AgroLife Scientific Journal - Volume 2, Number 2, 2013
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

BIODEGRADATION WASTE OIL PALM EMPTY BUNCH (*Elaeis guineensis* Jacq.) BY LIGNOCELLULOLYTIC FUNGI

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Abstract

Research has been conducted to determine the effectiveness of lignocellulolytic fungi biodegradation, either single or consortium, as well as the effective dose of inoculum in the process of biodegradation of waste oil palm empty fruit bunches. This research used experimental methods to Completely Randomized Design (CRD) 4 x 3 factorial in three replications. The first factor were species of fungi, Rhizopus oryzae (j1), Penicillium citrinum (j2), Aspergillus nidulans (j3) and consortium of Rhizopus oryzae, Penicillium citrinum and Aspergillus nidulans (j4). Second factor was inoculum dosage (D), 0% (d0), 5% (d1) and 10% (d2). The results showed that Rhizopus oryzae, Penicillium citrinum and Aspergillus nidulans singly gave the result by decreasing 35.78%, 27.02% and 21.60% C/N ratio, while a consortium of Rhizopus oryzae, Penicillium citrinum and Aspergillus nidulans gave the result by decreasing 23.40% C/N ratio. Decrease in C/N ratio suggests that Rhizopus oryzae and Penicillium citrinum either single or consortium is more effectiveused in the process of palm oil empty fruit bunches waste biodegradation. In addition, 5% was the most effective inoculums dosage during the biodegradation process by decreasing 33.73% C/N ratio 30 days.

***Key words**: biodegradation, palm empty fruit bunches waste, Rhizopus oryzae, Penicillium citrinum, Aspergillus nidulans, C/N ratio.*

INTRODUCTION

Lignocelulolytic fungus is one of a group of microorganisms that have an important role in the process of biodegradation of lignocellulose components. The ability of fungi to degrade lignocellulosic materials lignoselulolitik due to its lignocelulolitik enzyme system, so it can degrade lignocellulosic materials into more simple compounds (Zabel and Morell, 1992). Fungus of the class Basidiomycetes are the main group of degrading lignocellulose (Wymelenberg, 2006). Lignocellulose is an organic material which is abundant in nature and consists of three types of polymers, namely cellulose, hemicellulose and lignin. Empty fruit bunches of oil palm is a type of solid waste with lignocellulosic as the largest component which is difficult to degrade. Oil palm empty fruit bunches (TKKS) consists of cellulose (40-60%), hemicellulose (20-30%) and lignin (15-30%) (Dekker, 1991 in Wardani, 2012).

In 2005, oil palm empty fruit bunches generated by the palm oil industry is estimated at 9.9 million tonne. Utilization of oil palm empty fruit bunches at the moment is still very limited and almost no economic value. During the oil palm empty fruit bunch burned in incinerator for ashes and taken as a potassium fertilizer. This way will be banned in the future because it raises the problem of pollution, in addition to the high cost of treatment incinerator. Darnoko et al. (1993) explains that the oil palm empty fruit bunches have a C/N ratio is high, ie > 45, if the material is added directly to the soil, N in the soil will be reduced due to mobilize on the reform process of organic matter by soil microorganisms). One alternative in the treatment of oil palm empty fruit bunches are biodegradable solid waste into compost bioactive oil palm empty fruit bunches. The application of composting systems palm empty fruit bunches has technical and economic opportunities are sufficient to control the organic solid waste and increase the carrying capacity of oil palm plantations (Goenadi, 1997). According to Rao (1994), the biological activities such as root growth and microbial metabolism in soil plays a role in shaping the texture and fertility of the soil. One of the *lignocellulolytic* fungi that can help the process of soil formation is *Trichoderma* sp. Suhartatik et al. (1998) explained that the inoculation of *Trichoderma* sp. in the biodegradation process of rice straw, can reduce the ratio of C/N to C/N less than 20 in the fourth week. Based on the above, it is important to do research on the ability of the fungus lignoselulolitik either single or consortium in the biodegradation process waste oil palm empty fruit bunches. The purpose of this study is to obtain stren lignoselulolitik fungus that can decompose lignocellulosic oil palm empty fruit bunches and get a dose of inoculum that can effectively and quickly lower the C/N ratio of waste oil palm empty fruit bunches.

