The effectiveness of various compositions lignolytic and cellulolytic microbes in composting empty fruit bunch palm oil and sugar cane biomass

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Abstract. In sustainable agriculture, the balance of the use of inorganic fertilizers, organic matter and biofertilizers are highly recommended to increase crop production and at the same time maintaining or even improving the soil health and quality. Farm waste such as palm oil empty bunches and chopped sugar cane biomass are abundant and is a useful material to be returned to the soil to fertilize the plants. Constraints of both the material utilization is a very long process of decomposed because it contains lignin and cellulose and difficult to decomposed by microbes. Organic materials palm oil empty bunches and sugar cane biomass inoculated cellulolytic fungi, cellulolytic bacteria, lignolytic fungi, lignolytic bacteria consortia 1%, 2%, 3%, 4%, and without decomposers. The experiments were conducted in randomized block design (RBD) with four replications. Composting each 1 m³ of organic matter carried out aerobically for one month. Largest decrease in cellulose content of palm oil empty bunches and sugar cane biomass obtain on inoculation cellulolytic and lignolytic microbes that was 4% respectively to 64.92% and 61.12%, while the largest decrease in lignin content on palm oil empty bunches contained of 3% decomposer with cellulolytic and lignolytic microbes. While on sugar cane biomass obtained by 4% decomposers cellulolytic and lignolytic microbes. The addition of containing 2% cellulolytic and lignolytic most effective in decreasing the C/N either palm oil empty bunches or sugar cane biomass.

1. Introduction
One of the Green Revolution program launched by the Government of Indonesia in the early 1970s within the framework of self-sufficiency in rice is to optimize the means of production inputs, particularly inorganic fertilizer (chemical). Fertilizer plant construction has been carried out by the government at that time to support agricultural development. The government program has succeeded in making Indonesia the country achieve self-sufficiency in the year 1986. On the other hand, the success of agricultural development in Indonesia has encouraged the use of inorganic fertilizers/chemical massive and exaggerated at the farm level. At the end of the 1990s began to feel the negative impacts. The study results showed that 79% of productive land in Indonesia has been degraded due to excessive use of inorganic fertilizers which is one reason stagnation of agricultural productivity nationally.

In sustainable agriculture, the balance of the use of inorganic fertilizers, organic matter and biological fertilizers are highly recommended to increase crop production and at the same time maintaining or even improving the health and quality of soil. Organic materials such as compost that is...
created through a process of enzymatic decomposition by microbes has a number of favorable characteristics of the plants. Therefore, compost has been widely used in the sectors of food crops, horticulture and gardening. Including compost quality is determined by the composition of composted organic material. Besides the type of soil microbes involved in the composting process also determines the quality of the compost. Good compost is not only enriching nutrients in the soil, but also improve the physical, chemical, and biological soil [1].

Palm oil is the second most traded vegetable oil crop in the world, after soy, and over 90% of the world’s palm oil exports are produced in Malaysia and Indonesia. The development of the oil palm industry in Indonesia and Malaysia has brought economic benefits to both these countries. However it has also generated considerable environmental and social costs. Empty fruit bunch are accumulated, where the empty fruit bunch represents about 9 %. The empty fruit bunch is a lignocellulolytic material which typically contains 25% lignin, 50% cellulose and 25% hemicellulose in their cell wall. In the past years, empty fruit bunch was being used as fuel to generate steam in the palm oil industries [2]. Indonesia harvests about 400,000 ha of cane for centrifugal sugar, of which almost three-quarters is on Java. Most of the remainder comes from Sumatra, Kalimantan and Sulawesi. While a decade ago more than half of Java’s cane was irrigated, this area has declined reflecting a shift to the cultivation of more profitable crops. About 70 percent of the sugarcane areas are cultivated by farmers. The remainder is cultivated on sugar factory plantations, both in Java as well as on other islands where the dominant form of sugarcane cultivation is plantation-style. The processing of sugar cane produced fiber of sugarcane that can still be used as an ingredient organic matter to be returned to the soil

