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Studies on Composting Spent Coffee Grounds by *Aspergillus sp* and *Penicillium sp* in Aerobic Static Batch Temperature Control

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Abstract

Spent Coffee Ground (SCG) is characterized by high organic content, in the form of insoluble polysaccharides bound and phenol compounds. Phenol compounds are toxic to nature and are a cause of environmental pollution. Composting method of this study is aerobic static batch composting with temperature control with adding activators of some fungi such as *Aspergillus sp*, and *Penicillium sp.* The purpose of the research is to fill the research gap from previous studies of spent coffee grounds compost, which requires a long time in composting, so that if it is used directly on the soil and plants, the positive effect also requires a long time. The result of composting for 28 days with this method is that mature compost has black crumb and normal pH, with characteristics of C/N ratio below 10: C1 (7.06), C2 (6.99). This value is far from the control with a C/N ratio of 8.33. Decompose rate of macromolecule are above 40% for lignin and 70% for cellulose. Implementation of compost in radish plants, resulting Germination Index above 80% which indicates that the compost is ripe: control (92.39%), C1 (183.88%), C2 (191.86%). The results of the analysis with FTIR also showed that the compost was mature and stable, and rich in minerals. So, it can be concluded that this composting method can speed up composting time and optimize the results of compost produced.

Keywords

Spent Coffee Ground (SCG), *Aspergillus sp*, *Penicillium sp*, Aerobic Static Batch Temperature Control

1. Introduction

Around 9 million tons of spent coffee grounds are produced in the world [1].

Coffee is the favorite beverage in the world; therefore, this number always increases every year [2]. Consumption per ton of coffee beans can produce 650 kg of coffee grounds [2]. An estimated percentage of around 90% of the brewed coffee ends up though in the form of Spent Coffee Grounds (SCG). SCG is characterized by high organic content, 38% hemicelluloses and 9% cellulose, and 14% protein [2]. SCG still contains phenol compounds, tannins and caffeine [3]. This compound is toxic to nature and is a cause of environmental pollution [4]. Therefore, it is necessary to treat spent coffee grounds to reduce the toxic component by composting. Composting is a simple and efficient way to convert waste into stable, non-toxic and has good nutrition for soil and plants [5]. It is generally equipped with heating and agitating devices that promote biodegradation of organic materials by microorganisms under neutral to alkaline conditions at moderate to high temperatures (40˚C - 60˚C) [6] [7] [8].

In this study, composting of coffee grounds was added by the addition of some fungi as starter culture with temperature control. The starter is an additional material used in the early stages of the process fermentation. The starter is a culture of certain microbes that are grown inside substrate or medium for specific process purposes [9]. The requirements for the fermentation starter are pure, superior, stable and not pathogenic. According to Utama *et al.* (2013) requirements for fermentation, starters are safe to use and capable inhibits pathogenic bacteria [10].

The combination of some microorganism and growth optimization with temperature control is expected to further maximize the production of enzymes and the process of material degradation so as to accelerate the composting process. Fungi are the main decomposers in soil ecosystems [11]. In addition, fungi are also able to decompose lignocellulose, protein and amino acid very well. The types of fungi used in this study were *Aspergillus sp* and *Penicillium sp*. It was isolated from fertile soil. Fungal activity of combination *Aspergillus spp* and *Penicillium sp* can also speed up the composting process. Activators of this fungus can improve the biological, physical, and chemical properties of the soil, therefore that plants can grow better and are more resistant to pathogens [12].

According to Subowo & Corazon (2010) and Subowo (2015) that *Aspergillus spp.* and *Penicillium sp*. has the ability to break down lignin, as well [13] [14]. *Penicillium sp.* and *Aspergillus spp.* is a type of fungus that can grow on media containing lignin [15]. *Penicillium spp.* even can increase plant growth in peat soils because they help provide the elements nutrients for plants by degrading the organic matter (including lignin compounds) in peat soils [16]. Both of these genus fungi known to have a lignin degradation ability quite good compared to other fungus genera which has also been proven in other studies [13].

Penicillium sp and *Aspergillus sp* are soft rot fungi. Soft-rot fungi are mostly ascomycete fungi that can degrade polysaccharides in the surface layers of plants [17]. Gupta (2015) and Gupta *et al.* (2016) mention that soft rot fungi are no doubt the most efficient fungi to degrade lignin in mixed microbial population.

Besides that, the adaptation of soft rot fungi in various temperature, different pH, and limited oxygen is higher than other fungi [18] [19] [20].

However, still little is known about the degradation mechanism of lignocellulose by soft rot fungi [17]. Therefore, this study uses soft rot fungi as activator and learn this effect on quality of compost produced. This is because even though the degradation capability is low when compared to white rot and brown rot function as mentioned above in the previous work, soft rot fungi also have good degradation capability. In addition, because compost is one of the best places for the growth of soft rot fungi, apart from soils, piles of woodchip, and straw [21].

The addition of activator in the composting process has been widely studied by researchers. However, the use of specific combination types of fungi activators with temperature control for SCG composting is still very little studied. Yamane *et al.* (2014) studied the use of coffee grounds directly on plant growth, the results of which took a long time for the benefits of coffee grounds to function positively for the soil and plants [22]. The difference in the use of fresh and composted spent coffee ground is also studied and composted SCG is better for plants [23]. SCG compost using solid state fermentation was also studied by Echeverria *et al.* [24]. Because of the lack of reference composting of SCG using soft rot fungi with temperature control, therefore in this study studied the composting of coffee grounds using fungi especially by *Penicillium sp* and *Aspergillus sp.*

Activator using fungi is a very promising method for increasing agricultural production, as well as reducing the release of chemical pesticides into the environment. This is because they are able to change and release many nutrients that play an important role in the nutrition cycle and maintain vegetation [25]. In addition, in this study also added cow dung and chicken manure to supply extra carbon for microbial activity, balancing C/N ratio, and providing most of the nutrients, including nitrogen (N), phosphorus (P), and potassium (K).

