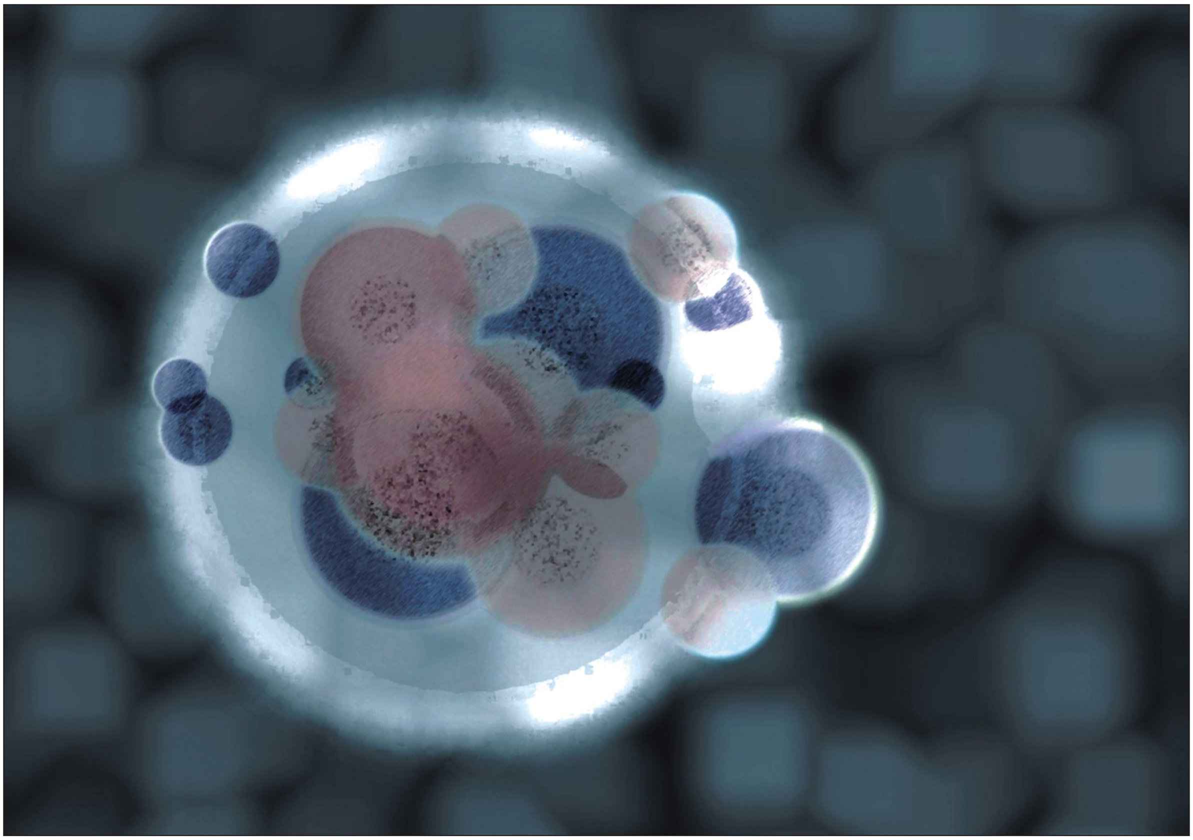

Journal of Biological Sciences

ISSN 1727-3048

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.21.27

Research Article

Making Organic Fertilizer Using Sludge from Biogas Production as Carrier Agent of *Trichoderma harzianum*

[1]Ambar Pertiwiningrum, [2]Edy Cahyo Budyanto, [3]Muslikhin Hidayat, [4]Rochijan, [1]Yudistira Soeherman and [1]Mochammad Fahmi Habibi

[1]Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3, Bulaksumur, 55281 Yogyakarta, Indonesia
[2]Vocational High School 1 Temanggung, Jalan Kadar Maron, P.O. Box 104, Sidorejo, Temanggung, Jawa Tengah, Indonesia
[3]Department of Chemical Engineering, Faculty of Engineering, Universitas Gadjah Mada, Jalan Grafika No. 2, Kampus UGM, 55281 Yogyakarta, Indonesia
[4]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3, Bulaksumur, 55281 Yogyakarta, Indonesia