Organic matter as compost material generally consists of plant material (plant residues) and animal waste. Thus, soil organic matter as a source of energy and nutrients the microbes have physical and chemical properties vary. In general, organic components derived from plants composed of cellulose (15-60% dry weight), hemicellulose (10-30%), lignin (5-30%), the fraction that is soluble in water include simple sugars, amino acids, and aliphatic acid (5-30%), the fraction that is soluble in ether and alcohol such as fats, oils, waxes, resins and pigments, proteins (nitrogen and sulfur), and minerals (1-13%). The above composition varies depending on the age and type of plant [3].

The main role of soil microbes is destroying the organic material, a process that releases CO2. The whole of the above components decomposed by microbes at different speeds. Water-soluble material is decomposed so that in the soils can not be found in large numbers, but materials such as lignin or cellulose are difficult to decomposed[4] so that there are in a large numbers. Besides determined by the characteristics of the material, the decomposition rate is determined by the level of organic matter in the soil, cultivation, temperature, humidity, pH, depth, and aeration. Decomposition already occur at temperatures below freezing, and will be accelerated with increasing temperature. Humidity affects the respiration of soil and sufficient water for microbiological process [5].

The decomposting process of cellulose and lignin is a naturally by soil microbial consortium activity. The fungus responsible for the degradation of cellulose because it has an efficient cellulosytic enzyme systems and have different kinds of cellulosytic enzymes. Multicellular microbes more "superior" than unicellular microbes (bacteria). The existence of causing fungus mycelium can cover a surface more quickly than do bacteria. The mycelium is able to penetrate the substrate and grow in all directions, so as to mobilize and transporting products of polymer degradation at a relatively far distance.

2. Materials and Methods

Cellulosytic and lignolytic microbial isolates obtained from the isolation on palm oil empty fruit bunches. There were produced 12 microbes isolates. These isolates were tested for their potential of degrading lignin and cellulose in lignin and cellulose amended media.

Oil palm empty fruit bunches obtained from oil palm plantations in South Sumatra while sugar cane biomass obtained from Jatitujuh sugar cane plantation in West Java. Results of a preliminary analysis of the content of cellulose, lignin, and C/N ratio of palm oil empty bunches was 72.6%, 3%, and 79; whereas sugar cane biomass were 65.6%, 3.1%, and 63 respectively.
The composition of the consortium cellulolytic bacteria and fungi, lignolityc bacteria and fungi at concentration 1%, 2%, 3%, 4%, and without decomposers. Composting is done aerobically and incubated for 1 month. At the end of incubation were analyzed contains of cellulose and lignin, and C/N ratio compost oil palm empty bunches and sugar cane biomass. Cellulose and lignin content were determined according to [6]. All experiments were conducted in randomized block design (RBD) with four replications. The data were subjected to analysis of variance (ANOVA) and tested for significance using Duncan test.

**Figure 1.** Orange and green lignolytic fungi isolates on lignolytic media after 3 days of incubation

3. 3. Results and Discussion

3.1. The content of cellulose in decomposed palm oil empty bunches and sugar cane biomass

Cellulose content of oil palm empty bunches and sugar cane biomass decreased after being decomposers and incubated for one month compared with the cellulose content of the raw material. Decrease in cellulose content vary affected by the given concentration of cellulolytic microbial and lignolytic consortium (Table 1). A decrease in the provision due to the cellulose content by cellulolytic and lignolytic decomposers seen very clearly in the raw material of oil palm empty bunches compared to sugar cane biomass. Giving 4 % cellulolytic and lignolytic microbial consortium appear produce the ultimate reduction in cellulose content in palm oil empty bunches. While on sugar cane biomass application of 4% cellulolytic and lignolityc microbial consortium in sugar cane biomass led to a high drop, but the effect is not different from application of 2 % and 3% consortium.