Composting method of this study is aerobic static batch composting with temperature control. Because can be generated in a short period of time [26] [27]. Temperature control is done to maximize the growth of microorganisms, especially *Penicillium sp* and *Aspergillus sp* during the composting process. This is because temperature is one of the most important environmental factors in the composting process. The objective of this study was to understand the effect of using combination fungi with temperature control in SCG composting.

2. Material and Experimental Methods

2.1. Preparation of Fungi Starter Culture

Fungi was isolated from fertile soil on Prefectural University of Hiroshima Japan, and then inoculated for 2 weeks in PDA (Potato Dextrose Agar) medium. A loop full of individual culture potential was taking up and inoculated in 100 g dried malt extract then added 1000 ml distilled water, autoclaved 121˚C, and 20

minutes. Then cooling around 10 minutes. Stirring using Mixer MG-600 100 rpm, 36 hours. Then incubated 4 weeks, 25˚C. That mix solution (**Figure 1**) was used as the inoculum or activator [28].

2.2. Preparation of Commercial Starter

Commercial activator that we used is pure, patented by the Bio Food Industry Research Center, and the Industrial technology center, Fukuoka Prefectural. Produced by the non-profit organization Eco cycle Kyushu/Okinawa Japan.

2.3. Preparation of Cow Dung and Chicken Manure

Cow dung and chicken manure were used are commercial manufactured by Green Plant Factory Japan.

2.4. Treatment and Composting Procedure

This study was conducted in Prefectural University of Hiroshima, Shobara campus, Japan. Each sample was produced in 1-liter plastic container with small holes for air circulation. 3 samples (control, C1, and C2) then put in incubator with temperature 30 degrees Celsius. Description of sample as explains below:

1) Control: SCG 150 g, chicken manure and cow dung each 100 g and adding water until Moisture content around 60%.

2) C1: SCG 150 g, chicken manure and cow dung each 100 g, adding commercial (dilution 100 times with water) to sample until Moisture Content around 60%.

3) C2: SCG 150 g, chicken manure and cow dung each 100 g, then adding fungi activator (dilution 100 times with water) to sample until Moisture Content around 60%.

The amount of activator used is 1 percent of the total weight of water. Spent coffee grounds were fermented Robusta coffee from Jember, East Java, Indonesia. Then dried in oven laboratory at 60 degrees Celsius to remove all the humidity and inhibit microbial processes. After, these materials were mixed and used in composting to achieve a mixture with C/N ratio below 10 (for horticulture plant), the idea being obtain compost rich in nitrogen for plant fertilization.

About 350 g dry material of each mixture was composted 28 days. The moisture was maintained around 60% of water content. The mixture was homogenized manually revolving each mixture almost every day. The temperature was controlled in 30 degree Celsius. Samples were taken on days 0, 7, 14, and 28 days. In all analyzes, 3 repetitions were performed for each sample. Sketch of the composting equipment can be seen in **Figure 1** below.

2.5. Physical and Chemical Analysis

2.5.1. Physical and Chemical Analysis of Raw Material

Before composting is carried out screening of raw materials first, including analysis of cellulose, lignin, caffeine, and protein. Analysis of lignin, which is 200

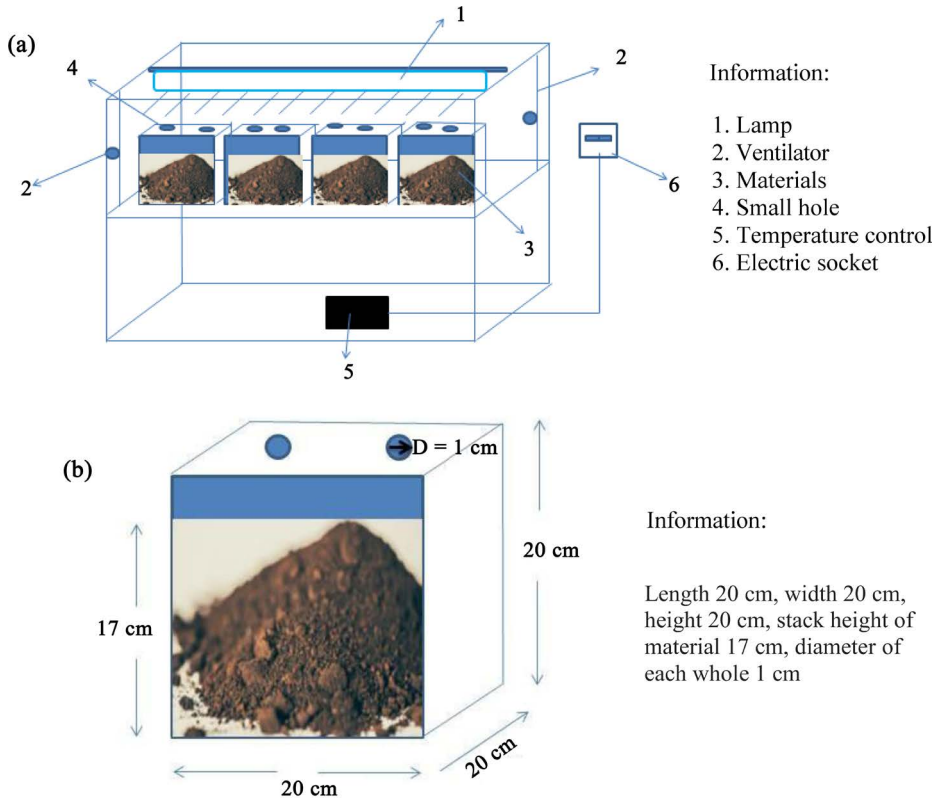

Figure 1. (a) Composting equipment with temperature control, (b) box of compost.