Abstract

Background and Objective: The sludge from biogas production can be used as organic fertilizer and carrier agent of *Trichoderma harzianum* which functions as plant growth stimulator and bio-fungicide. This study aimed to determine the effect of different volumes of *Trichoderma harzianum* inoculum on the concentration of nutrients and organic fertilizer as carrier agent of *Trichoderma harzianum*. **Methodology:** Rice husk charcoal and *Trichoderma harzianum* inoculum in broken corn were added to the sludge. This study applied four treatments with the addition of different volumes of inoculum and do repetitions of each treatment three times. Testing the ability of the mixture in storing *Trichoderma harzianum* tests 8 times once a week. Testing the ability of *Trichoderma harzianum* in decomposing organic materials until they are ready to use the test be repeated three times. The variables observed were the total volume of nitrogen, phosphorus, potassium and conidiophores. Data obtained were examined by ANOVA analysis and conducted the Duncan test if there are differences between treatments. **Results:** The result showed that sludge could not yet be used as organic fertilizer since the result was below 4. The addition of inoculum did not guarantee the higher amount of living conidiophores. On the contrary, the less inoculum was given the more conidia could survive especially in the 4th and 5th week. The mixture could keep *Trichoderma harzianum* optimally until the 6th week since it was incubated. The result of *in vivo* test to mustard green plant the result showed no significant difference. **Conclusion:** The conclusion of the study is the increase in concentration nutrient (N, P and K) and the formula which had the highest average value was T3 (with the addition of 112.5 g of *Trichoderma harzianum*). Every time a number of conidia/conidiophores in each treatment was measured, the results fluctuated. *In vivo* test showed no significant result from all given formulas.

Key words: Organic fertilizer, sludge, biogas production, biofungicide, *Trichoderma harzianum*

Received: November 01, 2016 Accepted: December 01, 2016 Published: December 15, 2016

Citation: Ambar Pertiwiningrum, Edy Cahyo Budyanto, Muslikhin Hidayat, Rochijan, Yudistira Soeherman and Mochammad Fahmi Habibi, 2017. Making organic fertilizer using sludge from biogas production as carrier agent of *Trichoderma harzianum*. J. Biol. Sci., 17: 21-27.

Corresponding Author: Ambar Pertiwiningrum, Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3, Bulaksumur, 55281 Yogyakarta, Indonesia Tel: +628112544699

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Half of the world's population-up to 95% in poor countries-rely on solid fuels, including coal and biomass fuels (e.g., wood, dung/faeces, agricultural residues/agricultural waste), to meet their energy needs[1]. The development of modern human society has relied heavily on fossil fuel. Large quantities of fossil fuels have been consumed and harmful emissions are being released into the environment by automobiles, especially in recent decades[2]. To solve the energetic and environmental dilemmas, the application of biomass-based fuels in automobiles is receiving increasing public and scientific attention[3]. As alternative fuels, biomass-based fuels, commonly known as biofuels (including biogas) have many advantages over fossil fuel[4].

The benefit of biogas is already well known in the community. Biogas is methane gas produced by anaerobic fermentation of organic materials. Biogas is made up of 50-70% CH_4, 30-50% CO_2 and some notable impurities such as NH_3, H_2S, siloxane and halides[5,6]. The concentration of each of these compounds depends on the composition of the raw materials and the process conditions used during the digestion[7]. Besides the methane that can be used as fuel, biogas plant also produces solid and liquid mixtures that come out of the digester's drain hole. According to Sutaryo *et al.*[8], methane production was strongly inhibited by increased levels of NH_3. Total VFA, isobutyric and isovaleric acid concentrations accumulated during ammonia inhibition, thus these parameters and the methane yield were useful indicators of ammonia inhibition.

The by-product of biogas plant that looks like mud, known as sludge, contains many nutrients. It can be used as fertilizer for plants[9]. The quality of residual sludge from biogas production process is better than the manure obtained directly from the cattle cage. It is because anaerobic digestion of organic material occurs in the fermentation process in the digester. It results in the increasing concentration of nitrogen, phosphorus and potassium. This condition makes the sludge ready to be used as organic fertilizer and it can be separated into the solid and the liquid one[10].