| Concentration Microbes Consortia | Cellulose Content (%) |          |          |
|----------------------------------|-----------------------|----------|----------|
|                                  | Palm Oil Empty Bunches | Sugar Cane Biomass |
| A= 1 % cellulolytic and lignolytic microbes consortia | 66.62 bc | 62.92 a |
| B= 2 % cellulolytic and lignolytic microbes consortia | 67.15 b | 61.20 b |
| C= 3 % cellulolytic and lignolytic microbes consortia | 67.35 b | 62.22 ab |
| D= 4 % cellulolytic and lignolytic microbes consortia | 64.92 c | 61.12 b |
| E= Without cellulolytic and lignolytic microbes consortia (control) | 70.65 a | 63.07 a |

Different letters in a column indicate significant difference at P ≤ 0.05 by Duncan Test

Cellulose is the largest part of the lignocellulose components of plants and is a basic component of plant cell walls as a constituent of the main structure of cells [4]. According to [7], the cellulose is always bonded to other polysaccharides, such as hemicellulose, pectin and lignin. The basic structure of cellulose is a carbohydrate polymer composed of glucose and 8000-12000 units linked by bonds β-
1,4-glycosidic molecular weight of 50,000 - 1000,000. A high level of plant cellulose content are not fixed, but depends on the age and type of plant. Palm oil empty bunches have a greater cellulose than sugar cane biomass (Table 1), so that cellulytic microbes will get a supply of more nutrients while they decompose palm oil empty bunches. Consequently cellulytic microbes work on palm oil empty bunches will be active and decreased cellulose better than sugar cane biomass. Besides, cellulose overhaul work will be accelerated by working cellulytic microbial consortium. According to [8], in the process of degradation of cellulose, the association of β-1,4-glycosidic the cellulose is broken down into glucose monomers chemically (acid hydrolysis) and biology (cellulase enzyme hydrolysis) is used as an energy and carbon source for a number of organisms. Cellulytic microbes that play a role in biological degradation produce cellulase enzymes produced by fungi, bacteria, actinomycetes. In cellulose degradation by fungi, selulolisis start the process, but when fungi begin to form colonies in materials containing cellulose, hemicellulose or lignocellulose, fungi always quickly joined by other microorganisms, mainly bacteria.

3.2. The content of lignin in decomposed palm oil empty bunches and sugar cane biomass
Lignin is a constituent material of plants tissue which are difficult destroyed by microbes. Only certain microbes that produce enzyme lignolytic which can degrade lignin. Lignin content in the ingredients palm oil empty bunches and sugar cane biomass ranging from 3 to 3.5% decreased significantly during the decomposition process by the activity of cellulytic and lignolytic microbial consortium with the addition of 1% to 5%. Whereas the sugar cane biomass, the reduction of lignin in the sugar cane biomass effectively is due to the addition of 3% and 4% cellulytic and lignolytic microbial consortium. At a concentration of cellulytic and lignolytic microbial consortium 3% in palm oil empty bunches showed a high decrease of lignin, while the sugar cane biomass required concentration of 4% cellulytic and lignolytic microbial consortium to produce a high reduction in lignin content.

Table 2. Lignin content on decomposition of palm oil empty bunches and sugar cane biomass after one month incubation with various concentrations of cellulytic and lignolytic microbes consortia

| Concentration Microbes Consortia | Lignin Content (%) | Palm Oil Empty Bunches | Sugar Cane Biomass |
|----------------------------------|--------------------|------------------------|-------------------|
| A= 1 % cellulytic and lignolytic microbes consortia | 1.85 b | 2.42 a |
| B= 2 % cellulytic and lignolytic microbes consortia | 1.97 b | 2.27 a |
| C= 3 % cellulytic and lignolytic microbes consortia | 1.07 c | 1.72 b |
| D= 4 % cellulytic and lignolytic microbes consortia | 1.27 c | 1.27 c |
| E= Without cellulytic and lignolytic microbes consortia (control) | 1.35 a | 1.67 a |

Different letters in a column indicate significant difference at P ≤ 0.05 by Duncan Test

According to [4], lignin is aromatic compounds associated with cellulose and hemicellulose are difficult to hydrolyze. Lignin contained in the cell walls of vascular plants, the second layer cell wall or sometimes in the middle lamella. [9] states that the middle lamella area containing 70-80% lignin (by weight) and help glue all wood cells become one. [4] told that the lignin molecules consist of three elements, namely carbon (C), hydrogen (H) and oxygen (O), but has a structure which is aromatic. Unit structure of lignin is phenyl propane joined together by a strong bond ether (-COC-) or via a CC bond [9].

