoshimura et al. 2002). In addition, a selective pathway and/ or absorption of metals from their food may contribute in the accumulation and distribution of the spesific elements on termite mandibles (Ohmura et al. 2007).

MATERIALS AND METHODS

Experimental site

The field site (20x20 meters in the Southern bloc of forest area, elevation ±516 m, annual rainfall >1600 mm, relative humidity ±78%, temperature ±23°C) was in the experimental forest of Hasanuddin University, eastern part of Indonesia (±30 km east of Makassar) as indicated in Figure 1. The topography varies from mild to steep slopes dominated by the plantation pine forest of *Pinus merkusii*. The endemic *Macrotermes sp.* is naturally found in the plantation forest.

Bait preparation

Baiting method, generally known as the way of controlling termite attacks on building constructions using bait materials containing a slow-acting toxic chemical, can be modified and applied in various approaches. This study employed an alternative baiting method using non-toxic bait formulation readily attacked by termites and physiologically converted into a biogenic structure useful for soil properties enhancement. The bait formulation was prepared by mixing equal quantities of cardboard, office paper, and decayed pine wood based on their oven-dried weights. The mixture was then put in a closed design of PVC-pipe (10 cm diameter and 10 cm length) with the target density of 0.5 g cm^{-3}. Prior to field test, the mixture formulation matrix was dipped in soybean boiled water to enrich the formulation with nutrients and moisture content of 50-70%. Soybean boiled water is actually wasted by-product of tofu making process readily available in many areas of Indonesia. In this study, it was obtained from the boiling process of 400 g of soybean in 6000 ml of water for about one hour. By that way, termites were expected to not only use the bait formulation as a food source, but also to survive and grow well on it for sustainable production of biogenic compounds, altering soil properties. An experimental unit were prepared for field assay by assembling four sets of PVC pipes containing food formulation using L-joint pipes. Each of the assembled pipes was holed at the bottom side (1