Different microbial systems participate in transforming organic residues in a number of reactions into biogas under anaerobic conditions, these include; hydrolysis-acidogenesis, acetogenesis and methanogenesis[11,12]. The sludge from biogas production can also be used as carrier agent of *Trichoderma harzianum*. According to Soenandar and Tjachjono[13], the genus *Trichoderma* serves as biological agent and plant growth stimulator. The fungal culture of *Trichoderma* sp., acts as bio-fungicide which can inhibit the growth of some fungi, such as *Rigidiforus lignosus*, *Fusarium oxysporum*, *Rizoconia solani* and *Sclerotium rolfsii*, that cause plant diseases. The culture of *Trichoderma* can also serve as bio-decomposer which can convert organic wastes into compost.

Trichoderma sp., is useful to prevent root diseases and stem rot that causes the wilting of plants. A study conducted by Umrah *et al.*[14] showed that *Trichoderma* sp., has the strongest antagonistic potential against *P. palmivora in vitro*. Biofungicide products are usually formulated in solid form. Formulations are made by adding the conidia of *Trichoderma* sp., into the carrier medium. *Trichoderma* sp., will have a dormant period if the environmental condition is dry and has a neutral pH (6-7). To that end, the carrier media that is usually used is kaolin, also called China clay, which has neutral pH (7) and is conditioned in a dry state.

The purpose of this study was to determine the effect of different doses of *Trichoderma harzianum* to increase the nutrient content (nitrogen, phosphorus and potassium) and the development of conidia (conidiophore) in the sludge from biogas production process with the addition of rice husk charcoal as the organic fertilizer that acts as the carrier of *Trichoderma harzianum*.

MATERIALS AND METHODS

Research materials: The ingredient used in this study was the dark brown solid sludge as by-product of biogas plant which has a pH of 7, total nitrogen concentration of 1.28%, total phosphorus concentration of 0.16% and total potassium concentration of 0.33%; rice husk charcoal with a pH of 7; inoculum of *Trichoderma harzianum* in broken green corn as the result of the fungal culture made in the Pests and Diseases Observation Laboratory, Temanggung, Central Java. The sampling method in this study used probability sampling which gave equal opportunities to each member of the population to be elected as part of the sample.

Research variables: The independent variable in this study was the composition of *Trichoderma hazianum* inoculum in broken corn with the concentration of 37.5, 75 and 112.5 g which each was added to the mixture of sludge and rice husk charcoal (300 g) to determine the increase of nutrient content (nitrogen, phosphorus and potassium) in the mixture of sludge and rice husk charcoal as fertilizer. Another variable was a number of conidia in each treatment and every testing time. The experiment also measured the ability of *Trichoderma harzianum* isolate in decomposing organic matters in the mixture of sludge and rice husk charcoal a planting medium.

Design of experiments: This study used an experimental method with a completely randomized design with single factor and three repetitions. The factor used was the concentration of *Trichoderma harzianum* isolate in broken corn (T).

T0 = Control sludge and rice husk charcoal
T1 = Sludge and rice husk charcoal plus 37.5 g of *Trichoderma harzianum*
T2 = Sludge and rice husk charcoal plus 75 g of *Trichoderma harzianum*
T3 = Sludge and rice husk charcoal plus 112.5 g of *Trichoderma harzianum*

In this study, done the addition of two control on a test *in vivo*, namely the mixture of *Trichoderma harzianum* in kaolin (Tk) and organic fertilizer from manure (POr). The figures after treatment code indicated the repetition of the above treatment.

Preparation of materials: The preparation of materials started by taking sludge from the biogas outlet as the main raw material of fertilizer. After that conducted the reduction of water content in the sludge by wind drying it until it reached a certain degree of moisture. While, waiting for the sludge to dry, then prepared water, sugar and the inoculum of *Trichoderma harzianum* in broken corn obtained from Pests and Diseases Observation Laboratory, Temanggung, Central Java. The unification process of the carrier and *Trichoderma harzianum* inoculum was shown in Fig. 1.