MATERIALS AND METHODS

Equipment and Materials

Apparatus and materials used include kjeldahl, flask, micropipette, microscope phosphoric acid (H_3PO_4) acid, sulfuric acid (H_2SO_4) acid, distilled water, CMC (Carboxy Methyl Cellulose), CuSO4, diphenynilamine, phenol sulfate 1N, 1N $FeSO_4$, standard HCl, potassium dichromate ($K_2Cr_2O_7$) 1N, $KMnO_4$, lignocellulolytic fungal cultures isolated from oil palm empty fruit bunches, lignin, fermentation medium include: waste oil palm empty fruit bunches (TKKS); starter medium include: rice, soy flour, flour oil palm empty fruit bunches flour and corn cobs; methyl red, 10% NaOH, and Potato Dextrose Agar (PDA) of OXOID®.

The method used in this research are descriptive and experimental. This study is divided into three stages, namely stage isolation, selection and identification; manufacturing phase inoculum (starter); stages of biodegradation of waste oil palm empty fruit bunches by using solid fermentation. Experimental methods used in the third stage, the stage of the biodegradation of waste oil palm empty fruit bunches by solid fermentation process with completely randomized design (CRD) 4 x 3 factorial pattern, which consists of three factors. The first factor is a type of fungus (J), the fungus *Rhizopus oryzae* (j1), the fungus *Penicillium citrinum* (j2), the fungus *Aspergillus nidulans* (j3) and a consortium of *Rhizopus oryzae* fungus, *Penicillium citrinum* and *Aspergillus nidulans* (j4). Factor II was inoculum dose (D), ie 0% inoculum dose (d0), 5% (d1) and 10% (d2). The main parameters measured include the number of fungi (Total Plate Count Method) and the measurement of the ratio of C/N (total carbon content by the Walkley and Black method, total nitrogen content by Kjeldahl method. The data obtained were statistically tested using analysis of variance test (ANOVA). Apabila berbeda nyata maka dilanjutkan dengan uji jarak berganda Duncan dengan taraf nyata 5% (Gomez dan Gomez, 1995).

If there are differences, then continue with Duncan's multiple range test with a significance level of 5%.

RESULTS AND DISCUSSIONS

Lignocellulolytic fungi isolated from Waste Oil Palm Empty Fruit Bunch

Three fungal isolates were selected because they have the highest index of lignolitik and cellulolytic which would then be used in the process of biodegradation of waste oil palm empty fruit bunches. Identification is done macroscopically and microscopically. Identification results, known as *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans.*

Growth of *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans* during Biodegradation Process Waste Oil Palm Empty Fruit Bunch

Fermentation is an activity of various microbial action under controlled conditions. Presence of the metabolic activity of microorganisms in the end will lead to a change on the substrate.

The changes that occur during the process of fermentation include physical and chemical changes (Buckle et al., 1987 in Darmawan, 2008). Fungal colony populations during biodegradation of waste oil palm empty fruit bunches were statistically analyzed using analysis of variants (ANOVA).

ANOVA results showed that there were interaction between type of fungus and inoculum dose factor in provide population of fungus colonies during the empty bunch biodegradation process.

The Duncan Multiple Range Tes at 5% significant level has been made to determine the numbers between colonies fungus population (Table 1).

It was known that the population of the fungus inoculum dose 10% of the population fungal equal to inoculum dose of 5%. This indicated that the inoculum dose 5% was as effective as inoculum dose of 10%. While the fungus *Penicillium citrinum* and *Aspergillus nidulans* both single and consortium produced by the same population. This indicated that the two species of fungi are synergies for growth and biodegradation.

C/N ratio during the process of Biodegradation of Waste Oil Palm Empty Fruit Bunch

There is a species of fungus interaction factor and inoculum dose produces a decrease in the C/N ratio of waste oil palm empty fruit bunches. I ANOVA results were analyzed by Duncan's Multiple Range test at 5% significance level.

Duncan test results (Table 2), shows that the biodegradation by *Rhizopus oryzae* with a 5% inoculum dose, *Penicillium citrinum* inoculum dose of 5% and a consortium of *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans* inoculum dose of 10% can reduce larger percentage of the ratio C/N.

However, biodegradation by *Rhizopus oryzae* and *Penicillium citrinum* singly with 5% inoculum dose of its equally effective to reduce the ratio C/N in the waste oil palm empty fruit bunches by a consortium of *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans* inoculum dose of 10%.

