



Changing Spent Mushroom Substrate into a Quality Vermicompost

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Abstract Safety food and healthy food gain widespread popularity nowadays. In Thailand, mushroom consumption is increasing because of its high protein content and role as an effective stimulant of various immune system functions. As mushroom farmers' respond to this demand, the need for sustainable waste management of mushroom cultivation has become important. Therefore, the study examined the growth and reproduction of earthworm and chemical properties of *Eudrilus eugeniae* vermicompost cultured under different ratio of spent mushroom substrate. The study found that application of bedding with spent mushroom substrate at the ratio 60:40 gave the highest growth rate of earthworms (2.5%) and had the highest increase in nutrient content (Total N, P, K, Mg, Ca) and had the highest decrease in the value of pH, electrical conductivity, organic carbon and C/N ratio significantly. Based on these results, application of bedding with spent mushroom substrate at the ratio of 60:40 seemed to be the most suitable for vermicompost production. In general, recycling of spent mushroom substrate through vermicomposting may reduce the environmental stress and can produce organic fertilizers with better chemical and biological properties.

Keywords spent mushroom substrate, mushroom, earthworm, vermicompost, vermicompost quality

INTRODUCTION

Safety food and healthy food gain widespread popularity nowadays. The effect to mushrooms consumption is increasing because of mushroom are a healthy food. The world production of *Pleurotus ostreatus* was about 4.1 million tons in the year 2019 (FAO, 2020). In Thailand has the potential to produce mushrooms in the industrial sector. The export data of mushroom products in 2011 showed that the total export volume was 939 tons, increasing from 2008 with 781 tons of export volume. The total mushroom production is 120,000 tons per year (Chanyuth, 2018).

The production 1 kg of mushrooms generates about 5 kg of spent mushroom substrate (Hrebeckova et al., 2020). The spent mushroom substrate (SMS) is a by-product generated from mushroom production, which contains a large amount of fungal mycelium and extra-cellular lignocellulosic enzymes along with various organic substances (carbohydrates, proteins, and fats), as well as a considerable quantity of inorganic nutrients such as ammonium nitrate, superphosphate, and potassium salts, (Lou et al., 2017; Gong et al., 2019). In the year 2017 there was about 20.5 million tons of SMS generated from *Pleurotus ostreatus* cultivation around the world. (Huan-Na et al., 2017; Wang et al., 2020). In Thailand found about 1.1 million tons of SMS in the year 2020. These SMS are

commonly sent to landfill or openly burnt at farms and could not be reused in the next cultivation due to the possibility of contamination which will consequently affect the mushroom production. As mushroom farmers' respond to this demand, the need for sustainable waste management of mushroom cultivation has become important.

Vermicomposting is a sustainable technique to convert organic wastes into a nutrient-rich humus-like product, important substances that are beneficial to plants such as plant growth promoters. through the joint action of earthworms and microorganisms under aerobic conditions (Edwards and Burrows, 1988). In addition, it can be easily done with low investment and high benefits. Can do both at the family and farm system. Therefore, this method is interesting for reduce SMS or organic waste and leading to produce organic fertilizers.

OBJECTIVE

This research aims to study the growth and reproduction of earthworm and chemical properties of *Eudrilus eugeniae* vermicompost cultured under different ratio of spent mushroom substrate.

METHODOLOGY

Materials for Vermicomposting

The SMS was obtained from the Sufficiency Mushroom Learning Center, Tha Phra, Mueang, Khon Kaen, Thailand. Its is waste from the cultivation of *Pleurotus ostreatus*. The SMS containing sawdust (80-90%), rice bran (5-10%), rice broken (1%), lime (2%), gypsum (0.5%), sodium sulfate (0.2%) and pumice (2%), at 65-70% humidity. All samples are dried in the shade for 7 days to feed earthworms and bedding was obtained from faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand including soil, cow dung, rice husk ash and vegetable waste at ratio 4: 3: 2: 1 dry weight respectively (Iwai et al., 2011).

Treatments and Experimental Design

In this study, the earthworm *Eisenia eugeniae* (density about 20 pcs/kg) was selected for vermicomposting because it tolerates a wide range of pH, temperature and moisture content. Plastic buckets of the diameter size 50 cm × height 18 cm were used to accommodate 4 kg of feed materials. Each container had a 0.2 m² of exposed surface covered with mosquito net to prevent hindrance from small insects and other microorganisms which could hamper the findings of this study. Punch holes in the bottom of the tank for good drainage and air. The materials used cultivating earthworms were cultured under bedding (B) with SMS in 6 different ratios were 100: 0, 80:20, 60:40, 50:50, 40:60 and 20:80 respectively. The study planned a Completely Randomized Design (CRD) there were 6 treatments and 3 replications with 45 days period.