mg of raw materials (Spent Coffee Grounds, cow dung, and chicken manure), added 1 M H_2SO_4 1 ml mixed and put to water bath shaker 1 h. Then it was autoclaved 1 hours 121 degree Celsius. Then it was filtrated using filter paper. Then put in oven 105 degrees Celsius 24 hours. Weighed sample as lignin. Total of Polyphenol analysis uses spectrophotometry method [29]. Analysis of protein use Lowry method [29]. In addition, screening of macro and micronutrient content related to their potential to be composted was also carried out.

2.5.2. Physical and Chemical Analysis during Composting

The following analysis was carried out in fresh compost samples: electrical conductivity (EC), and pH were determined by "Soil analysis" standard procedures [30]; Total carbon and total nitrogen were determined also in dry sample by Macro corder (JM 1000CN). Then, the C/N ratio was calculated.

2.5.3. Macro Nutrient Analysis

The concentration of element macro nutrient Ca, Mg, P, and K were measured in dry samples by ICP-OES (inductively coupled plasma optical emission spectrometry) (Hitachi, PS7800) after nitric per chloric acid digestion [30].

2.5.4. Morphology of Starter

Morphology of fungi was observed under microscope using slide culture. While commercial activator was observed using Scanning Electron Microscopy (SEM) [31].

2.5.5. Relative Enzyme Activity

Each Fungi isolate obtained were grown on the minimal agar medium supplemented with 0.5% CMC (carboxyl methyl cellulose), 0.5% caffeine, and 0.5% lignin (pH 7.0) at 30˚C for 4 - 5 days. The plates were then flooded with 0.33% iod solution followed. The appearance of a zone of hydrolysis around the colonies indicates synthesis of extracellular celluloses, xylenes by the microbes and fungus. Enzyme activity potential of the positive isolates was evaluated by measuring Relative Enzyme Activity (REA), *i.e.*, ratio of diameter of zone of hydrolysis to the diameter of the colony [32].

2.5.6. Germination Index

Percentage of seed germination, root growth and germination index (GI, a factor determined by both germination & root growth) were calculated based on the formula [33]:

Seed germination (SG %) = SG % in each extracts/SG % in control × 100.

Root growth (RG %) = mean root length in each extracts/mean root length in control × 100.

Germination index (GI) = multiplying SG % and RG %.

2.5.7. Functional Group Identification

Functional group analysis is carried out by means of FTIR-ATR (Attenuated Total Reflection-Fourier Transform Infra-Red) [34].

3. Results and Discussion

3.1. Chemical Composition of Raw Material

Spent coffee ground (SCG) contains many polysaccharides such as hemicellulose (39.75%), and lignin (23.1%). Cow dung and chicken manure also contain hemicellulose and lignin which are also high. Cow dung has 18% hemicellulose, and 8.9% lignin, while chicken manure has 11% hemicellulose and 13.75% lignin (**Table 1**). These values are comparable to the others reported in the literature for SCG [35]. A little difference with some of the results of other studies may differ the type of coffee, method of roasting and also brewing techniques. It is very suitable for composting, mixing with spent coffee grounds to prevent nitrogen loss. The composition of manure is highly variable, according to animal

Table 1. Chemical content in raw materials.

Chemical content	SCG (%)	Cow dung (%)	Chicken manure (%)
1. Hemicellulose	39.75 ± 0.007	15.6 ± 0.007	26.7 ± 0.007
2. Lignin	23.1 ± 0.007	9.05 ± 0.007	10.2 ± 0.007
3. Caffeine	1.83 ± 0.007	n/a	n/a
4. Protein	10.82 ± 0.007	n/a	n/a
5. TPC	1.61 ± 0.07	n/a	n/a

Data are expressed as mean ± SEM (standard error of the mean) of three replicates. n/a—not analyzed.

type, diet, type of housing, and the amount and type of litter, and spilling water used.

The high content of hemicellulose and lignin is the basis for choosing the type of activator fungi in composting, namely *Aspergillus spp* and *Penicillium spp*. Lignin is a major structural component of plants and is the one that is degraded the slowest. It has been claimed that humus is mainly formed from lignin, polysaccharides and nitrogenous compounds [36] [37] [38]. Therefore, in this study we choose specific fungus that can degraded lignin very well. The degradation of lignin is primarily accomplished by fungi. For hemicellulose, the main degrading enzyme is xylanase, produced by many bacteria and fungi [39].

This study also analyzed the caffeine content and total polyphenols in SCG. This is because both of these compounds are toxic to soil and plants [4]. The caffeine content in the SCG sample was 1.83%. This value is lower than previous research of Musatto *et al.* (2011), because the sample used is Robusta coffee that has been fermented [35]. This fermentation process causes caffeine to degrade and its value is lower than other references.

Hakil *et al.* (1998) mention that *Penicillium* and *Aspergillus* are the more frequent caffeine-degrading genuses [40]. It therefore seems logical that the majority of the studies done on caffeine degradation by filamentous fungi are related to *Aspergillus* and *Penicillium* genuses.