Decrease in C/N ratio due to carbon compounds in the fermentation substrate or compost is used as an energy source by decomposer microorganisms and subsequently released into the air in the form of CO_2. This resulted in the organic carbon content in the substrate continues to decline. According to Sa'id (1996), composting depends on the activity of microorganisms that require carbon as a source of energy and cell formation, as well as with nitrogen for the formation of cell protein. Graph C/N ratio of oil palm empty fruit bunches at the beginning and end of biodegradation can be seen in Figure 1. C/N ratio of waste oil palm empty fruit bunches at the start of biodegradation is at 99.06 (Figure 1).

Tabel 1. Duncan's multiple range test factor interactions fungi type and dose of inoculum on the number of fungi for biodegradation process waste oil palm empty fruit bunch

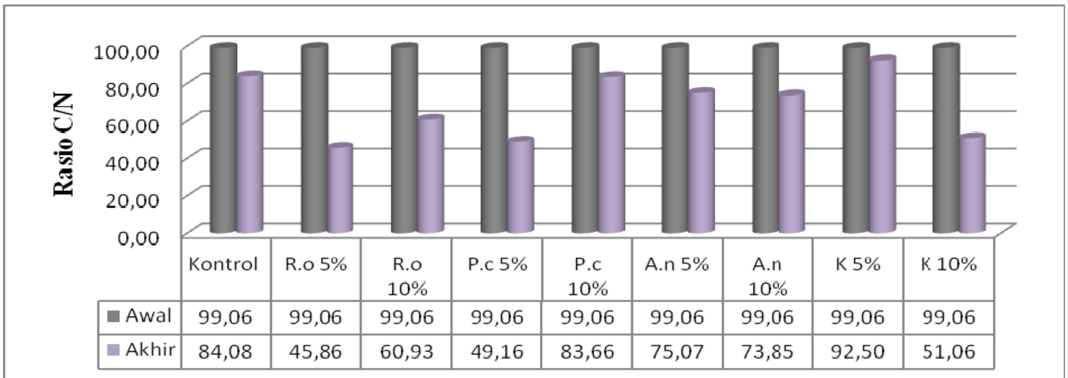
Type of Fungus	Inoculum Dose (D)		
	(d_1) 0%	(d_2) 5%	(d_3) 10%
Rhizopus oryzae	4.72×10^7 **a**	6.79×10^7 **a**	11.76×10^7 **b**
Penicillium citrinum	4.72×10^7 **a**	16.78×10^7 **c**	24.29×10^7 **e**
Aspergillus nidulans	4.72×10^7 **a**	18.62×10^7 **cd**	22.17×10^7 **de**
Consortium of *R. oryzae, P. citrinum* and *A. nidulans*	4.72×10^7 **a**	22.53×10^7 **de**	27.33×10^7 **e**

Note: small letter read all directions and the small letter indicates no different at 5% level

Tabel 2. Duncan's multiple range test factor interactions fungus type and dose of inoculum to the decrease in c/n ratio waste oil palm empty fruit bunch after biodegradation process

Type of fungus and inoculum dose	Percentage of reduction of C/N ratio (%)
Control	15.13 **abc**
Rhizopus oryzae 5%	53.71 **d**
Rhizopus oryzae 10%	38.50 **bcd**
Penicillium citrinum 5%	50.37 **d**
Penicillium citrinum 10%	15.55 **ab**
Aspergillus nidulans 5%	24.22 **abc**
Aspergillus nidulans 10%	25.45 **abc**
Consortium of *R. oryzae, P. citrinum* and *A. nidulans* 5%	6.62 **a**
Consortium of *R. oryzae, P. citrinum* and *A. nidulans* 10%	48.46 **cd**

Note: small letter read all directions and the small letter indicates no different at 5% level



Percentage of reduction of C/N ratio (%) (%)	**15.13**	**53.71**	**38.50**	**50.37**	**15.55**	**24.22**	**25.45**	**6.62**	**48.46**

Percentage of reduction
R.o : Rhizopus oryzae P.c : Penicillium citrinum A.s : Aspergillus nidulans
K : Konsorsium R. oryzae, P. citrinum* dan *A. nidulans

Figure 1. Graph C/N ratio of oil palm empty fruit bunches at the beginning and end of biodegradation