Measurements

The number of earthworms were monitored at the beginning and the end of process following the methods of Gong et al. (2019). The electrical conductivity (EC) and pH were determined in an aqueous solution (sample: water, 1:10) using a conductivity meter and a pH meter (Unuofin and Mnkeni, 2014). The wet oxidation method of Walkley (1934) was used for determination of organic carbon (OC). Total nitrogen was analyzed following a modified semi-micro Kjeldahl method. For the determination of total phosphorus, potassium, magnesium and calcium 0.2 g of dry sample was digested with 70% (v/v) sulfuric acid and 65% (v/v) hydrogen peroxide. Total phosphorus was measured by

Spectrophotometric molybdovanadophosphate method. Total potassium was determined using a flame photometer. Total Calcium and Magnesium measured with atomic absorption spectrophotometer. (AOAC, 2000). C/N ratio values were calculated and analysed.

Statistical Method Analysis

The reported results are the mean of three replicates with standard error (SE). Chemical properties were analyzed by repeated measures analysis of variance (ANOVA). Mean values were compared by Tukey's HSD method. Differences were considered significant, only when P values were lower than 0.05.

RESULTS AND DISCUSSION

Earthworm Growth and Reproduction

During the 45 day of vermicomposting, The number of juvenile earthworm significantly increased in all treatments ($p \leq 0.05$) and adult earthworm increased in B+SMS 50:50 and B+SMS 60:40 (Table 1).

Table 1 The growth and reproduction of earthworm at the beginning and the end of the vermicomposting process by using *Eudrilus eugeniae* in different ratio for 45 days

| Parameter | The number of adult earthworm | | | Juvenile earthworm |
|---------------|-------------------------------|-----------|--------------------|--------------------|
| | Initial | Final | Percent change (%) | |
| B + SMS 100:0 | 80 ± 0.00 | 77 ± 1.11 | -3.75 ± 1.38 | 4 ± 1.56b |
| B + SMS 80:20 | 80 ± 0.00 | 77 ± 2.00 | -3.75 ± 0.63 | 49 ± 11.78ab |
| B + SMS 60:40 | 80 ± 0.00 | 82 ± 0.89 | 2.50 ± 1.11 | 101 ± 7.00a |
| B + SMS 50:50 | 80 ± 0.00 | 83 ± 1.56 | 3.75 ± 0.63 | 49 ± 14.00ab |
| B + SMS 40:60 | 80 ± 0.00 | 76 ± 4.22 | -5.00 ± 1.88 | 69 ± 11.78ab |
| B + SMS 20:80 | 80 ± 0.00 | 80 ± 0.00 | 0.00 ± 0.00 | 62 ± 2.44ab |
| F -test | ns | ns | ns | * |

ns = non significantly different *, ** = significantly different at $p \leq 0.05$ and $p \leq 0.01$, respectively. Similar letter within the same column indicates no significant difference by Tukey's HSD test. The numerical value of \pm shows standard error. B = Bedding (soil: cow dung : rice husk ash: vegetable waste; 4:3:2:1), SMS = spent mushroom substrate.

These changes in amount of earthworm may have reflected the greater availability of food at the start of the incubation and the reduction of food over time. The substrate ratios added significantly altered *Eisenia eugeniae* number during the vermicomposting. The maximum individual number of earthworm and maximum individual growth rate significantly higher in all bedding with SMS addition treatments than in the control. Among all substrates tested, the growth rate of earthworm had the highest increase in the B+SMS 60:40 and B+SMS 50:50. However, low quality organic waste may cause earthworms to lose weight and increase mortality during vermicomposting. (Gong et al., 2019).

Physiochemical Change during Vermicomposting Process

The pH, EC, organic carbon and C/N ratio value at the end of the process significantly decreased in all treatments ($p \leq 0.05$). (Table 2) The decreasing of pH in the early stage can be attributed to the degradation of the organic compounds, which leads to the generation of ammonia, and the subsequent decrease in pH might be due to the production of intermediate organic acids (Hanc and Chadimova, 2014). The B+SMS 60:40 treatment had the highest decrease in the value of EC was 1.67 dS m⁻¹

(43.85%). The decreasing of EC during vermicomposting confirms by Lou et al. (2017) which spent mushroom compost for 12 weeks using earthworms *Eisenia andrei* and *Eisenia fetida*. It had (8580 $\mu\text{S cm}^{-1}$) decreased about 40% when compared to week zero (14,650 $\mu\text{S cm}^{-1}$).

