Influence of nano-activated carbon on biodegradation of bamboo paper in the soil

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Abstract. Paper made from natural fiber of amapel bamboo (Bambusa vulgaris) and nano-activated carbon from sawdust had been tested as food packaging and showed its ability to maintain freshness and nutritive value of foodstuffs. However, as a packaging material, natural degradability of this alternative natural-fiber paper is required to be tested. This study aims to determine the effect of nano-activated carbon on paper’s biodegradation properties. The results showed that paper treated with nano-activated carbon degraded faster in the soil compared to paper made of bamboo fiber only (control) after 8 weeks of observation. The microorganism population density analysis showed that the paper with nano-activated carbon had a lower microorganism density than the control which accompanied by a decrease in paper weight after 12 weeks of observation. This finding demonstrates the potential utilization of nano-activated carbon as an additive to be inserted into paper to accelerate the biodegradation rate of paper in the soil. The ability of paper to be degraded naturally is very important to support environmental sustainability.

1. Introduction
Environmental issues are currently becoming an important concern for paper-making industries. A part from a large amount of sludge produced by the pulp and paper mills, legality issue of the wood as raw material for pulp and paper industries, and capability of resultant paper products also received serious attention related to the environmental impacts. Furthermore, another crucial issue related to the paper products, especially those that are used as food packaging, is related to the problems of polymer-lifetime after being used, such as possibility be recycled, and/or discarded processes in the environment. Therefore the number of studies of polymers biodegradability is increasing [1].

Biodegradability refers to the potentiality of material properties to be degraded into simpler components through particular biological processes. The biological processes which is also known as ‘biodegradation’ can be evaluated and monitored under certain conditions and particular time of observation. The process involves microbial activities which releasing particular enzymes to effectively break down the chemical bonds of the polymers into simpler compounds [2].

Paper mainly consists of cellulose—a polymer which abundantly found in plant-based materials in nature. In order to maintain the equilibrium of the cellulose biogeochemical cycle on the earth, cellulolytic microbes that utilize cellulose for nutrition are also abundant in term of its numbers and biodiversity [3]. Most of them are fungi and/or bacteria that can produce various enzymes to degrade complexity of cellulose chain and convert it into digestible compounds to support the microbes’ life cycle as well as metabolisms of others surrounded living organisms [4]. Therefore, naturally, cellulose can be degraded by many microorganisms. However, the presence of paper in Municipal Solid Waste
(MSW) hinder the composting process of municipal waste. Alvarez and co-authors found that none of paper waste reached 70% of biodegradation after 45 days under controlled composting conditions [5]. This phenomenon indicates that natural biodegradation of paper waste does not always run smoothly. Therefore, biodegradability studies for plant fiber-based packaging materials are still required before these products are mass produced and widely utilised.

Our previous study revealed that paper from bamboo fiber (Bambusa vulgaris) which was used as food-wrapping paper was able to maintain freshness and nutritive value of the food [6]. The potential performance of this bamboo-activated carbon paper as food wrapping needs to be supported by information on its ability to degrade naturally. It is important to know the ability of paper to degrade naturally in the soil to determine its impact on the environment so that it can support environmental sustainability. Therefore, biodegradation rates of thus paper were observed and analysed to obtain more comprehensive information on recommending the use of nano activated carbon-bamboo paper as wrapping paper.

2. Materials and Methods

2.1. Materials

Paper with nano-activated carbon that used in this study were made from *ampel* bamboo (Bambusa vulgaris) and nano-activated carbon originated from sawdust waste. Plastic containers and composted soil were used to evaluate the paper degradation in the soil. NaCl 0.85% was used to suspend the microorganism. Vortex, plastic tube, porous lid, tissue, soft brush was used in experimental. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) in petri dishes were used to grow microbial colonies

2.2. Methods

2.2.1. Paper production. Paper with nano-activated carbon was prepared through series of process as described in our previous study [6]. Paper was made from *ampel* bamboo fibers which produced by semi-chemical method using hot soda process. Nano activated charcoal was produced from sawdust waste which carbonized in 400-500°C for 4-5 h and activated by water vapor in 800°C for 70 min. The resultant activated carbon was pulverized using grinder and filtered by mesh filter to get nano scale. Nano-activated carbon was added into pulp as additive by the proportion 20% to get the paper density of 60 gm⁻². Papers contain nano-activated carbon and native paper without nano-activated carbon denoted as CP and NP, respectively.