Method of data analysis: Data analysis in this research uses Completely Randomized Design (CRD) statistical analysis and SPSS software to determine the optimal composition of the mixture of *Trichoderma harzianum* inoculum, the sludge from biogas manufacture and the rice husk charcoal. This study also used Duncan test to examine the differences between the results.

Laboratory analysis: Laboratory analysis included the measurements of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) concentration. The determination of the N concentration uses the Kjeldahl semi-micro test, whereas mineral test was used to examine P_2O_5 and K concentration. In addition to testing the content of N, P and K, also done a measurement of the amount of *Trichoderma harzianum* colonies using serial dilutions. The next conducted the calculation of the conidia/conidiophores on haemocytometer tool under the microscope with

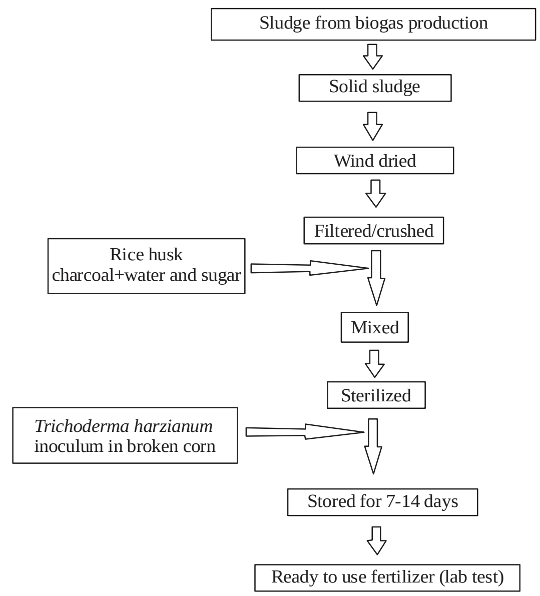

Fig. 1: Unification process of the carrier and *Trichoderma harzianum* inoculums

200 times of magnification, 8 times of observation that was done once every 7 days and started after 14 days of the unification of agent and inoculum.

Interpretation of laboratory results data: The research applied CRD with four treatments, which were the one without the addition of *Trichoderma harzianum* inoculum, the one with 37.5 g of inoculum, the one with 75 g of inoculum and the one with 112.5 g of inoculum. On each treatment is replicated three times. The variables measured were the total nitrogen, total phosphorus, total potassium, the number of conidia and the ability of *Trichoderma harzianum* inoculum in decomposing organic matters. To determine the effect of the treatment, then data obtained were examined by ANOVA analysis with Statistical Product and Service Solutions (SPSS) software and conducted the Duncan test if there are differences between treatments.

RESULTS AND DISCUSSION

Solid fertilizer: Anaerobic digestion has been used as an effective technology for converting different renewable organic residues into biogas; which is tagged with several advantages besides energy generation, including waste stabilization, production of biofertilizer or solid fertilizer and soil conditioners, reduction of greenhouse gas emission, decreased levels of deforestation, non-competitiveness with food crops and ease of the technology with no geographical restriction[12,15,16]. This study have obtained the results of an increase the solid fertilizer made of sludge from biogas installation and rice husk charcoal with *Trichoderma harzianum* inoculum in broken corn. The solid fertilizer also functioned as the carrier agent of *Trichoderma harzianum*. The concentration of N, P and K in total before the sludge was given the treatment were 1.3, 0.2 and 0.3%.

Total content of nutrient (N, P and K): Based on the laboratory test, after given the treatment, the solid fertilizer that was enriched with bio-protector using *Trichoderma harzianum* inoculum in broken corn with the volume of 0, 37.5, 75 and 112.5 g showed the average concentration of total nitrogen (total N) of 1.4, 1.9, 2.0 and 2.1%. With the addition of rice husk charcoal and sugar water, the sludge with 0 g of *Trichoderma harzianum* inoculum in broken corn showed an increase of the total N content. This increase was apparently due to the addition of sugar water. The addition of *Trichoderma harzianum* inoculum in broken corn (37.5, 75 and 112.5 g) and sugar into the mixture of sludge and rice husk charcoal resulted in the increase of total N. This was presented in Table 1. *Trichoderma harzianum* functioned as decomposer that can break down decaying organisms such as debris of leaves and twigs into compost[13].