C/N ratio of waste oil palm empty fruit bunches at the start of biodegradation is at 99.06 (Figure 1). Biodegradation by species of *Rhizopus oryzae* and *Penicillium citrinum* singly with a dose of 5% and a consortium inoculum *Rhizopus oryzae*, *Penicillium citrinum* and *Aspergillus nidulans* 10% inoculum dose affecting the percentage decrease in C/N ratio compared to other treatments, i.e. each - amounting to 53.71%, 50.37%, and 48.46%. It shows that *Rhizopus oryzae* and *Penicillium citrinum* either single or consortium has a high ability to degrade waste oil palm empty fruit bunches. C/N ratio of waste oil palm empty fruit bunches at the start of biodegradation is at 99.06 (Figure 2). Biodegradation by species of *Rhizopus oryzae* and *Penicillium citrinum* singly with a dose of 5% and a consortium inoculum *Rhizopus oryzae*, *Penicillium citrinum* and *Aspergillus nidulans* 10% inoculum dose. Increased the percentage decrease in C/N ratio compared to other treatments, i.e. each - amounting to 53.71%, 50.37%, and 48.46%. It shows that *Rhizopus oryzae* and *Penicillium citrinum* either single or consortium has a high ability to degrade waste oil palm empty fruit bunches.

According Karmakar and Ray (2011) *Rhizopus oryzae* produce extracellular enzymes endo - glucanase ith high activity for bioconversion of various agricultural waste such as orange peel, sugar cane bagasse, coconut shells and dried flowers. Saccharification percentage is very high, especially in the orange peel and sugarcane bagasse. *Rhizopus oryzae* is also known to produce extra cellular endoglucanase with relatively high activity when nurtured in both liquid and solid fermentation with CMC as the sole carbon source. As we know that a cellulase enzyme complex consisting of extracellular endo - β-1.4 - glukonase (CMCase, Cx cellulase endoselulase, or carboxymethyl cellulase), complex exo - β-1.4 - glukonase (aviselase, selobiohidrolase, C1 cellulase), and β-1.4 - glucosidase or selobiase. Endoglucanase (EC3.2.1.4) is a beta cellulase cuts randomly and generally in the amorphous parts. *Penicillium citrinum* isolated from soil also produces cellulase alkali tolerant and resistant to high temperature (Duta et al., 2008). *Penicillium citrinum* is one of the species of fungi that has the ability to degrade cellulose Based Kuhad research and Singh (1993), *Penicillium citrinum* used in the fermentation process of rice husk to produce cellulase enzyme activity 37 unit/g maximum that can decompose cellulose content of 70 rice husk % within 12 days. Cellulase enzyme produced by *Penicillium citrinum* is thermostable. *Penicillium citrinum* is a fungus that can produce endoglucanase and Fpase.

Based on the research Dutta et al. (2008), *Penicillium citrinum* can produce 1.89 ± 0.12 IU/ml endoglucanase after 168 h and 1.72 ± 0.14 IU/ml FPase after 72 hours, with wheat bran is used as a substrate in the SSC (Solid Substrate Culture).

Penicillium citrinum can also secrete enzymes lignin peroxidase (LIP) that plays a role in the overhaul of lignocellulose components (Bhende and Dawande, 2010).

Rhizopus oryzae and *Penicillium citrinum* on 5% inoculum dose also has the ability to adapt better compared with 10% inoculum dose. This is due to the lower inoculum dose, the microbes does have a chance to gain more nutrients with a low level of antagonism.

Penicillium citrinum is a microorganism that has a relatively rapid growth, and has the ability to suppress other microorganisms (Cayanto, 2010).

CONCLUSIONS

Based on the research results, it was concluded as follows:

1. Lignocellulolytic fungus can be obtained from waste oil palm empty fruit bunches are *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans*.
2. *Rhizopus oryzae, Penicillium citrinum* and *Aspergillus nidulans* singly waste in the process of biodegradation of oil palm empty fruit bunches can lower the C/N ratio of each - amounting to 53.71%, 50.37% and 24.22%, while the three consortia to reduce the ratio of C/N of 48.46%.
3. Inoculum dose effective solid waste in the process of biodegradation of oil palm empty fruit bunches are 5% inoculum dose that can lower C/N ratio with a percentage of 33.73% for 30 days.

ACKNOWLEDGEMENTS

The author addressed to Director General of Higher Education through research programs and decentralization that has been funding this research.

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