2.2.2. Biodegradability test in the soil. Papers of NP and CP were cut in strips of 3 cm x 1 cm. Those papers were dried in 75°C for 48 h and the weight after drying was calculated as Initial Dry Weight (IDW). Papers were placed into plastic container filled with the composted soil made from composted household-waste produced by ‘One Home Farm’ (a local farming industry) in 45% of humidity and covered by porous lid. The trial set consists of 12 repetition was observed for 12 weeks with 4-weeks interval (3 sampling times). The sampling time denoted as T1, T2 and T3. The initial condition was denoted as T0. At the time of sampling, pieces of paper are taken and cleaned by a soft brush. Description of biodegradation tests in soil as described in Figure 1. Biodegradability of the papers was evaluated by measuring the paper dry-weight loss after certain time of incubation and assessing microbial density and diversity that grown on the paper surface, as well as changes on microscopic morphology of the paper’s surface prio and post treatments.
2.2.3. **Measurement of dry weight for each sampling.** The cleaned papers were removed from the soil in plastic container and placed into plastic tube containing 10 mL of 0.85% of NaCl solution. The tube was shaken using vortex to get the microorganisms attached on the surface of paper were suspended in NaCl solution. After complete shaking, the paper was removed and dried in 75ºC for 48 h. The weight after drying (IDW) was measured using the formula 1, while the aliquots (NaCl solutions which were assumed containing microbial suspension from the paper surface) were subject to be isolated and enumerated to describe microbial density and diversity.

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\Delta DW = DW_0 - DW_x
\]  

Where: \(\Delta DW\) is the difference of dry weight, \(DW_0\) is initial dry weight before observation (biodegradation in the soil) and \(DW_x\) is dry weight after observation.

2.2.4. **Microbial enumeration.** Microorganisms density was determined by *plate count method*. The homogenized aliquots that have been prepared in the previous section (see 2.2.3) were then decimally diluted from \(10^{-1}\) to \(10^{-6}\). The colonies were calculated using *plate count method*. One ml of each resulting solutions was spread evenly into 9 ml either PDA or NA media. Plating was conducted with two replications for each dilution, on each media. After being incubated at 30ºC for 2-7 days in inverted position, the Colony Forming Units (CFU) of each plate ranging from 30 to 300 colonies was calculated.

2.2.5. **Microbial isolation and identification.** Various microbial colonies growing on the plates were then sub-cultured on to separate fresh agar plates based on their morphology, size and appeared colour to ensure its purity. The pure microbial colonies were subject for molecular identification to determined microbial diversity that may associated to the biodegradation process of the tested paper naturally.

Molecular identification of microbial isolates was conducted through series of process starting from DNA extraction, PCR amplification, DNA-bands visualization by electrophoresis technique and
captured using Biorad Gel-documentation. The PCR products were sent to the 1st Base Inc. (Singapore) for DNA sequencing. The sequence data were edited using BLAST program and then matched against the Genebank DNA-public database to obtain taxonomic information of the microbial isolates. The accession number of the Genebank database with the highest similarity index against the DNA sequence of the sample was pointed as the taxonomic identity of the isolate (Table 1). Detail procedures were described in the publication of Agustini and co-authors [7].

2.2.6. Microscopic observation of paper morphology. Microscopic observations were made using a stereo microscope and a Scanning Electron Microscope (SEM) to determine changes in paper morphology prior and after biodegradation testing in the soil.

3. Results and Discussion

3.1. Biodegradation test in the soil

Results of biodegradation tests in soil as described in Figure 2. At the first time of observation/T0, paper with nano-activated carbon (CP) seemed more difficult to degrade. However, paper with nano-activated carbon (CP) degraded faster than paper made of bamboo fiber only (NP) after 8 weeks of observation. It was indicated by the remained weight of CP (25%) which was lower than NP (42%) after 12 weeks of observation. The results of previous study have indicated that the presence of carbon or charcoal increased the microbial abundance in the soil [8]. Another research reported that the presence of 0.4% biochar and hydrochar in the soil promote the growth and enzyme activity of soil-resident ligninolytic fungi [9]. Porous structure of nano-activated carbon is supposed to be used as a locus for the viable microorganisms which then lead to enhance biodegradation process of paper in the soil. Therefore, the presence of nano-activated carbon in the paper increased the biodegradation rate of paper. Notably, paper containing cellulose fibers which consist of amorphous and crystalline regions, whose degradation takes place with different rate [10].

![Figure 2](image-url)  
*Figure 2.* The percentage of remained biomass of observed paper after 12 weeks of observation.

Paper with nano-activated carbon (CP) have been degraded more completely with a more brittle physical structure compared to papers without nano-activated carbon (NP) at the end of observations. About 50% of CP paper were degraded completely, meanwhile only 1 sample of NP paper was fully degraded during the biodegradation test in the soil test blocks. Paper appearance after 12 weeks of observation as shown in Figure 3. However, the ability of paper to degrade is also affected by the type of paper. The results of previous studies examining the biodegradability of various types of paper show that different types of paper have different biodegradability under controlled conditions. The study found that newspaper and tissue/paper handkerchiefs were more difficult to biodegrade unless the manufacturing process was changed [5].
Figure 3. Papers morphology after 12 weeks of observation: paper without nano-activated carbon (upper) and paper with nano-activated carbon (bottom).