Based on the data in Table 1, the laboratory analysis and observations showed that in solid fertilizer that was enriched with 0, 37.5, 75 and 112.5 g of *Trichoderma harzianum* inoculum in broken corn, the average of total phosphorus (total P) are 0.1, 0.2, 0.7 and 1.1%. After the addition of rice husk charcoal and water only, the sludge with 0 g of *Trichoderma harzianum* inoculum had a decrease in total P. The decrease was apparently due to the damage during the sterilization, the reduction of the sludge volume when tested and it did not increase despite the addition of sugar water. On the contrary, the addition of 37.5, 75 and 112.5 g of *Trichoderma harzianum* inoculum and sugar would increase the total P of the sludge and rice husk charcoal mixture.

As presented in Table 1, the laboratory analysis and observations also showed that in solid fertilizer that was enriched with 0, 37.5, 75 and 112.5 g of *Trichoderma harzianum* inoculum in broken corn, the average of total potassium (total K) were 0.2, 0.6, 0.6 and 0.9%. After the addition of rice husk charcoal and water only, the sludge with 0 g of *Trichoderma harzianum* inoculum had a decrease in total K. The decrease was apparently caused by a damaged during the sterilization, the reduction of the sludge volume when tested and it did not increase despite the addition of sugar water. On the contrary, the addition of 37.5, 75 and 112.5 g of *Trichoderma harzianum* inoculum and sugar would increase the total K of the sludge and rice husk charcoal mixture.

The mixture of sludge and rice husk charcoal enriched with *Trichoderma harzianum* inoculum in broken corn could not yet be used as solid fertilizer because the minimum standard as stated in 2011 Regulation of Agriculture Minister was 4% while the concentrations of N, P and K in this study were only 2.1, 1.1 and 0.9%. According to Moussa[17] that *Trichoderma harzianum* is the most effective biocontrol agent. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani* were observed by scanning electron microscopy. *Trichoderma harzianum* attached to the host by hyphal coils. Attitalla and Salleh[18] reported that *Trichoderma harzianum* not only prolonged the metabolic activity of the entrapped organism but also it promotes the slow release of microbial spores into the medium for successful enzyme production.

Conidia or spores test: According to study demonstrated by Howell[19], Kucuk and Kivanc[20], Harman[21] and Mir *et al.*[22] *Trichoderma harzianum* completely overgrew on the colony of the pathogens fungi. *Trichoderma harzianum* treatment reduced the mycelia growth of the pathogenic fungi due to the rapid growth of *Trichoderma harzianum* which colonized medium surface and a substrate. In this study determined the viability of *Trichoderma* conidia or spores that exist in the mixture of sludge and rice husk charcoal, then conducted serial dilutions (10^{-4}) using sterile water/distilled water.

Table 2 showed that T1 formula in the 1st observation or on the 14th day of incubation had a greater amount of conidia (5.29×10^9) than T2 (2.50×10^9) and T3 (2.50×10^9). Similarly, in 2nd observation or on the 21st day of incubation, a number of conidia in T1 was greater (5.00×10^9) compared to T2 (4.17×10^9) or T3 (3.33×10^9). However, this amount of T1 decreased when compared to the 1st observation, while the amount of T2 and T3 increased in 2nd observation. From all observations, it can be seen that T1 reached its optimal amount of conidia on the 4th and 5th observation or on the

Table 1: Average of total nutrient (total N, P and K) in solid fertilizer made from the mixture of sludge, rice husk charcoal and enriched with *Trichoderma harzianum* inoculum

Trichoderma harzianum inoculum in broken corn (g)	Concentration total (%)		
	N	P	K
0	1.4^a	0.1^a	0.2^a
37.5	1.9^b	0.2^a	0.6^b
75.0	2.0^c	0.7^b	0.6^c
112.5	2.1^c	1.1^c	0.9^d

$^{a-d}$Same letters in the same column indicate the absence of significant difference ($p>0.05$), while different letters in the same column show a significant difference ($p<0.05$)

35th and 42nd day of incubation and then decreased. The T2 achieved its greatest amount of conidia on the 2nd and 4th observation and then decreased. The T3 had the average amount of 2.50×10^9 from the beginning to the end of the observation. Changes in total conidia of solid fertilizer made from the mixture of sludge, rice husk charcoal and *Trichoderma harzianum* in each observation period has presented the data in Fig. 2.