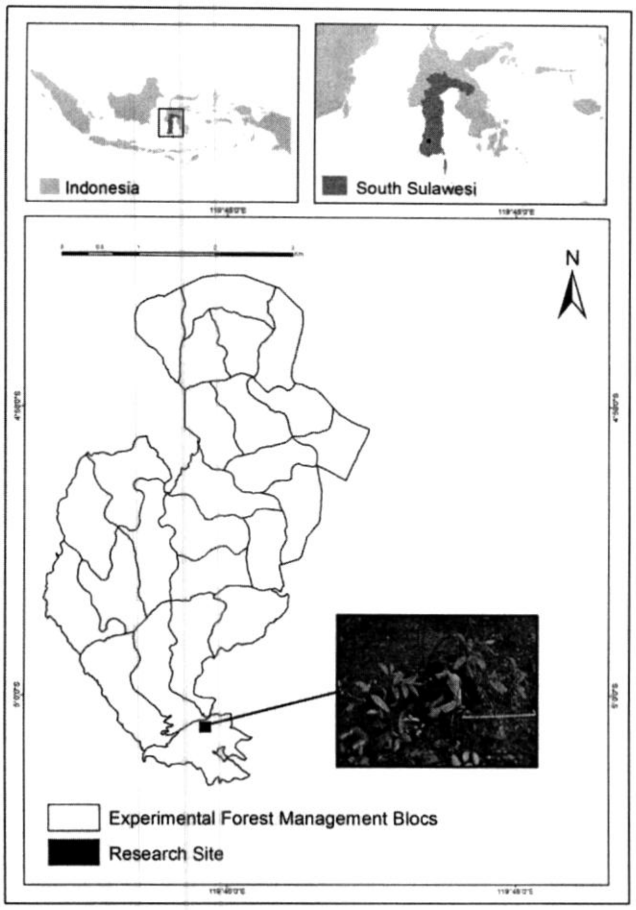

FIGURE 1
Research site

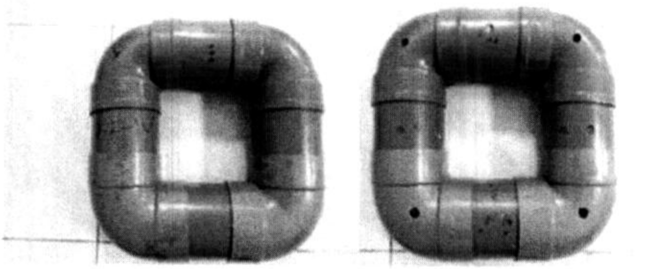

FIGURE 2
Design of an experimental unit
with 1 cm diameter holes at the bottom side

cm diameter) for access of termites as shown in Figure 2.

Field assay

To evaluate responses of active termites to the designed bait formulation and subsequent bioconversion products, the experimental units were placed onto an active *Macrotermes sp.* foraging site. The genus of active termites were previously identified using stereomicroscope with phototube camera ERc 5S. Field assays were conducted using five experimental units (each with four assembled samples) which were put underground and buried (top flush with soil surface) in 4-m distance of each experimental unit. The presence of termites in the experimental unit, as well as the physical and chemical properties of resulted biogenic structure, were periodically checked at 2, 4, and 6 weeks. Soil samples from the surrounding areas of the test units were also collected by using soil rings and analyzed for their structure and chemical properties as references. The pH of soil and biogenic products were determined in soil/ water suspension (soil:solution = 1:5). The biogenic structures consisting of bulk density and aggregated fraction distribution were evaluated based on the procedures published by Decaëns et al. (2001). The samples were also analyzed for their C-organic contents using the method of Walkley and Black (1934) and for their cation exchange capacities (CEC) by the sodium acetate procedure of Bower et al. (1952). In addition, the termite attack on each test sample was considered to be associated with the bait performance. Therefore, the degree of termite attacks were visually rated according to the available standard, namely American Wood-Preservers' Association (AWPA) standard E1-97 (10: sound, 9: light, 7: moderate, 4: heavy, 0: failure). The resulted data were compared by Tukey's test, with $P < 0.01$ considered statistically significant.

RESULTS AND DISCUSSION

Termite Attacks

The tested food formulation and matrix design were highly effective in attracting termites *Macrotermes sp.* Termites entered the experimental units through holes at the bottom and rapidly consumed the mixture formulation as indicated by the significant degree of termite attacks at two weeks exposure. The degree of termite attacks increased with the increasing exposure time as shown in Table 1. The termite feeding activity clearly indicates that all assembled samples within an experimental unit evenly attracted termites at similar rates in time and degree of attack. This result is in line with the fact that termites showed similar response to food sources with similar characteristics, including nutritional composition, size, density, and design (Evans and Gleeson 2006).

Termite attacks increased with the increased duration of bait exposure. The condition of bait matrix before and after six weeks exposure was shown in Figure 3. This result emphasizes the importance of regular inspection and the necessity of bait replacement after six weeks in the study site, which is humid tropical climate. The period of bait replacement may vary with the habitat and climate conditions because of different existing termite species and activities. In tropical Australian savanna during the transitional period from wet to dry seasons, it required the exposure time of two months (Dawes-Gromadzki and Spain 2003). It is probably due to its much drier environment with the average annual rainfall of ±800 mm and the temperature of ±35°C. The effect of envi-

TABLE 1
Visual rating of matrix formulation after exposure to field test

Experimental units *	Visual rating after exposure for the period of time (week) **		
	2	4	6
1	8.00 ± 1.15 a	4.75 ± 1.50 b	1.00 ± 2.00 c
2	8.50 ± 1.00 a	5.50 ± 1.73 b	2.00 ± 2.31 c
3	8.00 ± 1.15 a	5.50 ± 1.73 b	1.00 ± 2.00 c
4	7.50 ± 1.00 a	4.00 ± 0.00 b	0.00 ± 0.00 c
5	8.00 ± 1.15 a	4.75 ± 1.50 b	1.00 ± 2.00 c
Overall	8.00 ± 1.03 a	4.90 ± 1.41 b	1.00 ± 1.78 c