3.2. Microorganism density
Microorganism density on the paper surface were observed and the results as shown in Figure 4. Microorganism density increased faster at initial stage (T0 to T1). Microorganism density of both papers slightly increased after 4 weeks of observation. However, bamboo paper with nano-activated carbon revealed higher microorganism density than native bamboo paper after 4 weeks of observation. This fact corroborates the hypothesis that the presence of carbon stimulated changes in the abundances and metabolism of rhizosphere bacteria and fungi [8]. This microbial abundance accelerates the degradation rate of the paper which causes the degraded CP to have lower biomass than the NP (Figure 3). The abundance of microorganisms on The abundance of microbes on the CP might also be associated with the ability of porous carbon in capturing water [11] and providing suitable humidity to support microbial growth.

Surprisingly, the microorganism density of bamboo paper with nano-activated carbon decreased dramatically after 8 weeks of observation. This decrease corresponds to the remaining biomass. In contrast, native bamboo paper showed the increase of microorganism density until 12 weeks of observation. However, this increase of microorganism density did not indicate a faster rate of paper degradation referring to the weight of the remaining biomass as shown in Figure 4.

Figure 4. Microorganism density of bamboo paper only/ NP (blue) and bamboo paper with nano-activated carbon/CP (red).
3.3. Microscopic observation

Microscopic observation of observed paper as described in Figure 5. Surface appearance of bamboo paper with nano-activated carbon (CP) and bamboo paper only (NP) have differences in the distribution of fungal mycelium. The distribution of fungal mycelium was seen more widely on the surface of the paper with nano-activated carbon compared to native bamboo paper. However, the paper biodegradation rate by microorganism is dependent on the fungal species and conditions [12]. White or bleached appearance on the paper surface might indicate the presence of white rot fungi which characterized by their ability to degrade lignocellulose constituents by giving rise to a cellulose-enriched white material [13].

![Microscopic observation images](image)

Figure 5. Microscopic appearance of paper surface (50x magnification): (A) Bamboo paper with nano-activated carbon/CP prior to biodegradation test; (B-C) bamboo paper with nano-activated carbon/CP after biodegradation test; (D) bamboo paper only prior to biodegradation test; (E-F) bamboo paper only after biodegradation test. Yellow narrow correspond to fungal mycelium from soil. Inserted images were in 250x magnification.

3.4. Microorganism biodiversity from soil

The results of identification of microbial diversity showed that 2 genera of bacteria (Bacillus and Pseudomonas) and 2 genera of fungi (Scedosporium and Pycnoporus) were identified from the composted soil used in this study (Table 1).

| Isolate code | Similarity of DNA sequence | GeneBank Accession Number | Possible Name                          |
|--------------|----------------------------|---------------------------|---------------------------------------|
| K.A          | 99%                        | KY122853.1                | Scedosporium apiospermum              |
| K.B          | 99%                        | KP.132626.1               | Scedosporium apiospermum              |
| K.C          | 99%                        | KY.880974.1               | Bacillus cereus                        |
| K.E          | 100%                       | KY228945.1                | Bacillus sp.                           |
| K.F          | 100%                       | MF.480459.1               | Bacillus sp.                           |
| K.G          | 99%                        | KR.149623.1               | Pseudomonas aeruginosa                |
| GB.S1        | 100%                       | AF.363759.1               | Pycnoporus sanguineus                 |

*Scedosporium apiospermum* is a soil fungus that assimilates numerous aromatics or polycyclic hydrocarbons. It may cause human infection [14]. *Bacillus cereus* is a common opportunistic foodborne
pathogen [15]. It was described as flagellated, rod-shape, Gram-positive facultative aerobe and capable to degrade odorous organics dimethyl disulfide (DMDS) in aqueous solution under aerobic conditions [16]. Bacillus are rhizospheres microorganisms. They play a significant role in formation and enrichment of the soil due to their metabolite activity and known as degrading strain [17,18]. Pseudomonas aeruginosa is gram-negative bacteria may cause several acute and chronic infections [19]. Pycnoporus sanguineus is white-rot fungus. The genus Pycnoporus has the ability to overproduce high redox potential laccases as the ligninolytic enzymes [20]. These fungi may affect the biodegradation rate of observed paper due to fungi of the Pycnoporus are efficient degraders of lignocellulosic materials [21]. The presence of carbon or char in paper affected the microenvironment of soil e.g pH and the moisture which leaded to the changes of environmental conditions for soil microorganisms and N cycle, hence the presence of carbon in the soil resulted in the highest bacteria genes expression level [22]. Activated carbon is supposed to alter the carbon abundance in the composting substrate and increased the abundance of microbes [23]. The presence of Bacillus strains was in good accordance with the previous study that investigated the related soil isolates [24]. The presence of Bacillus strains indicated high-temperature fermentation process ends and oxygen concentration is adequate which give benefit for the growth of aerobic strains [21].

4. Conclusion
The effect of nano-activated carbon on bamboo paper has been observed. The existence of nano-activated carbon increased the rate of paper degradation seen from the final dry weight during soil test blocks. The effect of nano-activated carbon in the first 2 weeks of observation showed a slightly higher microorganism density on the paper surface. In general, the addition of nano-activated carbon slightly improved the paper biodegradation rate.

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