Figure 2 showed the changes that occur during 9 weeks of inoculation. Initially, the three formulas did not show an obvious trend of slow growth, rapid growth and static phase. However, each formula had a different trend. For T1 formula, the optimal trend in the 1st observation declined in the second and the third, but rose again in the 4th observation. There was no change in the 5th observation, while for the 6th, 7th and 8th observations, it had a static phase.

Table 2: Average conidia amount of *Trichoderma harzianum* inoculum in the mixture of sludge and rice husk charcoal

Observation time	Average amount (conidia mL^{-1}) T1	T2	T3
1	5.29×10^{9a}	2.50×10^{9b}	2.50×10^{9b}
2	5.00×10^{9a}	4.17×10^{9a}	3.33×10^{9a}
3	3.33×10^{9a}	3.33×10^{9a}	2.50×10^{9a}
4	5.00×10^{9a}	4.17×10^{9a}	2.50×10^{9b}
5	5.00×10^{9a}	3.33×10^{9b}	2.50×10^{9b}
6	2.50×10^{9a}	2.50×10^{9a}	2.50×10^{9a}
7	2.50×10^{9a}	2.50×10^{9a}	3.33×10^{9a}
8	2.50×10^{9a}	2.50×10^{9a}	2.50×10^{9a}

[a,b]Same letters in the same row indicate the absence of significant difference ($p>0.05$), while different letters in the same row show a significant difference ($p<0.05$)

The observation of T2 began with low growth trend which then rose in the 2nd observation, fell again in the 3rd, up again in the 4th, down again in the 5th observations and finally had a static phase in the 6th, 7th and 8th observations.

The trend of T3 was different, began with the lowest state, then climbed up in the 2nd observation. In the 3rd to 6th observations, it had a static state, then increased in the 7th observation and fell again in the 8th observation.

From the beginning to the mid of observation period, T1 had the greatest amount of conidia with the least addition of the inoculum compared to T2 and T3. All of the three treatments had relatively similar environmental conditions, especially humidity and the amount of food in the formulas. The addition of a small amount of inoculum in T1 made the food sufficient for its needs, while in T2 and T3, the greater amount of conidia given triggered the competition between them so those which had not get enough food would be dead and this decreased the number of conidia. After the 6th observation, all of the formulas (T1, T2 and T3) showed the same average amount of conidia. It was because the conidia were in an active state but they ran out of food. They indicated a static state and it would continue to decline if the environmental conditions were conducive to life but there was no food supply. For that, the changes by making the environmental conditions not conducive to life, but supported the dormant state. The environment should be made dry and have a neutral pH or alkaline state. This was in accordance with the opinion of Sitepu *et al.*[23], which stated that the fungi

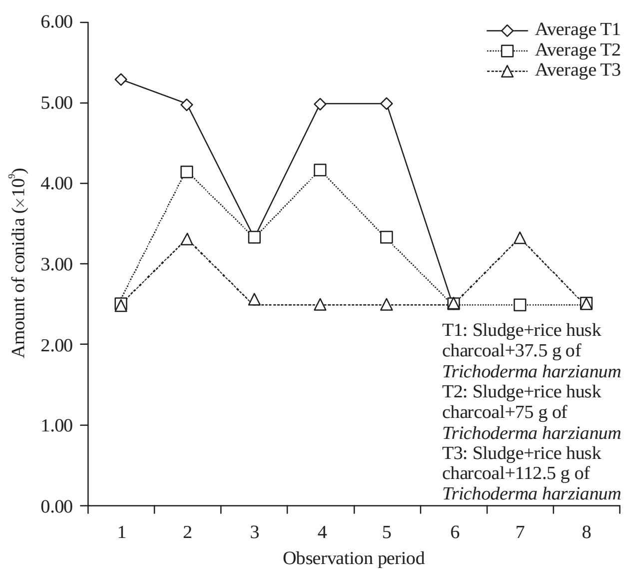

Fig. 2: Changes in total conidia of solid fertilizer made from the mixture of sludge, rice husk charcoal and *Trichoderma harzianum* in each observation period