Table 2 Chemical characterization of materials at the beginning and at the end of the vermicomposting process using *Eisenia eugeniae*

| Parameter | | B + SMS 100:0 | B + SMS 80:20 | B + SMS 60:40 | B + SMS 50:50 | B + SMS 40:60 | B + SMS 20:80 | F- test |
|---|---------------|------------------|------------------|------------------|------------------|------------------|------------------|------------|
| pH | Initial | 7.69 ± 0.11b | 8.06 ± 0.07a | 7.80 ± 0.08ab | 7.53 ± 0.10bc | 7.51 ± 0.01bc | 7.36 ± 0.05c | ** |
| | Final | 7.64 ± 0.02a | 7.56 ± 0.03ab | 7.35 ± 0.06ab | 7.30 ± 0.18ab | 7.30 ± 0.04ab | 7.24 ± 0.11b | * |
| | Change (%) | -0.65 ± 1.22b | -6.20 ± 0.79a | -5.77 ± 0.56a | -3.05 ± 1.20ab | -2.80 ± 0.70ab | -1.63 ± 1.00b | ** |
| EC (dS m ⁻¹) | Initial | 2.55 ± 0.05b | 2.48 ± 0.11b | 2.96 ± 0.16ab | 2.57 ± 0.11b | 3.14 ± 0.17a | 3.09 ± 0.13a | ** |
| | Final | 2.27 ± 0.15ab | 1.97 ± 0.13ab | 1.67 ± 0.09b | 1.57 ± 0.23b | 1.83 ± 0.08b | 2.84 ± 0.52a | ** |
| | Change (%) | -11.03 ± 1.13c | -20.30 ± 3.54bc | -43.85 ± 3.93a | -39.17 ± 6.36ab | -41.59 ± 3.43a | -23.69 ± 1.13bc | ** |
| Organic carbon (%) | Initial | 3.41 ± 0.19d | 5.12 ± 0.04d | 11.74 ± 1.24b | 8.46 ± 0.64c | 17.28 ± 0.18a | 18.27 ± 1.29a | ** |
| | Final | 2.69 ± 0.33d | 3.49 ± 0.27d | 4.62 ± 0.16c | 5.85 ± 0.16b | 6.95 ± 0.22b | 18.16 ± 0.52a | ** |
| | Change (%) | -20.63 ± 3.71b | -31.76 ± 5.75b | -60.14 ± 3.07a | -30.31 ± 6.25b | -59.77 ± 1.37a | -0.24 ± 4.76c | ** |
| Total nitrogen (g kg ⁻¹) | Initial | 1.17 ± 0.02 | 1.28 ± 0.02 | 1.17 ± 0.02 | 1.40 ± 0.00 | 1.52 ± 0.02 | 1.41 ± 0.02 | ns |
| | Final | 1.87 ± 0.02b | 2.45 ± 0.02ab | 3.03 ± 0.02a | 2.10 ± 0.02b | 2.33 ± 0.02ab | 1.75 ± 0.00b | ** |
| | Change (%) | 61.11 ± 7.41ab | 97.22 ± 12.50ab | 166.67 ± 2.38a | 50.00 ± 12.50b | 55.00 ± 13.33b | 30.56 ± 12.50c | ** |
| Total phosphorus (g kg ⁻¹) | Initial | 0.36 ± 0.00a | 0.34 ± 0.00ab | 0.30 ± 0.00c | 0.30 ± 0.00c | 0.32 ± 0.00bc | 0.25 ± 0.00d | ** |
| | Final | 0.79 ± 0.00a | 0.64 ± 0.00b | 0.64 ± 0.00b | 0.69 ± 0.00b | 0.65 ± 0.00b | 0.54 ± 0.00c | ** |
| | Change (%) | 119.63 ± 5.63ab | 83.56 ± 3.74c | 113.63 ± 3.05ab | 124.85 ± 6.79a | 100.09 ± 7.78bc | 113.09 ± 3.12ab | ** |
| Total potassium (g kg ⁻¹) | Initial | 2.19 ± 0.01ab | 2.10 ± 0.01ab | 1.83 ± 0.01b | 2.05 ± 0.01ab | 2.28 ± 0.01a | 2.02 ± 0.01ab | ns |
| | Final | 2.68 ± 0.01 | 2.97 ± 0.02 | 2.69 ± 0.02 | 2.46 ± 0.01 | 2.89 ± 0.01 | 2.44 ± 0.01 | ns |
| | Change (%) | 22.81 ± 8.81 | 42.11 ± 12.74 | 48.70 ± 12.58 | 20.35 ± 2.74 | 27.10 ± 3.04 | 20.78 ± 0.57 | ns |
| Magnesium (g kg ⁻¹) | Initial | 1.02 ± 0.00d | 1.35 ± 0.01cd | 1.52 ± 0.01cd | 1.65 ± 0.02bc | 2.18 ± 0.02b | 3.26 ± 0.02a | ** |
| | Final | 1.21 ± 0.01d | 1.49 ± 0.00cd | 1.67 ± 0.02cd | 1.97 ± 0.02c | 2.52 ± 0.01b | 3.68 ± 0.02a | ** |
| | Change (%) | 18.96 ± 0.15 | 11.07 ± 2.26 | 10.26 ± 3.85 | 21.07 ± 1.79 | 16.54 ± 5.74 | 13.84 ± 1.03 | ns |
| Calcium (g kg ⁻¹) | Initial | 0.80 ± 0.01d | 1.00 ± 0.00cd | 1.06 ± 0.01cd | 1.28 ± 0.00c | 1.79 ± 0.01b | 2.80 ± 0.02a | ** |
| | Final | 0.88 ± 0.01d | 1.09 ± 0.00cd | 1.41 ± 0.01bcd | 1.53 ± 0.01bc | 1.91 ± 0.01b | 3.96 ± 0.02a | ** |
| | Change (%) | 9.51 ± 4.69c | 9.30 ± 3.13c | 34.23 ± 4.38ab | 19.40 ± 3.55b | 7.05 ± 3.05c | 42.40 ± 3.36a | * |
| C/N ratio | Initial | 29.92 ± 4.75c | 40.61 ± 5.23c | 102.45 ± 5.04ab | 60.45 ± 4.59bc | 115.04 ± 9.83a | 135.68 ± 9.07a | ** |
| | Final | 14.63 ± 2.41c | 14.32 ± 0.76c | 15.46 ± 1.95c | 28.49 ± 4.19b | 30.02 ± 3.11b | 103.77 ± 2.94a | ** |
| | Change (%) | -50.01 ± 5.83b | -63.86 ± 5.95ab | -84.50 ± 2.96a | -52.60 ± 8.07b | -73.80 ± 2.54ab | -20.00 ± 6.47c | ** |