Microbes especially fungi play an important role in degrading lignin because it has enzymes lignolitik which is a very important role in the degradation of lignin include phenolic okidase (laccase) and peroxidases (lignin peroxidase / LiP) and manganese peroxidase (MnP) [10, 11]. Cellulytic
microbial consortium and lignolitik given on empty Bunches of palm oil and sugar cane biomass produces synergistic cooperation in reducing the lignin content of empty Bunches of palm oil and sugar cane biomass.

3.3. C/N ratio in decomposed palm oil empty bunches and sugar cane biomass

Carbon and nitrogen are the most important elements in biodegradation process as one or the other is normally a limiting factor [12]. C/N ratio is a reliable indicator and used as an index of compost maturity [13]. The content of the C/N by a consortium of decomposers after composting for four weeks decreased compared with no addition of decomposers and before decomposition. Addition of 2% cellulolytic and lignoliticyc microbial consortium produce a decrease in C/N ratio is high on sugar cane biomass although the effect did not differ significantly with the addition of 3% of the consortium. While the oil palm empty Bunches of 1 and 2% cellulolytic and lignoliticyc microbial consortium produce a decrease in C/N ratio tangible than other treatments. Addition of 2% cellulolytic and lignoliticyc microbial consortium had the higher ability to decompose oil palm empty bunches based on the C/N ratio, and exhibited the best potential for rapid composting.

Table 3. C/N ratio on decomposition of palm oil empty bunches and sugar cane biomass after one month incubation with various concentrations of cellulolytic and lignolytic microbes consortia.

| Concentration Microbes Consortia | Palm Oil Empty Bunches | Sugar Cane Biomass |
|----------------------------------|------------------------|-------------------|
| A= 1 % cellulolytic and lignolytic microbes consortia | 47.0 b | 41.5 a |
| B= 2 % cellulolytic and lignolytic microbes consortia | 46.0 b | 23.7 c |
| C= 3 % cellulolytic and lignolytic microbes consortia | 58.5 a | 27.7 c |
| D= 4 % cellulolytic and lignolytic microbes consortia | 50.2 ab | 35.0 b |
| E= Without cellulolytic and lignolytic microbes consortia (control) | 59.5 a | 47.2 a |

Different letters in a column indicate significant difference at P ≤ 0.05 by Duncan Test

Decrease in C/N ratio palm oil empty bunches (55.68%) due to the activity of microbial decomposers cellulolytic and lignolytic was greater than the reduction in C/N ratio in sugar cane biomass (48.94%). The initial content of the C/N palm oil empty bunches is higher than sugar cane biomass, a source of carbon and energy for heterotrophic microbial decomposers. The elements carbon (C) on palm oil empty bunches dominated by cellulose content is higher than sugar cane biomass, contrary to the content of lignin. While cellulose is the building blocks of plant, more easily degraded by microbes than lignin decomposers.

4. Conclusion

The largest decrease in cellulose content of palm oil empty bunches and sugar cane biomass obtained on inoculation 4% cellulolytic and lignolytic microbes consortia that is 64.92% and 61.12% respectively. Celulolytic and lignolytic microbes consortia contained of 3% have a largest decrease in lignin content on palm oil empty bunches, while on sugar cane biomass obtained by 4% decomposers consortia. The addition of 2% cellulolytic and lignolytic microbes consortia most effective in decreasing the C/N ratio either palm oil empty bunches or sugar cane biomass.

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