Table 3: Average result of *in vivo* tests on mustard greens plants using solid fertilizer as the carrier of *Trichderma harzianum*

Treatments	Average amount of leaves (piece)	Average weight of crop residue (g)	Average longest root (cm)
T0	7[a]	27[a]	9[a]
T1	6[a]	17[a]	11[a]
T2	6[a]	25[a]	8[a]
T3	6[a]	19[a]	9[a]
Tk	6[a]	14[a]	10[a]
POr	6[a]	22[a]	11[a]

[a]Same letters in the same column indicate the absence of significant differences ($p>0.05$)

of *Trichoderma harzianum* grown effectively on acid soil, while in neutral pH the germination was obstructed and in alkaline conditions, they did not even sprout at all.

***In vivo* plant test:** Table 3 shows the average result of *in vivo* tests on mustard green plant using solid fertilizer as the carrier of *Trichoderma harzianum*. The average of the maximum amount of leaves in T0 was 7 pieces, while other treatments get fewer results which were each 6 pieces. The experiment showed no linear result from each repetition in the same treatment. This suggested that there was no significant effect in each treatment.

The data of the average weight of crop residue showed that T0 had the highest value (27 g), while Tk had the lowest value (14 g). The experiment showed no linear result from each repetition in the same treatment. It showed no significant effect in each treatment. Table 3 also showed that the longest root average exists in POr and T1, each had 11 cm and the shortest roots were in T2. The experiment showed no linear result from each repetition in the same treatment. This suggested that there was no significant effect in each treatment. It was because *Trichoderma harzianum* incubated in the manure fertilizer were not yet capable decomposing the organic matter within the growing medium into a ready-to-use formula. This was due to the addition of organic material in the form of ready-to-use manure fertilizer for the entire treatment. This resulted in the lack of opportunity for *Trichoderma harzianum* to break down the undecomposed organic matter into ready-to-use nutrients. The growing medium was not yet adequate for the life of *Trichoderma harzianum*. The result of study Busko *et al.*[24] indicated that the potential of using of *Trichoderma* competitors for the control of mycotoxin production in grain as well as to reduce the toxigenic *Fusarium* inoculums levels in cereal debris it is considered a biocontrol strategy to reduce deoxynivalenol levels in crops residues. *Trichoderma harzianum* may be very useful fungi in biological control against cereal aggressive and toxigenic *Fusarium* species preventing *Fusarium* mycotoxin accumulation in plant tissues. This will happen when the growing medium is sufficient for the life of *Trichoderma harzianum*.

CONCLUSION

The conclusion of the study is the increase in concentration nutrient (N, P and K) and the formula which had the highest average value was T3 (with the addition of 112.5 g of *Trichoderma harzianum*). Every time a number of conidia/conidiophores in each treatment was measured, the results fluctuated. *In vivo* test showed no significant result from all given formulas. This was because *Trichoderma harzianum* incubated in the manure fertilizer were not capable decomposing the organic material in the growing medium. The ability of the mixture of sludge and rice husk charcoal as the carrier medium would increase the sale value of the sludge.

ACKNOWLEDGMENTS

We thanks to Head Laboratory of Leather, Waste and By-Products Technology, Faculty of Animal Science UGM for providing experimental facilities, materials and technical support throughout this study.