ns = non significantly different *, ** = significantly different at $p \leq 0.05$ and $p \leq 0.01$, respectively. Similar letter within the same column indicates no significant difference by Tukey's HSD test. The numerical value of \pm shows standard error. B = Bedding (soil: cow dung : rice husk ash: vegetable waste; 4:3:2:1), SMS = spent mushroom substrate. (-) = The percent change of nutrient decreased.

The SMS is comprised of easily degradable carbon substrates which tend to benefit the microbial growth, resulting in a greater organic matter decomposition rate. From the result, had the highest decrease in the percent change of organic carbon (60.14%) and C/N ratio (84.50%) at the ratio 60: 40 of bedding with SMS. The organic carbon loss is likely due to the mineralization of the organic matter

through time (Gong et al., 2019). Elvira et al. (1998) reported that during composting there was a loss of 20-43% of organic carbon in the form of carbon dioxide. Due to microbial respiration and from the ingestion of organic matter. The longer duration either for vermicomposting or composting indirectly enhanced the quality of final end product as a result of the loss of carbon and increased nitrogen content (Kaviraj and Sharma, 2003).

The nitrogen, phosphorus, magnesium and calcium content at the end of the process significantly increased in all treatments ($p \leq 0.05$). (Table 2) While, the percent change of potassium had the highest increased at the ratio 60:40 of bedding with SMS (48.70%). But The potassium content non significantly increased at the end of the process due to microorganisms present in vermicompost utilize bound nutrients in vermicompost for their survival. This results in increased microbial activity, which leads to quicker immobilization of nutrients, causing decreases potassium content in vermicomposting. The percent change of nitrogen had the highest increased at the ratio 60:40 of bedding with SMS (166.67%) due to the high growth rate of earthworm. Causing bacteria to play a role in the mineralization process. Moreover, the excretion of mucus, fluids, enzymes and dead tissue during digestion processes containing nitrogen effect the total nitrogen content increased (Suthar, 2007; Lou et al., 2017). Application of bedding with SMS at the ratio 50:50 gave the highest percent change of phosphorus (124.85%) and magnesium (21.07%) because of earthworm like a media to increased phosphatase enzyme content, which increased content by waste from the gut becomes earthworm effect to increased phosphorus content in vermicompost (Gong et al., 2019). The calcium content had the highest increase in vermicompost from bedding and SMS at the ratio 20:80 was 3.96 g kg^{-1} . Increasing SMS rate will be higher of calcium content. This is because in the process of mushroom production are added lime, gypsum, salt and pumite to be used as food and adjusted the acidity and alkalinity for good mushroom growth. This results cause a large amount of calcium in waste from mushroom cultivation.

CONCLUSION

Waste from mushroom cultivation could improve vermicompost quality. It is a biological method that relies on earthworms farming to convert organic matter into high quality organic fertilizer. It was found that the spent mushroom substrate contained high protein and carbon content as a food source for earthworms. It can increase the amount of nutrients and microorganisms that are beneficial to plants. Application of bedding with spent mushroom substrate at the ratio of 60:40 seemed to be the most suitable for vermicompost production in term of plant nutrition and earthworm production. In general, recycling of spent mushroom substrate through vermicomposting may reduce the environmental stress and can produce organic fertilizers with better chemical and biological properties.

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