* *Consist of four samples for each experimental unit*

** *Mean and standard deviation of four replicates (10: sound, 9: light, 7: moderate, 4: heavy, 0: failure). Values in rows with different letters are significantly different by Tukey's test (P < 0.01).*

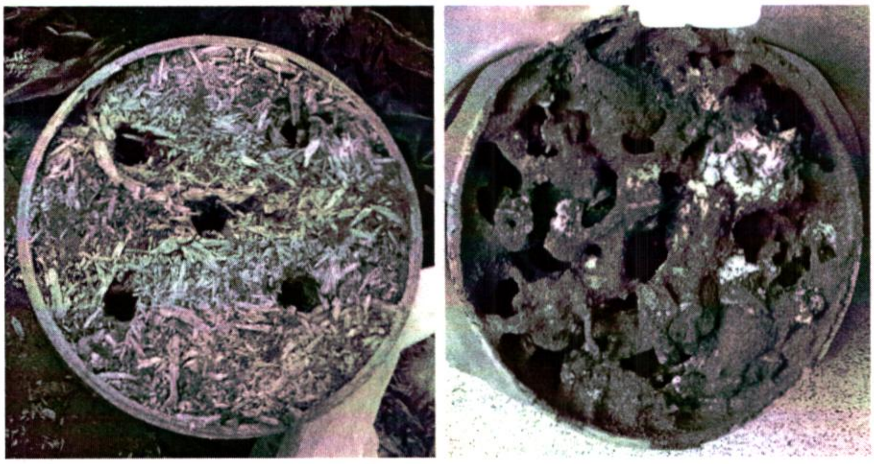

FIGURE 3
Conditions of samples before and after exposure in the field test

ronmental conditions such as temperature on bait efficacy was associated with subterranean termite feeding and molting rates (Smythe and Williams 1972; Sponsler and Appel 1991). Understanding of the bait replacement period in relation to the habitat and climate conditions is necessary in providing an appropriate quality of biogenic products when it is expected to transport to other sites, where available nutrients are limiting.

Biogenic Products

The characteristics of biogenic structure resulted from the paper-based food formulation by the biological activities of termite *Macrotermes sp.* were varied according to exposure time as shown in Table 2. The biological activities of termites enabling to convert the paper-based food formulation resulted in biogenic structures with lower density, higher pH, higher C-organic content, and higher CEC compared to surrounding soil. Soils with these biogenic products have significantly better physical and chemical properties with increased exposure time. Within the period of six weeks, the attacks of termites on the paper-based food source, which initially has the density of 0.53±0.03, reduced the density by about 40%. In addition, the termites' biogenic products increased pH value to around 7, C-organic contents more than 1.5-fold higher, and CEC more than 100%. The addition of soybean boiled water in this study may become one of supporting factors resulting in a high nutrient content of biogenic products since the wastewater resulted from boiling dehulled soybeans in water contained very high content of K (86.16 %), Ca, P, and Mg (Chaerun 2009).

The increased CEC value of biogenic products found in

TABLE 2
Physical and chemical characteristics of biogenic products resulted from paper-based food formulation by termite *Macrotermes sp.* with increased exposure time

Characteristics	Surrounding soil	Biogenic products after exposure for the period of time (week) *		
		2	4	6
Density (g cm^{-3})	0.66 ± 0.03	0.46 ± 0.05 a	0.37 ± 0.09 b	0.32 ± 0.08 b
pH	4.84 ± 0.49	7.39 ± 0.15 a	6.87 ± 0.24 b	7.23 ± 0.20 a
C-organic (%)	2.27 ± 0.54	5.37 ± 0.22 a	5.73 ± 0.40 b	6.10 ± 0.46 c
CEC (cmolc kg^{-1})	20.03 ± 1.60	28.55 ± 3.42 a	29.02 ± 3.42 a	41.33 ± 1.69 b