REFERENCES

1. Gordon, S.B., N.G. Bruce, J. Grigg, P.L. Hibberd and O.P. Kurmi *et al.*, 2014. Respiratory risks from household air pollution in low and middle income countries. Lancet Respirat. Med., 2: 863-860.
2. Lu, X., D. Han and Z. Huang, 2011. Fuel design and management for the control of advanced compression-ignition combustion modes. Prog. Energy Combust. Sci., 37: 741-783.
3. Akhtar, J. and N.A.S. Amin, 2011. A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. Renewable Sustain. Energy Rev., 15: 1615-1624.
4. Raheem, A., W.W. Azlina, Y.T. Yap, M.K. Danquah and R. Harun, 2015. Thermochemical conversion of microalgal biomass for biofuel production. Renewable Sustain. Energy Rev., 49: 990-999.
5. Yentekakis, I.V., T. Papadam and G. Goula, 2008. Electricity production from wastewater treatment via a novel biogas-SOFC aided process. Solid State Ionics, 179: 1521-1525.
6. Bakhov, Z.K., K.U. Korazbekova and K.M. Lakhanova, 2014. The kinetics of methane production from co-digestion of cattle manure. Pak. J. Biol. Sci., 17: 1023-1029.

7. Bond, T. and M.R. Templeton, 2011. History and future of domestic biogas plants in the developing world. Energy Sustain. Dev., 15: 347-354.
8. Sutaryo, S., A.J. Ward and H.B. Moller, 2014. Ammonia inhibition in thermophilic anaerobic digestion of dairy cattle manure. J. Indon. Trop. Anim. Agric., 39: 83-90.
9. Wahyuni, S., 2012. Menghasilkan Biogas dari Aneka Limbah. Agromedia Pustaka, Jakarta, ISBN: 9790063636, Pages: 104.
10. Simamora, S., Salundik, S. Wahyuni and Surajudin, 2006. Membuat Biogas, Pengganti Bahan Bakar Minyak dan Gas dari Kotoran Ternak. Agromedia Pustaka, Jakarta, ISBN: 979370294X.
11. Antoni, D., V.V. Zverlov and W.H. Schwarz, 2007. Biofuels from microbes. Applied Microbiol. Biotechnol., 77: 23-35.
12. Weiland, P., 2010. Biogas production: Current state and perspectives. Applied Microbiol. Biotechnol., 85: 849-860.
13. Soenandar, M. and R.H. Tjachjono, 2012. Membuat Pestisida Organik. Agromedia Pustaka, Jakarta, ISBN: 9790064195, Pages: 126.
14. Umrah, T. Anggraeni, R.R. Esyanti and I.N.P. Aryantha, 2009. [The antagonisticity and effectiveness of *Trichoderma* sp in controlling *Phytophthora palmivora* development on cocoa pod]. J. Agroland, 16: 9-16.
15. Parawira, W., 2009. Biogas technology in sub-Saharan Africa: Status, prospects and constraints. Rev. Environ. Sci. Bio/Technol., 8: 187-200.
16. Surendra, K.C., D. Takara, A.G. Hashimoto and S.K. Khanal, 2014. Biogas as a sustainable energy source for developing countries: Opportunities and challenges. Renew. Sustain. Energy Rev., 31: 846-859.
17. Moussa, T.A.A., 2002. Studies on biological control of sugarbeet pathogen *Rhizoctonia solani* Kuhn. J. Biol. Sci., 2: 800-804.
18. Attitalla, I.H. and B. Salleh, 2010. Improvement of carboxymethyl cellulase and xylanase production by alginate immobilized *Trichoderma harzianum*. Biotechnology, 9: 529-532.
19. Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis., 87: 4-10.
20. Kucuk, C. and M. Kivanc, 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol., 28: 111-115.
21. Harman, G.E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96: 190-194.
22. Mir, G.H., L.S. Devi, S. Ahmad, V.M. Kumar and P. Williams, 2011. Antagonistic potential of native isolates of *Trichoderma viride* on corm rot pathogen complex of saffron (*Crocus sativus*) in Kashmir. Plant Pathol. J., 10: 73-78.
23. Sitepu, H., U. Suryanti and S. Purwantisari, 2011. Eksplorasi jamur antagonis spesifik lokal untuk pengendalian jamur patogen penyebab busuk daun dan umbi Tanaman Kentang. Agromedia, 29: 50-57.
24. Busko, M., J. Chełkowski, D. Popiel and J. Perkowski, 2007. Solid substrate bioassay to evaluate impact of *Trichoderma* on trichothecene mycotoxin production by *Fusarium* species. J. Sci. Food Agric., 88: 536-541.