SCG also contains Carbon and Nitrogen ratios 19.5/1. Cow dung (12.36/1), and chicken manure (9/1) (**Table 2**) which approaches the C/N ratio of the soil, 20/1. Thus, SCG, cow dung, and chicken manure have the potential to be used as compost products because it has the macro and micronutrients needed by soil and plants.

3.2. Selection of Microorganism for Composting

2 fungal isolates from fertile soil were screened for their ability to produce celluloses. We also observed morphology of each strains. Fungi were observed under a light microscope with a magnification of 40× (**Figure 2** and **Figure 3**). This is because fungi have large cell sizes. From these observations, it is known that fungi are *Aspergillus sp* following characteristics: colony grows quickly, green color of colony, single row of phialides covering entire vesicle, conidiophore point out in all direction, and in variable length Rough, pitted, spiny. Besides, in activator also consist of *Penicillium sp*, with characteristic: old green color. Conidiophore hyaline upright, branched, tapered phialide, conidia pale green-shape

Table 2. Macro nutrient content of raw materials.

Macro nutrient content	SCG (%)	Cow dung (%)	Chicken manure (%)
1. Carbon	46.24 ± 0.18	29.56 ± 0.17	19.36 ± 0.18
2. Nitrogen	2.37 ± 0.014	2.39 ± 0.014	2.15 ± 0.02
3. C/N ratio	19.51/1	12.36/1	9/1

Data are expressed as mean ± SEM (standard error of the mean) of three replicates.

ellipse or sub globose and single celled. This is in accordance with morphology *Penicillium sp.* which was described by Watanabe (2002) [41].

For quantitative screening, Results present showed positive cellulose producers for plates flooded with Iodine solution. Clearing zones surrounding microbial growing colonies after incubating for a suitable period indicating their ability for cellulose production. Kasana *et al.* (2008) discovered that Gram's Iodine for plate flooding in place of hexadecyl trimethyl ammonium bromide or Congo red, gave a more rapid and highly apparent result [42]. Gram iodine was also used for the screening of cellulose producing microorganisms, *i.e.*, fungi [43] and bacteria [32]. After observing the clear zone produced, the Relative Enzyme Activity (REA) was measured by comparing the diameter of the clear zone and the diameter of the colony. The REA measurement results are presented in Table 3 below.

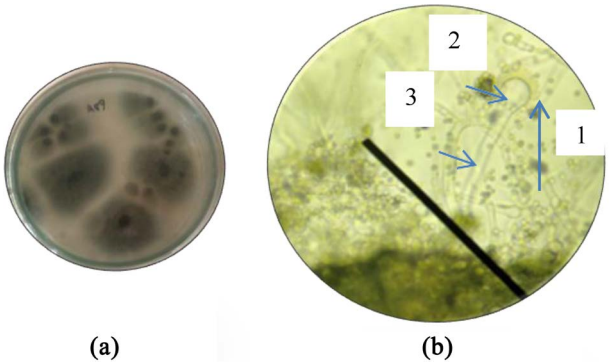

Figure 2. (a) Macroscopic and microscopic *Aspergillus sp*, green color of colony, (b) Morphology of Aspergillus with magnification 40× (1) conidiospore, (2) vesicule, (3) conidiophore.

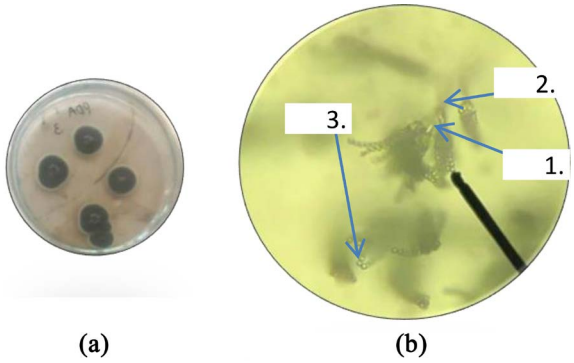

Figure 3. (a) Macroscopic and microscopic *Penicillium sp*, old green colour of colony, (b) Morphology of *Penicillium sp* with magnification 40× (1) Phialide, (2) conidiophore, (3) conidium.

Table 3. Relative Enzyme Activity of each fungi.

Sample	REA to degrade CMC	REA to degrade caffein	REA to degrade Xylan	REA to degrade lignin
Aspergillus sp	7.82 ± 0.61	5.17 ± 0.37	2.17 ± 0.008	10.5 ± 0.5
Penicillium sp	15.57 ± 0.81	13 ± 0.1	2.4 ± 0.038	18.35 ± 0.94

From the table above, *Penicillium sp* has a greater EAI than *Aspergillus sp.* combining these 2 isolates is expected to increase the production of cellulase enzymes so as to accelerate macromolecular degradation. According to Subowo & Corazon (2010) and Subowo (2015) that *Aspergillus spp.* and *Penicillium spp.* has the ability to break down lignin, as well [13] [14].

3.3. Chemical Properties Changes during Composting

In the composting process, to determine the maturity, stability and quality of compost is to observe chemical changes in the material. Chemical changes in the material during composting process are described below. Observation of each parameter is done for the initial sample, 7, 14, 21, and 28 days composting is carried out aerobically and the sample is left in a container at incubator 30 degree Celsius.