* *Mean and standard deviation of twenty replicates. Values in rows with different letters are significantly different by Tukey's test ($P < 0.01$).*

this study indicates the increase of total exchangeable cations, which is one of essential plant nutrients. The level of CEC is associated with the content of organic matters and depends on the pH level. The biogenic products after two weeks exposure with low organic matter has a lower CEC (28.55 $cmol_c$ kg^{-1}) compared to that after six weeks exposure with high organic matter (CEC greater than 40 $cmol_c$ kg^{-1}). It is in line with previous findings that the changes in organic matters enhanced exchangeable cations (Jouquet et al. 2004, 2005).

Chemical and physical properties of biogenic products are also thought to have a relation with particle size distribution. The termite activities had caused food and soil aggregation (Figure 4), which may result in lower density and perform benefits to soil physical properties, particularly aeration and soil porosity (Decaëns et al. 2001). The field test showed that *Macrotermes sp.* using the designed matrix formulation as a part of food sources had a greater impact on soil properties since the termite attacks leads to the construction of biogenic products with a low density, a high organic content, and a large fractionation. With the bigger particles, the biogenic products enable to have higher water holding capacity and porosity (Mora et. al. 2006). Results obtained from the present study demonstrated the role of termites in relation to the obtainability of organic matters, amount of pore space (bulk density), and soil aggregation.

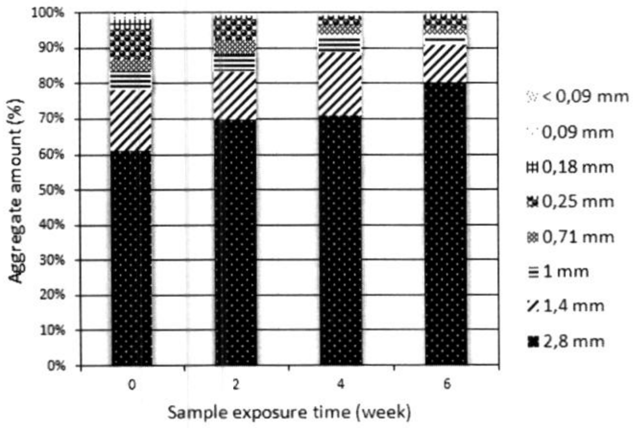

FIGURE 4
Aggregate fraction distribution of waste paper-based food formulation before and after exposure

The above mentioned characteristics of biogenic products will lead to the increase of soil fertility and production because the action of termites on bait formulations involves physical and chemical processes. However, the quality of soil after the application of the biogenic products on a time scale need to be further investigated. Some previous studies showed that termite Macrotermes was recorded to modify food sources to increase the contents of clay and soil organic matter in order to stabilize their nest structures including galleries, sheetings, fungus-comb chambers and epigeous mounds (Mora et al. 2003, Jouquet et al. 2004, Jouquet et al. 2011,). It was also found that the improved nest structure together with the increase of soil nitrogen had caused the increase of wheat yield by 36% from increased soil water infiltration, indicating their importance for agricultural sustainability in arid climates (Evans et al. 2011).

CONCLUSIONS

The use of mixture formulation of cardboard, office paper, and decayed pine wood wastes as food source for the production of biogenic materials by termites, especially *Macrotermes sp.*, was feasible in altering soil characteristics and land productivity. Preparing the mixture formulation in a closed design with the target density of 0.5 g cm^{-3} is an alternative way to attract termites and produce valuable biogenic products within six weeks exposure in the tropical land. The produced biogenic structures have significantly lower density, better fractionation, and higher content of organic components compared to the surrounding soil. These results seem to associate with the food source formulation, bait design, and the addition of nutrients and moisture content.

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