3.3.1. pH and EC

pH is monitored continuously every week because pH fluctuations indicate a degradation process organic compounds by microorganisms. Results of compost pH can be seen in **Figure 4**. pH conditions during the composting process change. On day 0 of composting, the pH was slightly acidic (6.72 - 6.75). On the 7th day, then the pH increased to reach pH 8 and more. Then the pH decreased to reach almost pH 7 or slightly basidic until in the end of the composting process. This is in accordance with the opinion of Marlina (2009) and Haga (1990) who stated that at the beginning of composting the pH of the compost material was acidic [44] [45]. With the continuation of the composting process, the number of microorganisms increases and produces organic acids thereby lowering the pH. Furthermore, microorganisms begin to convert inorganic nitrogen into ammonium which causes pH to increase rapidly and compost becomes basidic, in the first and second weeks. Kim *et al.*, 2007 in Caceres *et al.*, 2018 also stated that at the beginning of composting the pH rises Ammonia as a result of nitrogen decomposition which is also affected by temperature. Some of the ammonia is released or converted to nitrate, then nitrate is denitrified by bacteria so that the compost pH becomes neutral at the end of composting [46]. This indicates mature compost suitable for most cultivated crops [47].

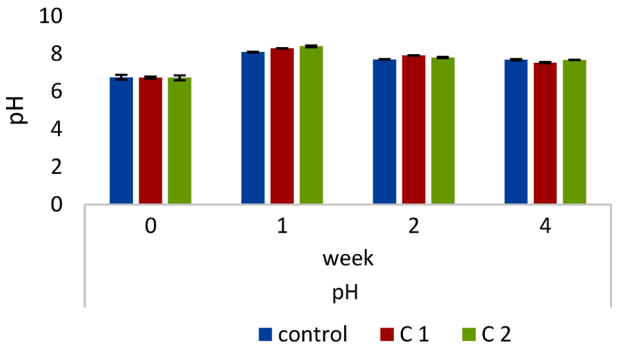

Figure 4. pH changes during composting.

Electrical conductivity (EC) is associated with the release of easily decomposable compounds into the solution and indicates if the account for the total soluble ions in composts may endanger the quality of compost used as fertilizer. The EC values of matured compost samples in the range of 2.19 - 9.32 ms/cm. The evolution of EC for the three samples of composts is presented in **Figure 5**. The general tendency of EC for all three samples of composts was to increase during the composting process. Usually a higher value of EC could be an indication of high nutrient elements presence, or a slower decomposition of the organic matter therefore a lower release of mineral salts into the solution in the process of biodegradation of biomass waste [48] [49].

3.3.2. C/N Ratio

The total of C content of the compost SCG was decrease during composting time. Because of the reduction in available carbon sources and synthesis reactions of the new complex and polymerized organic compounds or humification during the maturation phase [50], some researchers also indicated that the organic carbon content of compost samples has decreased during composting [51] [52] [53]. As shown in **Figure 6**, it can be seen that after one month the carbon has decreased. Compost sample with C2 has lowest total carbon average in final composting (6.99%), followed by C1 (7.04%) and control (8.33%). This means, C2 with fungi activator has the ability to degrade carbon better than others. Decreasing carbon of Sample C1 as much as 61.81%, while C2 60.56%. By using these three types activator, carbon reduction is better when compared to controls 56.51%. Our activator can compete with commercial. It is because the difference in the percentage of carbon reduction is only about 1.25%.

Decomposition rate of each compost can be seen in **Figure 6**. In the first week (mesophilic phase), C2 activator has a higher decomposition rate. Optimum temperature for *Aspergillus* and *Penicillium sp* growth is 28 - 37 degrees Celsius [54]. This indicates composting with a temperature of 30 degrees Celsius, very good for the material using a fungi activator. This species is also able to grow in a wide range of pH from 3.0 to 7.0 with maximal growth rates at 4.0, the optimum range being 4.6 to 6.8 [54]. At the end of composting, all three samples had almost the same decomposition rate of carbon.

Total Nitrogen (TN) includes both organic nitrogen and inorganic nitrogen

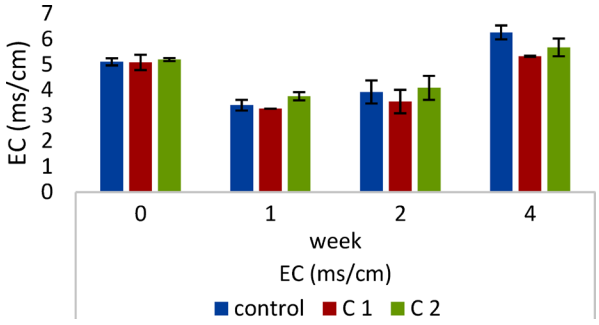

Figure 5. EC changes during composting.

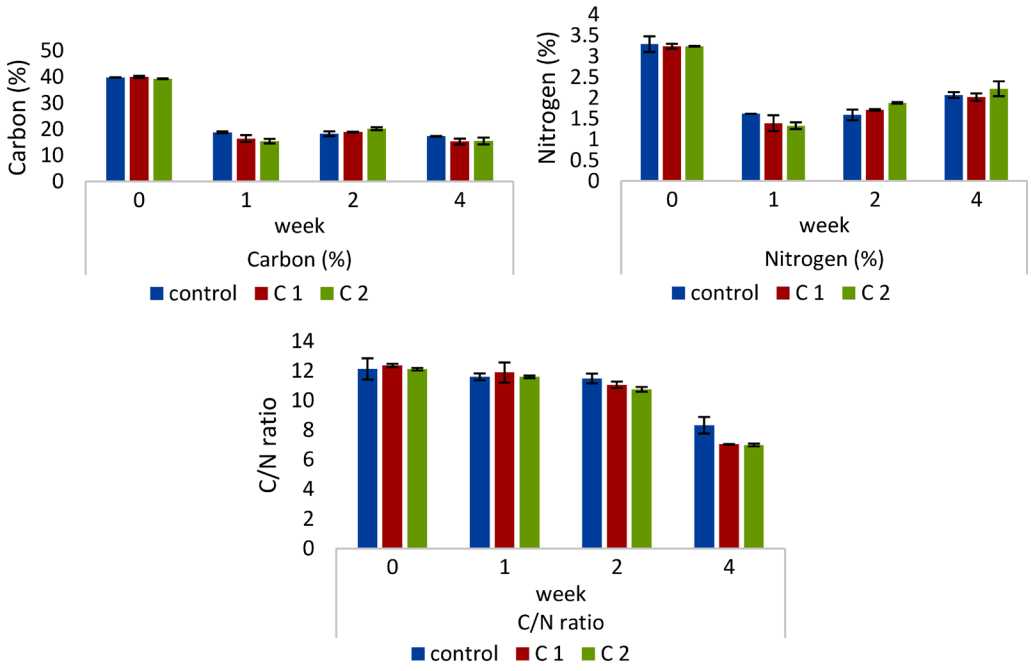

Figure 6. C/N ratio during composting.

(mainly ammonia nitrogen and nitrate) which are normally assimilated by microbes. The variations of TN for different treatments are also shown in **Figure 8**. In the first week when the pH is alkaline, so there is an ammonification process in which inorganic N turns into ammonium. In **Figure 8** below, nitrogen has dropped dramatically. However, in the second and fourth weeks, total nitrogen showed a slight increase. This is because the pH begins to return to near normal, there is nitrification where the ammonium changes to nitrate. Total N (TN) increased slightly as a consequence of concentration effect due to the mass loss during composting [50]. As NH_4^+-N could be easily transformed to organic forms under the metabolism of microorganisms or lost through NH_3 volatilization, there was decrease of NH_4^+-N concentration at the final of composting, which could be explained by volatilization and immobilization processes. As we can see in **Figure 8**, it shows the total nitrogen which has decreased compared to the initial.

3.3.3. NH_4^+-N/NO_3-N Ratio

Ammonium nitrate ratio is also one of parameter to check maturity of compost. The decrease of NH_4^+-N and appearance of NO_3^--N are good indicators of the maturation process [55].

NH_4^+-N/NO_3^--N ratio decreased gradually during composting and reached near 0.1 in the final compost (**Figure 7**), which was almost same with previous study, the value (<0.16) established by Bernal *et al.* (1998) for mature compost, but lower than the result found by Huang *et al.* (2004) [50] [56]. As different composting technologies and raw materials were used, the value varied at a wide range [50] [56] [57] [58].

3.3.4. Degradation of Lignocellulose

On the world lignocelluloses are the main part of biomass, because it is a renewable resource and the prominent structural component of plant cell wall as well. Cellulose is the dominant part of lignocellulose and consists of a linear chain of D-glucose linked by β (1-4)-glycosidic bonds to each other. The cellulose strains are connected to each other deliver cellulose fibril. A number of intra- and intermolecular hydrogen bonds are linked cellulose fibers together. Hemicellulose is the second plentiful constituent of lignocellulose, is comprised of diverse pentoses (arabinose, xylose) and hexoses (mannose, galactose, glucose) [59].

Previous study has explained about transformation of macromolecule such as cellulose, hemicellulose, and lignin as a consequence of biological activity during composting [60]-[69]. It has been claimed that humus is mainly formed from lignin, polysaccharides and nitrogenous compounds [36] [37] [38]. Another's studies also confirm the extensive loss of cellulose and hemicellulose, while confirming the increasing proportion of humus. Therefore, in this study we observed changes of cellulose, hemicellulose and lignin during composting.

Figure 8 summarized the rates of lignin, and cellulose decomposition during composting, and showed a relatively high initial proportion of lignin (around 41% - 46%) and a low cellulose content (around 4%). The greater lignin degradation in C2 could be explained by added to fungi when inoculating at t_0. This led to better global lignin degradation in C2 (40.28%) compared to similar rates in C1 (35.56%) and control (31.1%). For cellulose degradation, Sample C1 decreased 83.47%, Sample C2 74.39%. According to previous study, *Aspergillus*

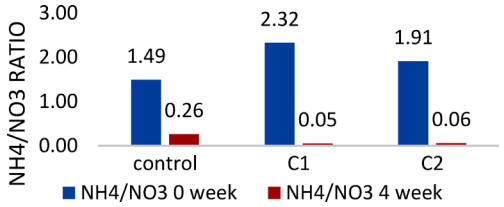

Figure 7. Ammonium-Nitrate ratio after composting.

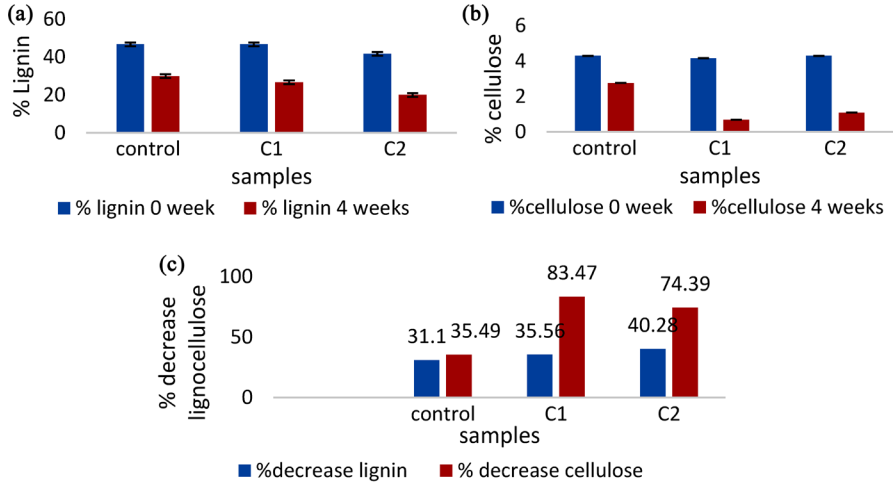

Figure 8. Decompose of lignocellulose.

and *Penicillium* is a soft rot fungus which show preference for cellulose and hemicellulose. Soft rot fungi can tolerate a wide range of temperature, humidity and pH conditions, and attack a variety of wood substrates [70]. They are usually thought to degrade mainly carbohydrates in soil, forest litter and compost, but they may also degrade lignin in these environments [71] [72] [73] [74]. Thus, some of them were found to be able to mineralize grass lignins [75] and e.g. *Penicillium chrysogenum, Fusarium oxysporum* and *F. solani* [71] mineralized in 28 days up to 27% of a 14C-labelled lignin prepared from milled wheat straw. Unlike model white-rot basidiomycetes such as *Phanerochaete chrysosporium* and *Phlebia spp.,* which degrade lignin during secondary metabolism [76] [77], the degradation by molds was maximal during primary metabolism [72].

Protein is the first degraded to peptides by the enzyme's proteases and then into amino acids by the enzyme's peptidases. These enzymes are produced by many species of Bacillus. Further decomposition yields NH_3, NO_3, CO_2, and water. This process (ammonification) occurs as a result of hydrolytic and oxidative enzymatic reaction under aerobic conditions by heterotrophic microbes such as fungi and bacteria. As shown in **Figure 9**, the percentage of decompose of protein samples C2 85.44% and C1 83.02% is higher than the control 81.82%.

3.4. Mineral

Phosphorus plays a role in cell division, fruit, flower and seed formation, plant maturity, stimulating the development of root hair, quality of crop yields and disease resistance [78] [79]. Enzyme activators, cell turgor regulation, nutrient transport and water and increase plant endurance [78]. Calcium is essential for the growth of meristems, and particularly for the proper growth and functioning of root tips. While magnesium is a specific constituent of chlorophyll, in which one atom of magnesium is bound to four pyrrole rings. Magnesium also plays a major role in numerous enzyme reactions [80].

Compost spent coffee ground has a high mineral element (**Table 4**) and is very useful for plant growth. This value is higher than before composting. This increase in concentration is due to a decrease in carbon during composting, as in previous studies in the literature [81] [82]. This is an advantage of spent coffee ground compost with high mineral elements.

3.5. Phytotoxicity

The seed germination index (GI) has been defined as a factor of relative seed

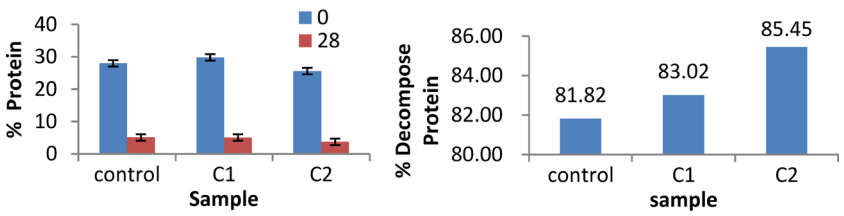

Figure 9. % Protein (a) and % Decompose Protein during Composting (b).

Table 4. Mineral element material and SCG compost.

Sample	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)
Initial material				
Control	2.71 ± 0.073	190.82 ± 0.04	9.59 ± 0.13	2.93 ± 0.05
C1	3.28 ± 0.014	190.76 ± 0.09	9.71 ± 0.09	2.53 ± 0.04
C2	3.25 ± 0.020	189.22 ± 0.04	9.95 ± 0.1	5.22 ± 0.064
Compost				
Control	2.42 ± 0.011	191.6 ± 0.011	12.36 ± 0.27	7.83 ± 0.59
C1	5.64 ± 0.065	192.5 ± 0.091	21.18 ± 0.025	9.537 ± 0.045
C2	3.29 ± 0.027	212.71 ± 0.024	40.28 ± 0.07	8.07 ± 0.005

Data are expressed as mean ± SEM (standard error of the mean) of three replicates.

Table 5. This is data of SG, RE, and GI of compost.

Sample	Seed Germination (SG) (%)	Root Elongation (RE)	Germination Index (GI) (%)
Control	90 ± 0.000	102.66 ± 0.049	92.39 ± 0.028
C1	95 ± 0.007	193.56 ± 0.098	183.88 ± 0.09
C2	100 ± 0.000	191.86 ± 1.42	191.86 ± 0.78

Data are expressed as mean ± SEM (standard error of the mean) of three replicates.

germination and relative root elongation [83]. It was reported that immature and unstable composts cause phytotoxicity affecting seed germination and root growth and thus result in lower seed GIs [84] [85]. Previous research efforts show that a GI value of 80% indicates maturity of the compost and the absence of phytotoxicity [56] [86]. All of samples showed non-phytotoxic (>80%) on germination test using Radish seeds (**Table 5**).

The results obtained indicated that all composts have matured within one month. By comparison, it was far faster than the normal duration of traditional composting (6 months to 1 year), vermicomposting (3 - 6 months) and Takakura home composting method (3 months). From **Table 5**, Sample C2 with Germination Index 191.86% is the best compare with others. While C1 have germination index 183.88%. This indicates from this method; all compost can produce healthier compost compare with compost control.

4. FTIR Analysis

Infrared spectroscopy is based on interactions of infrared light with molecules or molecule groups. An infrared spectrum reflects the chemical composition of the sample like a chemical "fingerprint". Theoretical assignments of absorption bands to functional groups have been summarized by several authors (Smith, 1999; Socrates, 2001; Bosh *et al.*, 2002; Madejova, 2003). FTIR analysis results of the material with fungi starter after 1 month of composting can be seen in **Figure 10**.

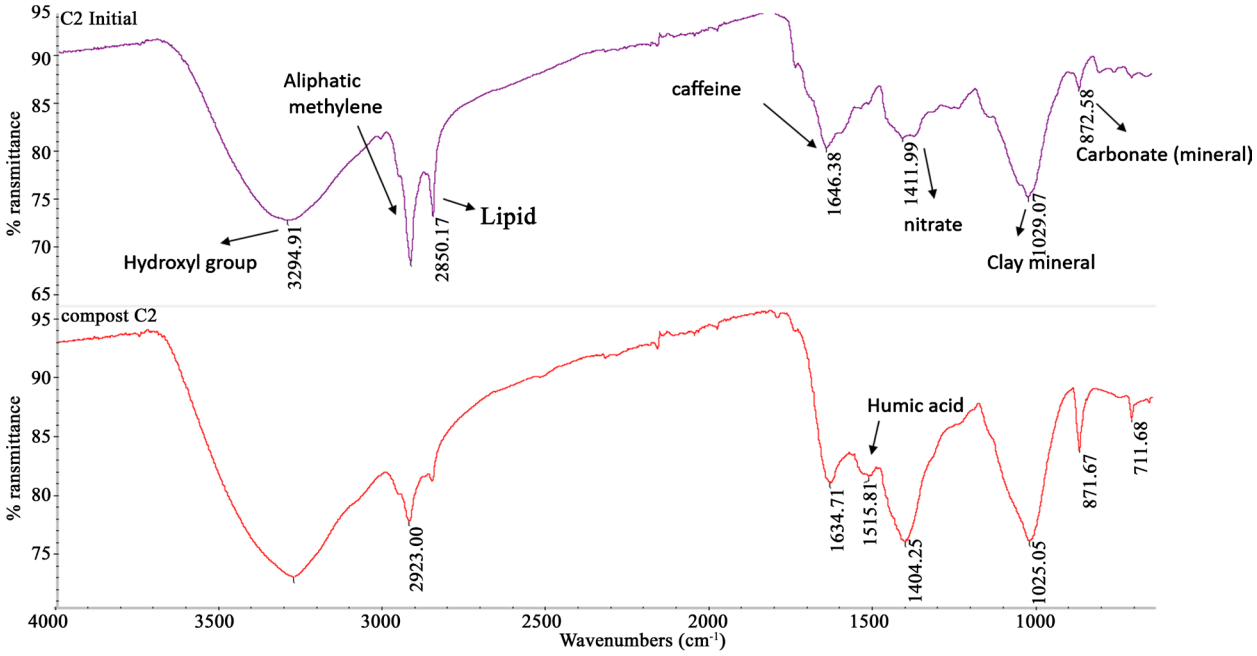

Figure 10. C2 initial and compost C2.

Compost control and C1 also have almost same functional group with C2 like **Figure 10** above. However only a little bit different concentration. The results of the analysis using FTIR were then compared with previous studies. In the spectra of compost the absorption bands at 2850 - 2892 cm^{-1} are attributed to aliphatic methylene groups and assigned to fats and lipids. Lipids are an important fraction of compost that can influence the water retention capacity of amended soils, their structural stability and the biodegradation-humification balance in soils [87]. All three samples not have resources in that range, which means all four samples are mature and stable. While all sample initial have wavenumber 2849.24.

The strong band at 1650 cm^{-1} can be assigned to amide I, carboxylates and C = C from aromatic and alkenes [88]. Of the four samples, C2 had slower peaks and lower wavenumber. This indicates that the aromatic structure is both lower than control and C1.

Components rich in proteins of compost can be identified by a strong band between 1570 and 1540 cm^{-1} [88]. From **Figure 9** above, all three samples, control, C1, C2, indicate reduced protein. Wavenumber 1515.57 at compost control is lignin. This is consistent with previous research by Kacurakova *et al.* (2002) [89], namely absorption bands in the region 1500 - 1600 cm^{-1} could be assigned to the aromatic rings of lignin. Peaks at 1510, 1460, 1420, 1270, 1230, 1130 cm^{-1} are typical for lignin [90] [91]. C1 and C2 not have this wavenumber, it is mean that compost C1 and C2 lignin degraded by microorganism.

In wavenumber 1384 - 1400 is a nitrate [88]. Sample C1 and C2 indicate the presence of a functional group N-O stretch which is nitrate. The intensity changes of bands at 1030 - 1040 testify the decomposition processes of organic compo-

nents and can be used to evaluate the composting processes [92]. In another reference mentioned, in the region 1080 - 1010 cm^{-1} is assigned to C-O stretching of polysaccharides or polysaccharide-like substances, Si-O of silicate impurities, and clay minerals possible in a complex with humic acids [93] [94].

5. Conclusion

Combination of some fungi and bacteria activators with temperature control in composting SCG can improve quality compost produced, with the physical characteristics of compost black and crumb, and normal pH. While the chemical characteristics of compost produced is a C/N ratio below 10 with and far difference from the control. Compost is also rich in minerals, such as phosphorus, potassium, calcium, and magnesium, as well as rich in humic acid as shown from the results of the FTIR analysis. Addition of a combination of activator fungus such as *Aspergillus sp*, and *Penicillium sp* can compete with commercial activators, likewise with the use of activator *lactobacillus sp.* This is also evidenced from the results of the phytotoxicity analysis, where the Germination Index of the compost sample with the addition of fungi activator (C2) is 191.86% greater than the commercial activator (C1) 183.88%.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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